Parvin Mehdipour *Editor*

Cancer Genetics and Psychotherapy

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یک شعر علمی منعکس کننده اسرار توسط مو لانا (672-604 هجری قمری)

نفی آن یک چیز و اثباتش رواست

چون جهت شد مختلف نسبت دو تاست

A scientific poem reflects secrets, by Moulana
(1225–1293: Thirteenth century, AD)
Ignorance is an insight, but proving, is logic
When direction competes against, ratio would be dual

Inside Complexity of Human (P. Mehdipour, 1990)

This book is dedicated to:
–My family
–Thoughts feeder
–Those who believe in global sharing
–Improver of the patients' future
–Those who have initiated, developed, and paved the ways towards discovery in cancer
Rain of Love on the Heart-pathway of cancer patients

Parvin Mehdipour

Preface

Philosophical Flavors

a. It may be possible to bridge Philosophy to Cancer Science and Cancer Medicine. The Philosophy of existence harbors a complicated territory and is based on the order of codes. According to the *Moulana* (known as Moulavi), to succeed an evolutionary and fundamentally movement within the style of planning, and for any translational crusade, the following items through an inspiration mode are required:

   - Directional changes.
   - Sudden evolutionary insights.
   - Translatable broad and innovative thoughts and ideas.
   - Reason to process an evolutionary insight.
   - Not facing the words' barrier which restricts us to reach to influential/eternal thoughts.
   - Breaking through the solid walls and limitations.
   - Promoting a movement through a motivator to emerge the valuable and translatable achievements from layer to layer of our thoughts.
   - An evolutionary crusade towards fertilizing the ideas and philosophy of existence.

   The nature of MOULANA's thoughts is reflective of:

   - Sea of demands.
   - Demand is an option to unmask the life origin.
   - Continuous efforts.
   - Tracing the hidden facts and realities.
   - A remarkable gap between demands and obtainability.

b.

یک شعر منعکس کننده پیچیدگی هستی توسط شیخ محمود شبستری (718-692هجری قمری):

جهان را سر به سر آیینه ای دان

به هر یک ذره در صد مهر تابان

A poem by S.M. Shabastari, is reflective of the existence complexity (1313–1339: Fourteenth century, AD):

Each element of the existing globe is a mirror.
Each element is reflective of hundreds of shiny suns.

He has delineated the world of existence in which each tiny element is engaged with a key duty. Each element harbors the entire global positive spirit. In another word, they have the same philosophy of achievements in common, but with diverse spectrum.

Scientific Insights of This Book

Initiation of neoplasms is originated from a single cell and development of multi-clones requires further interactive processes including metastasis (es) which is according to the programmed scenarios through the patients' life. These events are negatively supported by environmental hazards without an available systematic control system. Most importantly, predisposition is an essential requirement for initiation of different maladies including cancer. In fact, the major concern is that "how many predisposing genes have been discovered so far?" Or "will be unmasked in the future?" By considering a clear perspective, the most attractive vocabulary includes innovative/insightful/applicable, but it is peculiar to be acquainted with two important concerns; (1) what does inspire the cancer world? And (2) how would the inspiration be influential? Cancer has a traditional style and cancer management is formed by a quadrant shape including early detection, prediction, prevention, and prognosis. Such delineation would lead to design an appropriate and personalized diagnosis and therapy through a pedigree-based insight. All these are depending on the interactive information about clinical aspects, genetic, tumor cell biology, and therapeutic innovations. Besides, due to the tumor heterogeneity, the road map of the translatable paradigm is rather unclear. So, the complementary insights would, relatively, guarantee more applicable and practicable strategies.

This Book Presents 26 Chapters with the Following Contents in Brief

An introduction to pave the ways entitles basic principles of medical ethics and deals with the top issue in science and clinic which focuses on the fundamental facts and its application in Medicine and cancer patients by emphasizing on the rules and traditional insights in different individuals. The manner of managements for cancer patients and considering their lifestyle are also highlighted.

Chapter 1 "Psycho—Oncology: The Relationship Between Psychology, Personality and Cancer" has considered other medical/noncancerous complications as well. Regarding the supportive managements, etiology, diagnostics and prevention were also highlighted.

Chapter 2 "Psychotherapy in Cancer" highlights the importance of social supportive systems in different populations. The role of involved factors in cancer therapy and the alternative medicine was also explored. Besides, the impact of influential therapy on the patients' style of life was considered in this chapter.

Chapter 3 "New Developments in Psycho-Oncology" provides the relevant psychological factors in cancer patients by highlighting the declining side effects of routine therapies offered by oncologists and radiotherapists. In this chapter, it is tried to create a link between psychology and oncology in order to balance the interaction between body and psychological status of the cancer patients. To manage this matter, an organized program is required.

Chapter 4 "Psychiatric and Psychological Aspects of Breast Cancer: Diagnosis and Treatments" emphasized on Psychiatric and surgical diagnosis and treatments. The authors have focused on the spectrum of surgery, including cosmetic surgery of breast, and considering the required managements by psychologists. The major concern is revealed to be the lifestyle in breast cancer patients as the result of different therapeutic strategies. The authors have highlighted an interactive manner between the application of psychiatric and oncologic-based therapies.

Chapter 5 "Cancer Genetics at a Glance: The Comprehensive Insights" provides the basic approach and the involved machinery at the molecular and cellular levels. In this chapter, different aspects of genetic, miRNA, epigenetic etiology in different neoplasms such as brain and CNS, breast, alimentary system, oral, retinoblastoma, colorectal cancer, and hematopoietic disorders were also delivered. The major concern is the personalized cancer managements which require the complementary functional approach and informative follow-up studies.

Chapter 6 "The Relevance of Genetic Factors in Tumor Therapy and the Underlying Pharmacogenetic Principles" is reflective of the application of Pharmacogenetics in cancer therapy. The major items include, (1) the impact of genetic characteristics on the therapeutic managements, (2) targeted base, gene, and personalized therapy, (3) the benefit of anti-mutagenic components, and (4) experimental approach including *in vivo* and *in vitro* studies.

Chapter 7 "Genetic Counselling for Cancer Susceptibility" provides the significant fundamentals of genetic counseling, and the required molecular diagnostic test as an inherited susceptibility gene in the proband affected with cancer by considering the proband' relatives. The required information on the pedigree analysis is also provided. The complementary and supportive clinical managements, by considering the ethics, are highlighted too. The author has also emphasized on the basic exceptional molecular status such as "rare high penetrance gene mutations and less common variants of low penetrance," and the influential factors including style of life under environmental territories.

Chapter 8 "Cancer, Psychotherapy and the Airway" presented a dual insight which lead to a dramatic influence on the lifestyle of the patients affected with air way cancer. The author has provided an interaction between genetic field and psychotherapy by considering the psychological disorders such as "anxiety, depression and mood disturbance."

Chapter 9 "Female Reproductive System and Cancer" provides an interactive manner between gynecological malignancies and the psychosocial scene. This chapter focuses on epidemiology, histopathology, diagnosis, and therapy of the reproductive system. Genetic counseling is considered as a directive tool for the prevention of hereditary susceptibility of reproductive system. Author has also highlighted the importance of HPV vaccination, "Psycho-neuroimmunological" and special attention to the psychological status of patients. The therapeutic approaches in different stages of pregnancy by considering the health of mother and her offspring were also emphasized.

Chapter 10 "Cancers of the Endocrine System" reflects a multidisciplinary insights including classification, diagnostics, genetic, screening, and genetic counseling in endocrine system.

Chapter 11 "A Comprehensive Look at Oromaxillofacial and Laryngopharyngeal Cancers" is reflective of an exploration on head and neck cancer (HNC), clinical aspects, and histopathological classifications of HN. In this chapter, diagnosis, clinical managements, therapeutic aspect, targeted chemotherapy, and a cross talk between practice and psychosocial field as the beneficial impact on the survival of patients were presented. This chapter is based on a systematic insight in which the patients' education, the related managements including an early detection, based on the most informative diagnosis and most influential therapeutic strategies were highlighted.

Chapter 12 "Gastrointestinal Cancers" includes different aspects of classifications, diagnostics, clinical managements, and molecular genetic aspects in esophageal, gastric, and colorectal cancers.

Chapter 13 "Genetic and Cellular Complexity of Brain Tumors" is reflective of molecular genetics, functional aspects, and the role of psychological therapy in different types of brain tumors. The major aim in this chapter is to overwhelm the unsatisfactory status of the patients' style of life by focusing on the application of both molecular genetics and tumor biology at diagnosis and through the medical care by a systematic follow-up/cell-based study. This may facilitate to access the

way towards an early detection and clinical managements of both adult and infant group of patients affected with brain tumors.

Chapter 14 "Genetics, Hematologic and Psychological Aspects of Leukemia" includes information on the fundamental genetic and clinical aspects of leukemia. The authors have emphasized on the key points of hematologic, molecular, cytogenetic, psychological aspects in leukemic patients, classification, and also bone marrow transplantation. They have underlined the screening strategy of patients' psychological disorders, as a supportive strategy.

Chapter 15 "Cancer Stem Cell: from Conjecture to Reality" discusses the capability of the rare cancer stem cells (CSCs), in proliferation, tumor initiation, self-renewal, and invasive behavior in tumor metastasis. Author has also referred to the chemoresistance and recurrence as two notable characteristics of CSCs. However, diagnosis and therapeutic aspect of CSCs is still the main concern.

Chapter 16 "Cancer Immunotherapy: Friends or foe of Mental Health?" provides both negative and positive impacts of immunotherapy in cancer patients and their lifestyle. The authors, by providing examples, showed that the application of Interleukin-2 and IL-4 could lead to diverse depressive mode in patients. Besides, they have emphasized on the dual effects of "T-cell directed immunotherapies, dendritic cell vaccine and cytokine-induced killer cell" by destructing and improving life quality of cancer patients respectively which is a challenging item.

Chapter 17 "Challenges of Endocrine Therapy in Breast Cancer" focuses on the psychotherapeutic strategy in breast cancer patients who have received such therapy. Besides the fundamental insights of estrogen, the mechanisms involved in cell proliferation, the molecular based resistance to endocrine therapy, and the final outcome of estrogen therapy are also provided.

Chapter 18 "Skin Cancer: Genetics, Immunology, Treatments, and Psychological Care" is contemplative of different aspects in the fields of genetics, immunology, psychology, and therapy in patients affected with different types of skin neoplasms. The involved risk factors including positive family history of cancer, key gene mutations, and the immunosuppressive complications due to organ transplantation were also discussed. Besides, the epigenetics and immunologic alterations and environmental hazards are also considered as the pathogenic factors of skin cancers. Furthermore, different aspects of clinical managements and the patients 'psychological status are also discussed.

Chapter 19 "Alternative or Complementary Medicine: History and Legacy" focusses on the background, definitions of alternative, or complementary medicine. The common facts in "tradition of religious medicine" and alternative medicine are highlighted as "spiritual healing methods." Furthermore, the healing aspects in Asia (India and China) are discussed. Author has underlined the initiating movement of a global link between academic and alternative medicine.

Chapter 20 "Pharmacotherapy of Cancer from the Perspective of Traditional Iranian Medicine" explores the historical insight and application of natural anticancer agents based on the Persian traditional medicine (TPM) in cancer. The historical paradigm of TPM is known as an ancient medical application written by Avicenna in eleventh century and by Razi in tenth century. Besides, the

corresponding information and the anticancer agents' prescription of TPM were also provided.

Chapter 21 "Nutrigenomics, Epigenetics and Pain in Cancer" deals with a thoughtful complication which is a challenging paradigm. The author has focused on physiological, cellular, epigenetic, central nervous system, nutrients/phytochemical, and chemical-based components in this chapter. By introducing different signaling pathways and considering the machinery of cellular/molecular biology and nutrigenomics, the focal aim of the author was to shape an interactive territory through which the available natural elements may play influential roles in healing process against pain in cancer patients.

Chapter 22 "Metastatic Breast Cancer at a Glance: Scenarios of BC Brain- and BC Bone- Metastasis by Illustrations" provides the viewpoints on the machineries and the interactive processes between different territories in metastasis. Breast cancer brain metastasis and breast cancer bone metastasis are the focal points in this chapter. The road map in metastatic BC, the essential managements, involved genes, impact of stem cells, growth factors, and circulating cancer cells are presented. Impacts of the cellular and molecular targets on prognosis, prediction, and survival in these metastasess, and the personalized managements are also highlighted.

Chapter 23 "New Three-dimensional NLS-Bio-feedback Bio-resonance Approaches in Site Specific Diagnosis of Cancer" presents the application of "Non-linear-system and entropy method" to evaluate the body/environmental interaction. The authors have emphasized on the harmonization of internal organs and constant interaction between environment and biological machinery. Furthermore, they have stated that three-dimensional NLS is more suitable than 2D NLS for research purposes.

Chapter 24 "Human Cancer Cell Lines: Potential to Evaluate the Therapeutic Efficacy of Anticancer Agents" focuses on the application of cell line in evaluation of novel cancer drugs. The key information in such assessment includes the involved biological and molecular facts, and effectiveness of cancer drugs. Finally, the authors have highlighted the Aurora kinases as a target for research on cancer therapy.

Chapter 25 "Biostatistics Methods in Cancer Research: Cluster analysis of Gene Expression Data" deals with application of biostatistics in cancer including the modeling and classification of the relevant genes in cancer. Clustering is revealed to be a useful tool for classification of the genes involved in cancer at functional level. Besides, the authors have provided examples through which the suitability of methods in data analysis is elucidated.

Chapter 26 "Horizon of Cancer Genetics and Psycho-Art" is introspective of *Cancer, Culture, and Psycho-Art.* A summary of all chapters is provided, followed by some basic biological facts including the therapeutic topic and music therapy. In viewpoints, the peculiarities in cancer research have been delivered. Besides, further aims and tasks in cancer research are also included.

Two episodes are included which are reflective of experiences that may be useful as supportive elements for cancer patients.

As the concluding words, the question is How advisable is the personalized cancer managements to the cancer patients? The clinicians and scientists have negotiated and tried their best to achieve the strategy for personalized aims for cancer patients. Now we may ask: (1) where are we now? At the beginning or at the middle of HOPE-ROAD? And (2) are we far beyond we are hoping for? I have been cognizant of the evolutionary passageways' convolutions in cancer territory since 1978, but I am still exploring to reach to the main road. Is it required to consider the outlying restraints?

With an incredible hope to achieve our goals in cancer, let us change science to a value for cancer patients and those with benign tumor as well by trusting that *Success in cancer research is the leading bridging system to the cancer clinics.*

I do appreciate to receiving your comments.

Tehran, Iran
May 2017

Parvin Mehdipour

Contents

An Introduction to Pave the Ways

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Basic Principles of Medical Ethics

Abstract The factors addressed in the present outline of the basic principles of medical ethics are of importance to patients with tumor illnesses. However, these issues are also of relevance to ill people in general, irrespective of whether the illness is congenital or exogenous and the age at which it occurs. The outline has purposely focused on these generally applicable factors in order to emphasize and highlight their broad relevance.

Keywords Definition of health and disease • Interaction between patient and physician • Fundamental patient rights • Therapy and research • Ethnic considerations • Biopolitics, -law, and -ethics

The Relationship Between Medicine and Ethics

Medicine and ethics have always been very closely connected, since both concern human behavior. In its early history, medicine was defined as the art of healing. The word medicine derives from the Latin word ars medica (medical technique), which is a translation of the Greek terminus techne iatrike. As a medical practitioner, the physician is competent in, and responsible for, medical management. The word "techne" encompasses more than the word "skill": it also implies artisanry and science.

The word "ethics" is also Greek in origin. It derives from the word ethos, which refers to habituation, habit, or character. Ethos concerns the good life, or the sense that life is satisfying and worth living. In contemporary scientific discourse, a distinction is drawn between ethics and ethos. *Ethics* is standard science. As a thematization of the good life, the concept of *Ethos* is distinct from theoretical models of procedural judgment. Both medicine, understood here as human

medicine, and ethics concern the actions and attitudes of people. Consequently, in both disciplines, the correct action involves reflection, a consciousness of action.

Ethics considers two issues. The first concerns scrutiny of action, and the question of what constitutes the appropriate thing to do. The main question is: How should I act? Here, human action is oriented on rules, standards, and norms. As viewed from this perspective, ethics deals with rules and norms. In this context, ethics is understood as a theory of action. On the other hand, ethics considers the issue of life orientation. Here, the question is: How do I want to live? This issue can also be denoted as ethos.

From antiquity to the modern era, tradition viewed medicine as the art of healing and medical technique. This assumed an understanding of nature: medicine advanced observationally in this respect, i.e., empirically. Nonetheless, such knowledge was generated through the *experience* of physicians. The target of this knowledge was healing and therapy. Indisputably, medicine strove for rationality. Medicine should be a theoretically substantiated and well-founded practice. In the modern age, however, medicine is determined by the scientific, natural science method. The role of experience has been replaced by the experiment, by the verifiable and repeatable process. Medicine has been transformed into an applied science. Meanwhile, emphasis has been placed on evidence-based medicine. A concurrent medico-psychology perception of life does, of course, persist. From this perspective, health and disease impact all aspects of life. This viewpoint is accentuated in different ways, for example, as alternative medicine or salutogenesis. Particularly within psychiatry, this viewpoint also invariably assumes that health and illness impact human fate (Honecker 1995).

In the conflict between medical methods and approaches, one sometimes hears the maxim: "He who heals is right." Moreover, disease phenomena can be interpreted in different ways. With regard to both diagnosis and therapy, it must be borne in mind that illness *per se* does not exist, only ill people. Even the question "When is a person ill, when is he healthy?" is not always self-evident and amenable to a precise response. Is every deviation from a medically defined norm an illness? The concept of illness comprises four aspects. These are: the illness, the finding (Nosos); the ill person (the patient); the physician; and the society, the healthcare system. The ill person feels subjectively unwell, he is suffering. The physician and society are obliged to help the suffering person. The ill person, therefore, consults the physician, who elicits clinical signs. The physician establishes the presence of disease, the "Insanitas". For the physician, the ill person represents the target of his medical assistance. He, thereby, assumes personal responsibility for the ill person. In addition, the ill person needs, and lays claim to, assistance from society. As an ill person, he requires support and provision. Society is responsible for the healthcare system. In modern, differentiated societies, healthcare systems have been expanded and specialized. This refers, for example, to the organization of the healthcare system, the establishment and running of hospitals, insurance cover, and social welfare benefits. Besides the financing and organization of the health care system, state legislation is also necessary. Societal duties also include the training of physicians, the regulation of state medical examinations, and the issuing of

certificates of appointment. The formulation of rules and regulations for state medical examinations is a governmental task, despite the fact that medical privilege is widely accepted. Mention should also be made of medical research, and the establishment and control of research facilities. Society is, therefore, involved in medicine on many levels. A perpetual triangle exists between the patient, the physician, and society.

On the basis of such a differentiation, three ethical dimensions to the analysis of illness can be distinguished. The first dimension is that of *Individual Ethics.* This refers to the concern and responsibility of the individual for his own health and illness. Each person is responsible for himself. The second dimension is *Personal Ethics,* which concerns interpersonal relationships. Primarily, personal ethics concerns the relationship between the physician and the patient: it relates to their encounter as people. However, personal ethics also concerns caregivers, fellow human beings, relatives, and loved ones. These personal relationships render interpersonal communication obligatory. Furthermore, in the case of patients who lack capacity for informed consent, other persons are responsible, for example, parents, relatives, or carers. The third dimension, *social ethics,* is the ethics of social structure. This covers the institutions and organized procedures of the healthcare sector.

The organization of healthcare systems is now a major economic factor in industrial societies. As an employer, the organization that is responsible for health employs a large number of people, and operates an elaborate system of regulations and standards. The issue of social structure ethics within medicine is highly complex, and is not dealt within the present article. Neither will this article discuss spectacular and widely discussed topics of public interest from current research, or new methods and interventions, such as genome analysis, gene therapy, stem cell research, synthetic biology, advances in neuromedicine and brain research, or organ transplantation. Instead, the article will focus on the fundamental and general principles of medical ethics.

These principles are nonetheless complex. Fundamental to this from an ethical perspective is an alternative position, which is commonly denoted by the terms deonotology and teleology. The term *Deonotology* is derived from the Greek word deon, which means duty or obligation. This normative ethical position is based on immutable principles. These principles are absolute. For example, a person should never harm anyone. Interference in the development of human life and personality changes in the brain are taboo and prohibited on principle. Active euthanasia, and to some degree passive euthanasia, is immoral. Deontology refers to age-old principles and convictions. In contrast, *Teleogolgy,* which derives from the Greek word telos or aim, is oriented on consequences and results. Here, judgment is made according to the usefulness and success of an action. For this purpose, the potential benefits and disadvantages of an action are weighed-up. *Teleology* is therefore also referred to as consequentialism or impact assessment. Absolute deonotology and absolute teleogolgy are mutually exclusive and non-reconcilable. Moderate deontology and teleology, on the other hand, are often compatible. In this scenario, the weighing-up

of pros and cons is taken into account, but does not waive recognition of, and respect for, the fundamental values of life.

The considerations outlined so far refer to ethical dimensions and arguments. They purposely avoid discussion of the ethical theories and problems of fundamental ethics, since medical ethics is an *applied*, rather than a theoretical, form of ethics. An alternative term for applied ethics is *Field Ethics*. As with other types of field ethics—for example, political ethics, economic ethics, or cultural ethics—medical ethics is a traditional, classical system of field ethics. Medical ethics is now often equated with bioethics. *Bioethics* analyzes and evaluates the impact of science on life in a broad, holistic sense. This also includes all nonhuman life. Bioethics comprises the subdisciplines medical ethics, animal ethics, and environmental ethics. An extension of medical ethics to bioethics is also useful in that medical activity is often not restricted to that of a single, specific physician; instead, it commonly includes laboratory investigations, the assignment of diagnoses via technical apparatus and specialized diagnostic methods, genetic analyses, and even research on human subjects. The use of procedures and methods of this nature also gives rise to ethical problems. However, medical ethics is a core element of bioethics, as it concerns the personal stages of life. Therefore, within the context of bioethics, only topics of relevance to medical ethics will be included and discussed in the present article.

Health and Illness

Health is a fundamental and primary asset for all members of a society.

However, a clear delineation between health and illness is difficult, since both are dynamic processes. The understanding of health and illness is dependent on cultural beliefs and attitudes. No purely objective, natural science concept of disease exists. When every deviation from a defined norm of health that interferes with the well-being of a person is termed illness, the concept of disease is over-stretched. According to the World Health Organization definition, health is a "state of complete physical, mental, and social well-being and not just the freedom from disease and infirmity." Health is, therefore, the overall well-being of the person. But what exactly is well-being? Is it even equated with happiness? If so, then every unhappy person would have to be considered ill, since their well-being is impaired. Is health the same as fitness to work and productivity? Or is it the capacity for enjoyment, vitality?

No real or nominal definition of health is possible. It is only possible to describe phenomena that a person deems healthy from his own personal perspective. This has consequences in terms of the discussion of illness. *Illness* is indeed any disorder of the normal condition or normal activity of the body. However, when one adds the condition that illness only refers to a disorder that can be eliminated or improved, this definition excludes the disabled and individuals who require long-term nursing

care. This exclusion has financial and social grounds, since when there is no further possibility of cure, the health insurance provider is no longer obliged to remain involved, and the responsibility falls instead to other authorities. In either case, the person is ill if the coordinated cooperation between the physical or psychological or psychophysical functional components of the organism is disturbed, either subjectively or by a clinical finding, to the extent that the person requires medical or general support. Conversely, a person can also be deemed healthy when he can integrate into his life an unalterable disorder of his body, or of his psychological and social well-being, to the extent that he is fulfilled in all areas of life, has no loss of self-esteem, and remains healthy as a person.

Health is, therefore, more than, and yet distinct from, the absence of illness. However, one must appreciate the danger of "pathologizing" life when one says, for example, "Everyone is sick." If that were the case, the only difference between people would be the degree to which they are ill.

In religion, a connection has been made between *healing* and *salvation*. Health is the experience of salvation. One even speaks today—polemically—of health religion. Through this, health becomes the medium of a cultural grand narrative. It becomes identical to well-being. To counteract this, demystification of this overestimation of health is appropriate (see Aaron Antonowsky, Salutogenese: zur Entmystifizierung der Gesundheit 1997). Health is more than a physical condition. The term salutogenesis indicates this, and rightly so. In any case, one should not define illness and its antonym health too narrowly. Illness refers to dysfunctional states of a physical or psychological nature. Thus states of illness can be identified using correlated parameters, which can be verified empirically.

Understanding illness, therefore, combines subjective experience, objectively demonstrable findings, and social and cultural interpretation. In the majority of cases, illness has a biological basis. For this reason, illness cannot be viewed as a purely social construct. From an exclusively social science perspective, illness would be viewed only as a time-limited perception or as an ideological interpretation. The appraisement of illness is not, however, of purely theoretical significance, since it has considerable practical consequences in terms of everyday life. However, illness cannot simply be reduced to physically detectable deficits and impairments.

At the same time, many of the phenomena that are classified as illness have psychological or social causes. This raises the question of whether phases of grief experienced in response to bereavement should be classified as an illness, namely depression. A number of symptoms may occur, such as listlessness, sleep disturbance, lack of concentration, or anxiety. However, a naturalistic perspective leads to a narrowed view. For example, the issue of whether sexual deviation, or nicotine or heroin addiction, should be classified alongside cancer and diabetes as disease states cannot be decided on a purely biological basis. An exclusively naturalistic view of disease is one sided. However, it is equally one-sided to regard disease as a solely social and sociocultural paradigm, since many causes of disease are inherent to the nature of the human species.

The key to linking the different aspects of the concept of illness is the interpretation of the person who experiences his condition as an illness. The loss of control over one's own body or body parts, or over one's way of life, is a fundamental experience that triggers anxiety or a yearning for assistance. The illness affects both the person as an individual, and his self-determined lifestyle. Psychiatric disorders, in particular, are accompanied by a pronounced loss of equilibrium. In psychiatry, a disease concept cannot be formulated without reference to the subject. In everyday life, health and disease are relative terms within the sociocultural milieu. Personal life history represents the background to the illness, which often involves the interplay of social and historical causes. Ecological aspects are also relevant, such as environmental damage, noise, and climate pollution. The valence of illness also varies across cultures. From antiquity onwards, people with leprosy were excluded from society for many centuries. In contemporary society, people with HIV/AIDS often experience discrimination. In some illnesses, such as dementia, inextricably linked physical and psychological factors intermingle. On the other hand, serious diseases such as cancer can also trigger psychological effects.

Each individual should take precautions against illness. Everyone is personally responsible for their own health. An unhealthy lifestyle—for example, alcohol abuse, smoking, consumption of illicit drugs—triggers health problems. Prevention and health care can prevent illness. However, a difficult and controversial question is whether blame should be apportioned for an illness-provoking lifestyle and a lack of health care. In addition to disease control, however, disease prevention is an important medical and social task. A subjective right to health is not possible, since neither medicine nor society can provide and guarantee this right. However, a right to health protection and health care is possible. Even though a clear delineation between health and illness is problematic, illness prevention is a medical mandate.

Nature offers no definitive standards for illness and health. The state of health or illness is first recognized through contact with other people. The self-perception of the ill subject calls for medical action in the form of diagnosis, therapy, alleviation, and prevention. Here, the ill person takes center stage. Physicians and therapists are consulted. By applying their medical expertise, these parties objectify the subjective experience of illness. This requires communication between the physician and the ill person. Ideally, this serves not only as a form of diagnostic and therapeutic assistance, but also to facilitate understanding on the part of the ill person and thus enable him to interpret the illness for himself.

The Physician–Patient Relationship

The central interaction in medical practice is the relationship between the physician and the patient. The very ancient origin of this interaction can be traced back to the spontaneous or instinctive, magically religious or methodically established,

interaction between the help-seeker and the helper, or between the ill person and the healer. This form of care is found in many cultures and religions. Contemporary medico-ethical discourse addresses adequate and appropriate modes of explanation and decision-making. Here, respect for the autonomy of the help-seeking person and his right to self-determination must be respected, since an inevitable information gap exists between the medical expert and the patient. This gives the physician a monopoly on interpretation, and he has both the possibility and the ability to control the decision of the patient. The physician should, therefore, be prudent and preserve affective neutrality. In the twentieth century, the role of the physician was altered by the success of science-based medicine, the expansion of hospitals and other institutionalized healthcare facilities, and the establishment of a publicly financed healthcare system. Added to this was the professionalization and differentiation of the medical profession, which has promoted an asymmetrical relationship between physician and patient. In addition, an increasing tendency towards the economizing of medical activity is apparent, which manifests itself, for example, in demands for the rationalization and rationing of both medical services and the contribution of the physician.

In view of such developments, the normative concept of *informed consent* was introduced into clinical practice. On the basis of comprehensive and comprehensible information concerning the diagnosis and options for therapy, the patient should be able to make an independent decision. The decision must be made voluntarily and without manipulative influence. However, a problem arises with respect to both the comprehensibility of the medical information and the receptive capacity of the person who receives the information, since a discrepancy exists between medical terminology and everyday language. This forms the basis of the conventional paternalistic approach, whereby the physician makes a unilateral, paternalistic decision concerning treatment. This is also termed the "Hippocratic model," or a "priestly stance." This type of paternalistic approach is substantiated by the primacy of the physician's expert medical knowledge, whereby the paramount consideration is the welfare of the patient, "salus aegroti." Here, the principle of benefit, benevolence, takes precedence over the principle of autonomy.

The contemporary counter thesis is that the sole basis for a decision concerning treatment is the patient's unfettered self-determination. He alone decides, having received appropriate information. The will of the patient is decisive. "Voluntas aegroti suprema lex esto," the will of the patient is the highest and only standard. This view can be termed the "consumer model" but also the "contract model." The values of the physician should neither determine nor influence the decision. In the communication between physician and patient, a compromise between paternalism, and a decision made exclusively by the patient, is the goal of joint integrative decision-making. The medical consultation should also identify common values. Here, the personal values of the patient and the health-related values of the physician should be named and communicated, and then weighed-up.

This should lead to a consensus judgment. The control over the decision remains with the patient. To this extent, this model represents the ideal joint decision-making concept, although a medical consultation of this nature requires

time and mutual trust. In a crisis situation, however, the physician must decide and act spontaneously in order to preserve life or prevent and reduce harm to the patient. In certain situations, the physician may be unfamiliar with the patient and unable to ascertain their preferences in life-threatening circumstances, such as following an emergency admission to hospital or at the scene of an accident. This demonstrates the context-dependent limitations of the informed consent process. The use of new technologies also raises new questions, for example, the impact of the Internet on the information-seeking- and participation behavior of patients. The implications and consequences of telemedicine and the reduced level of personal contact between the physician and the patient also remain unclear.

In this context, the professional ethos of the medical profession becomes all the more important. This form of professional ethos has existed for millennia. The leadership of the medical profession has weighed-in on the behavior of its members and their behavior towards others. The most prominent example of this is the *Hippocratic Oath.* This states: "I will apply the principles of living to the best of my knowledge and conscience to heal the sick, but never to their detriment and harm." Since antiquity, the physician has pledged to respect the precepts of the art-of-medicine and to preserve medical confidentiality. Integrity in the male physician requires that he does not engage in sexual acts with female patients. Certain aspects of this "oath" arose from the circumstances prevailing at the time, such as the renouncement of surgical therapy for lithiasis. The Hippocratic physician does not perform bloody interventions; he leaves that to others. The question is whether medical ethics is based on particular moral principles. Unique moral principles of this kind are neither evident nor justified. Medical ethics is a field ethics. It does not concern a particular ethics, an ethics distinct from generally binding ethics, but rather the ethics of acting in a particularly demanding situation. The physician meets people during periods of disease and suffering, even during the phase of dying. He cannot guarantee a cure. On the other hand, through his medical knowledge and scientific reflection, he can strive to achieve the best possible outcome in terms of diagnosis and therapy.

Medical practice is, therefore, complex and inherently uncertain. Very few patients have the knowledge required to exercise control over their medical management. Professional organizations and representative bodies, such as medical associations, are limited in terms of the degree to which they can monitor physicians. Although medical practice guidelines can be formulated, their implementation cannot be fully monitored. A professional moral code, whose key maxim is the well-being of the client, thus expects an ethos in the physician that ensures trustworthiness and integrity. This is the only way through which personal trust in the individual physician can develop into a trust in the system.

A modern version of the Hippocratic Oath is formulated in the Geneva *Physician's Oath.* The latest revision of this from the 2006 General Meeting of the World Medical Association in Sun City states: "The health of my patient will be the highest commandment of my profession." The consent of the patient is a prerequisite for a health-related intervention. The formulation of the Geneva Physician's Oath in 1948 was also a reaction and response to the abuse of medical knowledge

and skills by physicians during the German National Socialist era. The purpose was to establish a barrier against a "medicine without humanity," which was the subject of criminal proceedings in the Nuremberg trials. For this reason, the Physician's Oath states: "I will not allow religious, national, racial, party, or class considerations to come into conflict with my duty to my patients." "Even threats will not induce me to use my medical knowledge in violation of my duty to others." Areas of dispute, which were not decided on during the formulation of the medical vows, include the prohibition of abortion—the pledge only obliges the physician to an unconditional respect for life from conception onwards—and assisted suicide. Besides these obligations on the part of physicians, an ethical obligation is now required from all professionals involved in medicine and medical bioethics.

The Rights of Patients

Besides consideration of patient–physician duality, and of medical ethos as a professional ethos, specific consideration is warranted for the position of the patient. In a modification of the words of Jesus, "The Sabbath was made for man, not man for the sabbath" (Mark 2:27): "Physicians are there for the sake of the sick, not the sick for the sake of physicians." Besides the natural science component, the anthropological component is therefore important. Illness can also be conceptualized as a life crisis, which can become a "metamorphosis." The subject in the relationship between physician and patient is therefore not the physician, but the patient. The patient is expected to collaborate with treatment, i.e., he is expected to demonstrate *compliance*.

One area of difficulty is how somatic illnesses are to be explained in terms of purely psychological experiences and pressures. The biographical history is often implicated. However, drawing an association between illness and guilt, or even punishment, is somewhat dubious, since this threatens to burden the patient with moral or religious blame in addition to the illness. This renders the patient doubly unhappy: not only does he have a serious illness; he is also accused of having been the cause of it. In addition, patients deal with illness in different ways. Religious beliefs can contribute to the mental stabilization of the affected person. By imbuing the ill person with a "positive attitude," this can facilitate "coping," as in the handling of conflicts and ailments. Another variable is the capacity to withstand stressors.

A more recently introduced term for this is *resilience.* The word resilience is derived from the Latin verb resilire: to jump back, rebound. The concept of resilience is also used in the context of reactions to ecological risks. In medicine, it refers to a psychological resistance that is based on recourse to personal and socially mediated resources. Resilience is a coping strategy. It is also referred to in the arguments of salutogenesis. Interindividual differences in the capacity to withstand stressors are indeed apparent. Although the phenomenon of resilience is

widely observed, no adequate explanation has yet been formulated. Is it attributable to individual disposition, e.g., genetic factors? Or is it acquired through the experience of coping with traumatic events? Due to individual differences of this nature, the physician must occasionally reckon with conflicts in terms of values and decisions within his relationship to the patient. A classic example is the issue of communicating the truth to patients at the bedside. Should a patient be informed of a poor prognosis in a matter-of-fact and blunt manner? Clearly, the patient should not be lied to. But when informing a patient about their diagnosis, the rule of empathy still applies. Particularly in the case of serious disease, informing a patient about the diagnosis and disease severity is a communication process. It should also be noted that the disclosure of disease status, for example, cancer, may trigger a state of shock in the patient. A particular problem is the communication of genetic disorders, especially in cases where no treatment is available. The physician is rightly expected to demonstrate truthfulness and authenticity. This is the intention behind the concept of informed consent, since informed consent is the prerequisite for the admissibility of a medical procedure. However, the issue of whether this should involve the disclosure of all conceivable risks is contentious. This can become a problem: total enlightenment concerning the potential risks of a procedure can lead a patient to become completely uncertain and unable to consent. However, the informed consent procedure presupposes that the patient is capable of consent, i.e., that he has the cognitive understanding required to consent to the intended therapy. Of equal importance to the concept of informed consent is the traditional ethical principle of the avoidance of harm. An absolute principle of self-determination can lead to patient injury. Informed consent and a moderate degree of paternalism can certainly be combined.

A further question is whether the concept of informed consent is valid across cultures, or whether it only applies to the concept of individualism found in cultures within the West. Consequently, in a pluralistic society, cultural tensions and conflicts may arise. Muslims, for example, are guided by the conviction of the omnipotence of God, and this applies whether they are the patient or the relative. They are therefore convinced that human life is the property of God, and that it cannot, and should not, be disposed of by man. In a secular society, this can lead to reservations among Muslims, for example, as regards passive euthanasia. In the clinical setting, this can result in differences of opinion between physicians on the one hand and Muslim patients and their relatives on the other. In the case of such conflicts, the involvement of an intermediary is recommended, for example, an Imam.

In the twentieth century, the introduction of modern interventions such as mechanical ventilation, artificial feeding, and resuscitation led to fears of targeted attempts at the artificial prolongation of life. In the 1970s and 1980s, this led to the design of the *advance healthcare directive,* which was made available as a printed form. The *advance healthcare directive* was first introduced in the USA, and then spread to other countries. Its introduction is attributable to the fact that the enormous increase in the potential to maintain human life, even under difficult conditions, does not only generate expectations; it also engenders fears.

Medical advances do not always provide benefit to the patient; time and again they lead merely to the extension of suffering and a prolongation of the process of death. An advance care directive can help the physician in extreme situations to formulate a management plan that is based on the wishes of the patient. However, the directive must refer to specific treatment situations. Prerequisites for the preparation of an advanced care directive are consultation, explanation, and the capacity for informed consent on the part of the patient. In place of an advanced directive, a legitimate caregiver can also make decisions for the patient. Advance directives are an expression of self-determination. Each patient has the right, in accordance with his autonomy, to refuse any treatment he does not want. However, critics of the advance directive draw attention to its limits and dangers, and point out the inability to predict future concrete possibilities for treatment at the time of its formulation. Critics also fear that with the refusal of treatment, the advance directive could open the door to active euthanasia. Advance directives are thus indicative of the patient's will, but are not the ultimate solution to a real problem, since in a decision-making situation, besides the patient's right to self-determination, the medical obligation to protect life can also be relevant.

Up to this point, the impression has been that the *only* issues of importance are ethical criteria and the patient's will. In the healthcare sector, however, criteria such as efficiency and profitability are also important. Evidence-based medicine, in particular, is considered appropriate. The effectiveness of a therapy should be demonstrated, since economic efficiency and the issue of cost must be considered. Efficiency enhancement through *Rationalization* is one strategy. In realistic terms, medical services are limited in scope and are in short supply. Rationalization is intended to facilitate the fair distribution of scarce resources. An unresolved and controversial question is which criteria should, and could, be applied to limit services. The issue of who should make decisions concerning these restrictions is thus a social and political one.

A new buzzword is the expression "wish-fulfillment medicine." Here, treatment is not triggered by the affliction of the help-seeking individual, and it is not aimed at reducing suffering. Categories of wish-fulfillment medicine include beauty operations in aesthetic medicine, pre-implantation diagnostics for the selection of gametes and embryos, and doping in sport. In wish-fulfillment medicine, a medical indication is neither the reason nor the trigger for the intervention. This changes the goals of medicine. Differentiation between necessary and non-necessary measures becomes obsolete. One example of this is *enhancement.* In bioethics, enhancement or improvement is understood as the use of pharmacological or biotechnological means to improve or enhance a healthy individual. The widespread desire for optimization is illustrated by the use of doping in sport, anti-aging agents, or substances to improve cognitive abilities such as memory performance or concentration capacity. One even speaks of neuro-enhancement. This raises difficult ethical issues. These concern health risks and side effects on the one hand, and social impacts such as competitive advantages on the other. In the context of wish-fulfillment medicine as a whole, the question also arises as to who should bear the costs.

This development also implicitly changes medical ethos. The *autonomy* of the wishes of the patient becomes the foundation of medical intervention. This also has consequences in terms of the understanding of illness. The social aspect of illness becomes dominant. In principle, wish-fulfillment medicine is without limits. Dynamic offers for treatment lead to aggressive marketing and commercialization. The purpose of offers for wish-fulfillment medicine is personal financial gain. Patients are converted into customers and consumers. A sweeping assessment of this development, whether as a clear affirmation or as a decided rejection, does not, of course, do justice to the complexity of these new possibilities. Each case must be evaluated and analyzed on an individual basis. This also entails consideration of the impact of research on diagnosis and therapy.

Therapy and Research

Research is a prerequisite for improvements in the treatment of disease. Before improved medications or novel invasive surgical procedures can be applied for the general benefit of patients, a clinical trial must be conducted. Basic research is also carried out within the field of medicine. The desire for knowledge is a fundamental human attribute. A person wants to be aware of what he does, and does not, know. Curiosity is a basic human drive. However, there are limits to what a person is entitled to know. This applies, for example, within the interpersonal realm. Personal privacy is, quite rightly, protected. No one has a right to knowledge of the private life of another person. Privacy is an asset considered worthy of protection. The confidential nature of the postal and tax systems is intended to serve the protection of privacy. Since the 1970s, data protection law has become established as an independent legal discipline. Personal curiosity is bordered by personal rights. A physician is not allowed to disclose the medical data of his patient to unauthorized third parties. The maintenance of patient confidentiality is an inherent aspect of the medical code of conduct.

In the case of scientific curiosity, i.e., the basic drive of a researcher, the situation is different. Insights generated through research should be made publically available, since they are of general interest and common value. This general rule applies in particular to medical research.

In the present context, ethical reflections on research involving substances or animals will be neither discussed nor evaluated. The sole focus here is on research involving people, human research. Hippocratic medicine was based on observational methodology. Active experiments were the exception. As is well known, for a long time, the church opposed the dissection of corpses. The performance of autopsies for teaching purposes first commenced in the fourteenth century. Thus, it was within the field of anatomy that the first steps towards scientific experimental medicine were taken. In the sixteenth and seventeenth centuries, Francis Bacon and Galileo developed a new method of scientific research. The maintenance of

tradition was replaced by the use of active, empirically based methods. Experience and tradition were replaced by the controlled and repeatable experiment. This gave rise to scientific medicine in its modern sense. The art of the physician was transformed into experimental, science-based medicine.

This development also led to the performance of morally dubious human experiments. Thus from the end of the nineteenth century, attempts were made in Prussia to regulate experiments involving humans. In 1931, comprehensive legislation concerning research into human subjects ("*Reichsrichtlinien zur Forschung am Menschen*") came into force. However, this legislation could not prevent the conduct of harmful, large-scale human experiments in National Socialist concentration camps. The victims of these experiments were either killed or subjected to severe, irreversible damage. Some of the physicians responsible for these experiments were prosecuted and convicted at the Nuremberg trials. Part of the judgment was incorporated into the "Nuremberg Code" for medical experiments. Human experiments for military purposes were also carried out in other countries, such as Japan and the USA. The unethical nature of a syphilis study from 1932, which was brought to light by the *New York Times* in 1972, generated a debate on research ethics. This resulted in a codification of research ethics standards. The 1964 "Helsinki-Tokyo Declaration on Biomedical Research," provides recommendations for physicians engaged in biomedical research involving human subjects. The most recent version was adopted in October 2013 in Fortaleza, Brazil by the General Assembly of the World Medical Association. The latest version is valid. Similarly, the Council of Europe has adopted the "Oviedo Convention." This "Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine: Convention on Human Rights and Biomedicine" was formulated in 1997 (Beauchamp and Childress 2013).

In research practice, considerable differences are found in terms of study design. For example, randomized trials involve the random assignment of subjects to different treatment groups in order to investigate the efficacy of substances or new forms of therapy. A condition for the performance of randomized trials is that the participants must be fully informed, provide voluntarily consent, and be able to terminate their participation at any time. In addition, the research must focus on the acquisition of knowledge. In German-speaking countries, debate has led to the establishment of the tripartite concept "Human experiment—experimental therapy —curative treatment." Curative treatment is directed at the individual well-being of the patient. The aim of the human experiment is the acquisition of knowledge, whereby the participant is the experimental subject. In the case of curative treatment, a licensed physician is responsible. In contrast, the human experiment is performed by a researcher who does not necessarily have to be a physician. Human experiments must be scrutinized and approved by an ethics committee. In the case of experimental therapies, the physician is free in terms of the choice of treatment, but remains bound by codes of medical conduct and the fundamental principles of medical ethics (Sass 1989; Kreß 2003).

Since the role of the physician and the role of the researcher are often combined in one person, the issue of whether the approach represents an attempt at a curative

treatment, or a human experiment, can be contentious. Prerequisites for research in human subjects are: respect for the self-determination of the subject (informed consent); the performance of a risk–benefit assessment; and the fair selection of subjects. A particularly sensitive area in this respect is research involving *non-competent subjects*. These include newborns and individuals with dementia. In some cases, the research goal will provide no benefit to the individual proband, but rather to groups or third parties. The inclusion of noncompetent subjects in research that is aimed at the benefit of third parties is highly controversial, and thus the subject of heated debate. On the one hand, these subjects are particularly vulnerable. On the other, these groups should not be excluded on principle from research, since this precludes insights into therapy. It is imperative to minimize the risk of harm, and to restrict investigative measures to a minimum. As a matter of basic principle, the use of humans as experimental objects represents an ethical problem. To determine the limits of action, the justification and necessity for research involving human subjects must be subject to continuous reflection.

Immutable limits are the respect for *human dignity* and *the prohibition of instrumentalization*. The latter refers to Kant's Formula of Humanity, which argues that a person should not treat either himself or other people as a means to an end. The Formula of Humanity, which has been a matter of extensive debate, advocates respect for the dignity of the person. People have moral status. This includes the concept of human dignity. This dignity is inviolable. Inviolable does not mean that it is not, or cannot be, violated; it means that it *should not* be violated. Respect for human dignity is mandatory. This instruction also protects ill persons. It prohibits the use of a patient for higher purposes, e.g., research purposes and the acquisition of knowledge, rather than providing him with treatment in accordance with his own will. In the former case, the patient is intentionally instrumentalized, possibly against his will. It is precisely those who are vulnerable or susceptible who are protected by the respect for human dignity. In bioethics, vulnerability is usually characterized by the absence of, or a reduction in, the ability to protect oneself or one's interests from injustice and harm.

Whether such a threat is caused by a loss of autonomy or by the social context requires clarification. Irrespective of the causes, it is incontrovertible that vulnerable people are in particular need of protection. Frequently, vulnerability is associated with the inability to formulate an informed opinion. Vulnerability is, of course, an inherent aspect of the human condition. Everyone is susceptible to injury. This basic fact must be taken into account both in human research and in medical ethics as a whole.

Individualism and Cross-cultural Bioethics

While the considerations outlined so far demonstrate the disparity between the different situations of medical action, they also show that irrespective of the various

standards and rules, the individuality of a given person must be respected. Characteristic features of most modern societies are individualism and pluralism. The individual pursues his own interests and demands autonomy and self-determination. That is his right. This implies that the requirements of societal solidarity must occasionally be resigned. One consequence of the emphasis on individuality is the emergence of a multitude of interests, needs, and goals. Due to differing expectations and requirements, pluralism becomes evident in society. While the right to individuality should in no way be criticized or questioned, individual rights must relate to the solidarity of the community. Freedom obtained at the expense of solidarity is not legitimate (Stempin 2014).

Individualism and pluralism in society also impact medicine. A positive example is the programmatic call for individualized or personalized medicine. In medical management, the therapy should take into account the individual disposition of the patient, and not represent merely a standard therapy. Every measure involved in the therapy should be justified. The appropriate pharmaceutical agent, gender-specific effects, and molecular constellations can be determined using biomarkers. In oncology, it is important to determine how patients are responding to therapy.

Mention should also be made of *palliative medicine.* The word palliative is derived from the Latin word pallium ("a cloak"). This refers to the management of patients who have entered the last phase of life due to a non-curable disease. Thus the aim is not healing and the restoration of health, but rather the assessment and treatment of pain and physical symptoms, e.g., respiratory distress, as well as the management of complaints of a psychosocial and spiritual nature. The intention is to improve quality of life for both the patient and their family. Closely related to palliative medicine is the hospice movement. Their goal was and is to provide support and palliative care to patients with non-curable diseases for a foreseeable period of time until death. Palliative care physicians oppose euthanasia and medically assisted suicide. Palliative medicine transcends the focus on biological-technical aspects otherwise found in modern medicine.

A consequence of individualization and pluralization in modern societies is that *cross-cultural ethics* becomes apparent. In the context of routine medical practice, challenges are faced in the physician–patient relationship. Christian, Jewish, and Islamic bioethics do not agree in every respect; they are not always the same, homogeneous. Differing ideas are shaped according to the respective religious–cultural background. This applies to the determination of the beginning of life, the termination of pregnancy, and the end of life, with regard to the prolongation of life and euthanasia. Questions also arise concerning the use of heart valves from pigs in replacement surgery, or the use of preparations containing pig gelatin, since in Islam, pigs are considered to be unclean. An issue of dispute is whether the bioethical conceptions of Western individualism are universal. This is also reflected in perceptions of the explaining of medical interventions and informed consent. In other societies, social inclusion is emphasized, especially in Muslim societies. Occasionally, the decision-making right of the family, and not of the patient, is claimed. Cultural contexts thus enter into medicine. Different values have to be considered. Further bioethical controversies exist concerning the kosher

slaughtering of animals and male circumcision. There can also be difficulties concerning the treatment of men by female personnel.

Thus the general question is whether a *universal* bioethics is at all conceivable. The question arises not only due to intercultural comparison; it also arises within a given culture. In Western societies, different religious and ideological convictions coexist. In medicine, it is impossible to exclude the everyday context on principle and completely. This is manifest in the particularization of ethics, including bioethics. The counter position is that of a strict universalism. Complete individualization leads to radical relativism. The alternative of absolute particularization, with the consequence of an absolute relativism and an absolute universalism, would lead to pervasive opposition and would be fatal. For moderate particularization and moderate universalism, the situation is different, since both can address the question of the common and binding nature of bioethics. Thereby, the primary issue is not the specific regulation of each specific case, but rather agreement concerning a regulatory framework (Sturma and Heinrichs 2015).

Biopolitics, BioLaw, Bioethics

In terms of outlook, only one further aspect should be discussed, namely the political and legal implementation of bioethics. When discussing an across the board, generally binding regulation of bioethical matters, the concepts of biopolitics and biolaw should be at least briefly mentioned. *Biopolitics* comes into play when a targeted political decision must be made. This can be goal-oriented, or even scientifically orientated. This was exemplified in a negative sense during the Third Reich, when eugenics was legitimized through the introduction of state legislation. Contemporary biopolitics concerns the appropriate protection of life. A broad concept of life refers to the environment, the climate, and animals. A narrower concept of life refers to human life. Politics and the state take a certain degree of control over life in both cases.

Biopolitics leads to legislation. Classical areas of biolaw are health law and medical law. Medical law concerns regulations relating to medical treatment, such as medical practice law, pharmaceutical law, medical product law, but also bodily injury, medical negligence, organ trafficking, and the unlawful handling of embryos. Legislation also exists concerning hygiene and contagion. As a rule, modern medical law and biolaw are mainly concerned with the prevention of, and the imposition of penalties for, abuse.

Novel technical and scientific developments in medicine and in the biosciences render regulations necessary. Hereby, the performance and validity of traditional standards are questioned. In addition to respecting and acknowledging existing standards, the identification of new standards is necessary. New standards can only be determined by reaching a consensus agreement via appropriate discourse.

On the one hand, juridification concerns the guaranteeing of at least objective security. On the other, the legal definition implies guidance. Such guiding principles are not abstract and rigid norms. Furthermore, it is not possible to deduce such norms from a given ethical theorem. No sole, general basis can be provided by virtue ethics, utilitarianism, deontological/ teleological ethics, or discourse theories. For this reason, the pragmatic principles formulated by Tom Beauchamps and James Childress have become widely adopted. These are the four principles of autonomy, beneficence, non-harm, and justice in the sense of fairness.

These four guiding principles are considered to be prima-facie in nature, i.e., they are immediately apparent, and intended to apply across cultures. In each specific application, they are to be interpreted in a culture-specific and culturally sensitive manner. In addition to this, the basic rights of the person are protected by bioethics and biolaw (Stempin 2014; Sturma and Heinrichs 2015).

Despite an acknowledgment and tolerance of the unique nature and specific requirements of medicine, an inseparable link exists between ethics and medicine. This connection also forms a bridge between the everyday circumstances of the patient and the mission and scientific conceptions of medicine.

Summary

Medical ethics has a long tradition. Even in antiquity, the so-called art of healing was based on the prevailing ethical regulations and norms.

Besides natural-science-based empirical therapy, a medical–psychological perception of life, health, and illness has always played an essential role in the management of ill persons. This point of view, which has been termed alternative medicine, influences all aspects of the individual's lifestyle. However, the ill person is assisted not only by the physician, but also by society and the healthcare system.

In dealing with ill persons, immutable ethical principles must be taken into account. These concern the principles of fundamental ethics, and thus medical ethics is a form of applied ethics.

It can be assumed that the concepts of health and disease depend on cultural beliefs and perceptions. They are therefore defined differently in different cultures.

The physician–patient relationship is based on a form of contact that may be characterized by natural-science-based medicine. However, in many cultures and religions, this contact also involves magical–religious concepts. The conduct of the physician is characterized primarily by an adherence to a professional ethos, the best-known example being the "Hippocratic Oath" and its modern equivalent, the Declaration of Geneva ("Physician's Oath").

However, the culture-specific attitudes of the physician and the patient must also be taken into account, since these can lead to differing personal values.

From this follows the need to determine what is common and binding in order to establish new standards. These standards will be based on the principles of autonomy, well-being, and justice.

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Chapter 1
Psycho-oncology: The Relationship Between Psychology, Personality and Cancer

Shahriar Shahidi

Abstract This chapter focuses on the relationship between psychology, personality and cancer. There has been a long tradition in psychological research linking personality characteristics to a wide range of physical illnesses including, coronary heart disease and cancer. This will be critically reviewed in the context of methodological and cultural issues, arguing that most of these studies have been retrospective and that the few prospective studies linking cancer to personality have failed to control other important causal factors properly. However, problems with the expression of emotions remains a strong characteristic of cancer patients albeit the fact that a causal relationship remains doubtful. Furthermore, because of the nature of cancer, there is a strong cultural bias towards the illness. This may have consequences for treatment of cancer as well as psychological wellbeing of patients.

Keywords Psychology · Personality · Cancer

Abbreviation

PTG Posttraumatic growth

Doctor Thomas sat over his dinner,
Though his wife was waiting to ring,
Rolling his bread into pellets;
Said: 'Cancer's a funny thing.
Nobody knows what the cause is,
Though some pretend they do;
It's like some hidden assassin
Waiting to strike at you.
Childless women get it,

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And men when they retire;

It's as if there had to be some outlet

For their foiled creative fire,....'

W. H. Auden, Miss Gee (1930s) Quoted in Sontag (1978).

'What is not fatal is not cancer'. Wilhelm Reich (1893–1957), Austrian psychoanalyst.

1.1 Introduction

According to the National Cancer Institute's definition, cancer is the name given to a collection of related diseases. In all types of cancer, some of the body's cells begin to divide without stopping and spread into surrounding tissues. Cancerous tumours are malignant, which means they can spread into, or invade, nearby tissues. In addition, as these tumours grow, some cancer cells can break off and travel to distant places in the body through the blood or the lymph system and form new tumours far from the original tumour (National Cancer Institute 2014). In a recent publication by the American Cancer Society, over 14 million new cancer cases were diagnosed worldwide in 2012 and well over half of these cases occurred in economically developing countries. Moreover, eight million deaths worldwide in 2012 were because of cancer. It is predicted that by the year 2030, death toll worldwide due to cancer, will rise to 13 million out of an increased 22 million of new cancer cases. It is estimated that an even larger proportion of this will be shared by developing countries because of expected increases in lifestyles that are known to increase cancer risk, such as smoking, poor diet and physical inactivity (Ibid 2015). One thing about the mentioned statistics which may be considered ironic is that the ratio of new cases of cancer and number of deaths as a result of cancer is not expected to change significantly in the next two decades. There is no doubt that there have been striking advancements in both early diagnosis and treatment of cancer, as well as our better understanding of the etiology of cancer. The latter includes a clearer understanding of both environmental factors or the so called 'teratogens' and 'carcinogens', but also the genetic mechanisms through which vulnerability for cancer is created and transmitted from one generation to the next. The reason for this is an increase in unhealthy life styles, in particular inactivity and unhealthy diet as well as environmental pollution and stress which has made cancer the second major cause of death in the world. This is particularly so in developing countries.

Psycho-oncology is a growing scientific field dealing with psychosocial aspects of prevention, etiology, diagnostics, treatment, and rehabilitation of cancer. It is an interdisciplinary field involving the cooperation between medical and nonmedical professionals in particular psychologists. Psychosocial distress in the form of negative affect and even psychiatric disorders are seen in 30–60% of cancer patients (Grassi and Watson 2012). Hence one important aim of psycho-oncology is to increase the subjective quality of life of cancer patients.

1.2 How Does Cancer Affect People Psychologically?

In this chapter we deal with the psychology of cancer and we pose a twofold question: On the one hand how does cancer affect people psychologically and on the other, to what extend do psychological factors and characteristics affects the development of cancer. The first question may at first sight seem easy to answer: People react to cancer in much the same way that they react to any serious illness. The literature abound with clear evidence that a wide variety of negative feelings and emotions flood in once cancer is diagnosed in the individual and throughout the course of the illness. Feelings of shock and fear is very common when diagnosis is made. Cancer is a serious illness and most people are afraid and feel unsure about the consequences of cancer and chances of treatment. What side effects are there? What is the impact on children and the family? Diagnosis of cancer causes anxiety and depression. In a recent study, Borjalilu, Shahidi, Mazaheri and Emami (in press) investigating the psychological impact of cancer on children and their parents, found that spiritual crisis may be a key psychological reaction to cancer. When children are diagnosed with cancer, they may raise difficult questions to which they try to find the answer, such as why they are inflicted with the illness and why they suffer. Indeed, the first question that arises when a person suffers is 'why'; therefore, he or she may make attempts to search for the meaning of their suffering. They may make enquiries about God and why He has caused them to suffer. Children may also ask questions about death and its nature. In such cases, individuals need purpose to deal with crisis and the distress caused by these misfortunate events is rooted in one's inability to find meaning and purpose in life. As such, spiritual crisis may lead to lack of energy, anxiety, depression, unexplained pains, sorrow, lack of control over thoughts and emotions, and a sense of disengagement and isolation. Other consequences may include guilt feelings, anger, denial, helplessness and frustration. All these negative feelings have been reported in a variety of studies. For a comprehensive review see Foster and Wright (2009).

1.3 Cancer and Culture

In her classic book, 'Illness as Metaphor', the late writer and teacher, Susan Sontag examined the fantasies concocted around cancer. By fantasies, she meant the unreal and often punitive uses of illness in general and cancer in particular, as a figure or metaphor in our culture. Her point is that illness is not a metaphor and any such metaphoric thinking should be avoided. Cancer as well as other illnesses (She wrote a similar treatise on AIDS, Sontag, 1989), is strongly identified with death and dying. She challenged the cultural tendency which leads to the mentality that the patient should be somehow blamed for having caused the illness.

At the time, a popular belief based on apparent scientific and medical evidence, was that there is a cancer prone personality characterised by repressed emotional expression and depression and the current fashionable alternative cancer treatment was psychotherapy for the patient's supposed "cancer personality". According to these proponents, patients brought cancer upon themselves by having a resigned, repressed, inhibited personality. By undergoing the often blame-filled psychotherapy offered by some groups, the patient would overcome cancer by consciously choosing to give up the emotional benefits he or she created the cancer for, and be healed (Olson 2002: 160–169). Others have taken her idea further, showing not that there is a real "cancer" behind the metaphors, but that all we have is metaphor to understand the behavior of a disease that remains mysterious (Jain 2013).

1.4 Cancer and Personality

The role of personality in the causation of cancer has been controversial. Nevertheless, the role personality factors may play in causing cancer has long been hypothesized. The Greek physician, Galen (130–200 A.D.) observed that melancholic women were more prone to cancer than were women of a sanguine temperament. In 1962 Kissen and Eysenck in a classic study of the association between personality and cancer, reported that patients with lung cancer were more likely to be extraverted and less likely to be neurotic as compared with hospital controls.

The findings of subsequent studies until the late 1980s, examining the association between personality and cancer incidence or mortality have been inconsistent (Grossarth-Maticek et al. 1988). Nearly 70% of retrospective studies have found statistically significant associations between higher scores for extraversion, lower scores for neuroticism and lower scores for trait anxiety and increased risk of cancer of the breast, lung, and all sites combined. Other studies have found no association between personality scales and cancer. 50% of prospective cohort studies *have* found statistically significant associations between "Type 1" personality (under stimulation) or hopelessness and total cancer and between anti-emotionality (an absence of emotional behavior or a lack of trust in one's own feelings) and breast cancer (Nakaya et al. 2003). It must be pointed out, however, that the majority of these studies have had methodological limitations, including the use of a retrospective design, a small number of cancer cases (ranging from 29 to 249), and failure to control sufficiently for potentially confounding variables such as smoking and alcohol use. The validity of the so called cancer prone personality has been under spot light from the onset. Wellisch and Yager (1983) opposed the notion based on methodological issues. They pointed out that until a hypothesis such as the existence of a cancer prone personality, can predict who will and who will not develop cancer, it has very little value. They argue that this has not been conclusively shown. Further the authors argue that a large portion of positive findings in

the area may be due to chance: 'If one looks at enough variables, by chance alone, some (about one in 20) may occur to be statistically significant' (Wellisch and Yager 1983, p. 148). It may also be that the changes in the immune and hormonal systems under stress, which have been put forth to explain the mechanisms by which personality may cause cancer, may result from differences in smoking, drinking, diet or other readily observable behaviors that can also change in times of pressure.

In a population-based prospective cohort study in rural northern Japan, Nakaya et al. (2003) examined the possible relationship between illness and personality in over 30,000 Japanese rural residents who completed a Japanese version of the short form of the Eysenck Personality Questionnaire-Revised and a questionnaire on various health habits. There were nearly 700 prevalent cases of cancer at baseline, and 1000 incident cases of cancer were identified during seven years of follow-up.

No association was found between any of four personality subscales measured by the Eysenck questionnaire (extroversion, neuroticism, psychoticism, and lie) and the risk of either total cancer or cancers of the stomach, lung, colorectum, and breast. It is important to note that the study had several methodological advantages compared with previous studies of personality and cancer, including a prospective design, recruitment of subjects from the general population, and extensive control for potentially confounding variables such as smoking, alcohol use, body mass index, family history of cancer, and education (a measure of socioeconomic status).

Some of the findings may provide clues for interpreting the discrepancies among previous studies on personality and cancer. For example, a statistically significant positive association between neuroticism and prevalent cases of cancer at baseline and with cancer cases diagnosed in the first three years of follow-up was found. A neurotic tendency among subjects with prevalent cases of cancer may be a consequence, rather than a cause, of having been diagnosed with cancer. Similarly, a neurotic tendency of subjects with cancer diagnosed in the early years of follow-up may be a consequence of subclinical symptoms caused by cancers that were present but undiagnosed at baseline.

So is there a cancer-prone personality? The results seem inconclusive and still more research is needed and much more information gathered before the debate could be settled. Some studies have shown that there may indeed be a link between behavior and personality and from this it may follow that the onset of and recovery from cancer may be associated to behavioral and/or personality factors. We know, for example that emotions such as depression, anger, and hostility make us more prone to illness and disease in general. Furthermore, new directions in the field of positive psychology has shown that positive attitudes such as hope, optimism, and happiness strengthens our immune system and may in fact protect us from illness (Peterson and Seligman 2004). Hence some psychologists point to two personality 'types' that seem to make us either cancer-prone or cancer resistant.

1.5 Cancer-Prone Personality Types

- Repression of emotions in particular anger, hostility and resentment.

One of the most important issues regarding the link between personality and cancer is the tendency to repress both positive and negative emotions. In particular repression of anger, resentment, or hostility towards self and others has been reiterated (Gubar 2016). Sandra Thomas and her colleagues have argued that extremely low scores in anger in cancer patients represents repression, suppression and restraint of anger, suggesting that lack of proper expression of anger and hostility is at least a salient characteristic of cancer patients (Thomas et al. 2000). According to the National Cancer Institute, the release of stress hormones (in particular cortisol) into the blood stream, as a resuly of stimulation from the nervous system, can adversely alter important cell processes such as DNA repair and the regulation of cell growth which protect the body against cancer. Since cortisol is released during stress in general and anger in particular, a causal link has been suggested by some researchers.

- Acceptance of extra duties and responsibilities, even under stress. A large body of research suggests that there may be a link between too much workload and work stress and cancer. Masahiro et al. (2001) in a study in Japan found that there may be a link between stress caused by perceived workload and oxidative DNA damage (a major cause of cancer). Similarly, Rintala et al. (2002) reported women in Finland who were diagnosed with breast cancer reported experiencing too much overload of duties and responsibilities at home and at work.
- Adverse reactions to life changes and not coping well with these changes. The term "coping style" has been used by psychologists to refer to a wide range of cognitive, psychological and social strategies used by an individual to reduce the adverse effects of stress in life. These are ways that help us deal with stress. It has been argued for a long time that there may be an association between cancer onset and some particular coping styles. In particular, strategies which are mainly emotion-focused (as opposed to problem-focused) were more associated with cancer patients. This lead some researchers, as early as the 1980s, to think that there may be an association between the onset of cancer and how people cope generally with stress (Temoshok 1987). This assertion is nowadays not accepted by most researchers. However what is clear is that patients who are diagnosed with cancer show certain distinctive patterns of coping compared to their healthy counterparts. It has been found that patients suffering from cancer tend to show more confronting, escape—avoidant styles and to use less self-control, accepting—responsible and positive repraisal. They seem to seek more social support and use a less planned problem solving style (Sprah and Sostaric 2004). Furthermore, coping in the caregivers has been found to affect patients' cancer symptoms. Greenberg and Stopplebein (2007) found that cancer symptoms in children decreased as a result of their parents using problem

appraisal and an emotional regulation strategy (social support). However these symptoms increased as a function of using avoidant coping (substance use and self-blame).

- Negative or pessimistic attitude. Depending on a societ's cultural context, those suffering from cancer and their families may feel guilty about their emotional responses to the illness. As is the case with most illnesses, there is often some pressure to be strong ans resilient at all times. Personal experience shows that there may be little difference between western and non western cultures in this matter. Although a lot of this pressure is in fact unrealistic, it nevertheless exists and can come from within the individual, from other people, or both. Feelings such as sadness, depression, guilt, fear, and anxiety are all normal parts of grieving and learning to cope with major life changes. When the individual tries to ignore these feelings or not talking with others about them, it can increase the emotional burden in the person with cancer and make him or her feel lonely. In Iran, there is a great deal of cultural emphasis on the role played by family members when cancer is diagnosed (Borjalilu et al., in press). Hence negative attitudes and emotions are seen even more in family members. Psychological therapy and intervention with family members seems to be a logical priority in cultures similar to Iran. Another cultural aspect with influences cancer patients is that chronic disease may be controlled by the mind. Although there is ample evidence that positive attitudes and high motivation does in fact have an effect on the onset and the progress of any illness, this belief may result in negative feelings when people with cancer don't do well and thus blame themselves.

To learn more about attitude and survival, researchers looked at the emotional well-being of more than 1000 patients with head and neck cancer to find out whether it affected survival. Over time, those who scored high on emotional well-being showed no differences in cancer growth or length of life when compared with those with low scores (Coyne et al. 2007). Based on what we know now about how cancer starts and grows, there's no reason to believe that emotions can cause cancer or help it grow.

- Susceptibility to depression and feelings of hopelessness as well as feelings of excessive worry about others and need for approval from others has also been argued as causes of cancer and part of a cancer prone personality. Research as well as clinical evidence clearly suggests that patients diagnosed with cancer, as is the case with most chronic illness, do experience significant amounts of depression and anxiety as a result of their illness compared to healthy individuals. However, the evidence relating these factors to the etiology of cancer is very sketchy and out of date. In a comprehensive review of psychological factors in patients suffering from head and neck cancer in 2013, the research team headed by M. Bryant Howren concluded that there was no conclusive evidence suggesting that either depression and anxiety may result in cancer. However, these is some evidence pointing to the fact that these factors may play

a negative role in treatment and thus to some extent survival of cancer patients. This was particularly so when considering anxiety and some cognitive and personality factors. These were notably fear of cancer recurrence and some illness cognitions such catastrophizing.

1.6 Cancer-Resistant Personality Type?

Evidence based on research and clinical experience shows that there is ample evidence suggesting that certain characteristics may help some cancer patients to deal better with the slings and arrows of chronic illness in that some patients may become immunized or at least be able to experience a higher quality of life if some or all of the factors above are present:

- Expression of emotions in a positive and constructive way.
- Control of anger and positively resolving anger issues.
- Knowing when and how to say no.
- Coping well with stress and feeling in control of situations.
- Optimistic and hopeful attitude.
- Resistant towards depression.
- Creation and maintenance of social support networks.
- Lack of excessive worry.
- Exhibition of empathy and development of relationships based on healthy attachment.

There are two important points to be made here. First, there is little evidence that the above assertions applies to all or even the majority of cancer patients or those so called resistant to the illness. Even, as we have seen, there is inconclusive proof that these 'types' should even merit statistical significance. Psychology abounds with examples of this kind. Theories which attempt to generalize often face the counter attack by evidence which offers exceptions: some of the most optimistic and positive people will get cancer, and some of the angriest and most hostile will always continue to lead a healthy and cancer free life. Nevertheless, there is clinical evidence suggesting that such traits may play a role in cancer, especially when considered in combination or interaction with other risk factors, especially life style and genetic factors (Peterson and Seligman 2004). Moreover, when a cancer patient is told that his or her disease is terminal, those who adopt cancer-resistant traits tend to live longer because their newly acquired behaviors seem to boost immunity (Taylor and Sherman 2004).

The second point is that even if we accept (with reservation) the salient role a 'cancer prone personality' may play in the etiology of cancer, one must then turn one's attention to its "usefulness". What use is the construct in the prevention and treatment of cancer? Sontag (1978) makes this point very clearly in her classic work mentioned previously. When cancer is associated so strongly with death and dying

and the bleak images of illness and disease is reflected not only in literature and politics but also in every day language as metaphor, then the notion of a cancer prone personality with all its negative connotations can only help to create hopelessness, guilt, depression and anxiety in patients which in turn may go a long way in deteriorating the course of the illness. Recently, psychological research has yielded an important insight into the relationship between personality, behavior and cancer: Positive beliefs may actually help people to come to grips with health threats and adopt better health behaviors. It has been reported, for example that women diagnosed with breast cancer, reported a greater appreciation for life in general and health in particular as a result of their illness. Also they reported improved close relationship and empathy and adopted a healthier life style (Taylor and Sherman 2004).

There is no doubt that psychological wellbeing may play a more decisive role compared to psychological factors and characteristics per se in a person's reactions to cancer. In other words, strengths and virtues are more significant than weaknesses and vices (Peterson and Seligman 2004).

1.7 Cancer and the Concept of 'Suffering'

Cancer as an illness is a stressful experience. This is because, like all chronic illnesses, cancer causes suffering. Suffering may be defined as the process of undergoing or feeling pain or distress and to sustain or endure injury, disadvantage, loss or death or anything unpleasant. This suffering is not only experienced when cancer is diagnosed, but the experience of suffering is apparent during various stages of treatment. Most of these treatments necessitate the endurance of great amounts of pain and discomfort on the part of the patient and his or her family. The suffering includes not only physical disruptions and problems but also many psychosocial problems, reactions such as anger, denial and guilt which in turn may lead to disruptions in the normal course of life as well as the quality of life.

Very little research has been done into the role of suffering in cancer patients. It has been found that suffering may exist in three dimensions: Physical, psychological and social. The physical dimension of suffering includes feelings of fatigue, pain and the possible side effects of chemotherapy. Psychological suffering most often includes depression and loss and all the negative feelings and emotions associated with cancer and the physical changes resulting from the illness. Considering the inevitable social and cultural association between cancer and death, this psychological debilitation is particularly important as it causes withdrawal and isolation leading to the third dimension, namely social suffering.

Ellis et al. (2015) conducted a qualitative study on terminally ill cancer patients and concluded that a transformation through suffering, predominantly spiritual in nature, leads to greater and deeper understanding of oneself. This spiritual understanding enables the patient to cope, understand and accept the suffering he or she is experiencing as a result of cancer. Hence a more lucid self and a deeper sense of

meaning is achieved (Ellis et al. 2015). For some patients, this develops into a more religious transformation. The authors correctly argue that although not all patients are religious, most (if not all) are spiritual.

Research into the area of suffering has opened new doors into our understanding of how and why cancer patients react to illness and more importantly, how some patients can be helped to utilize the suffering they experience to their advantage. In other words it is argued that cancer patients may learn to become stronger as a result of their suffering. This is what some psychologists have labelled 'posttraumatic growth' or PTG (Foss 2005). PTG is characterized by a change in general perception, self-perception and attitude to life including philosophical/spiritual changes. It has been found that many patients experiencing PTG gained a powerful spur for personal growth which was not due to adjustment and coping strategies alone (Ellis et al. 2015) but involved positive life changes and new inner strength.

1.8 A Note on Psychotherapy

The use of psychotherapy with cancer patients will be discussed in much more detail in the next chapter. Here, with regards to what we have already said, a few points needs to me made. During the last two decades of the 20th century, psychotherapy with patients suffering from cancer began to flourish in a systematic manner. It was realized that psychotherapy alongside medical treatment could in fact increase life expectancy in patients. This was reiterated in several studies (for example, Spiegel et al. 1989). The researchers studied the effect of psychosocial intervention on time of survival of 86 patients with metastatic breast cancer and found that compared to a control group, the intervention group survived 18 months longer. Other research reported improvements in psychological wellbeing including self-concept, health locus of control and self-confidence as well as physiological improvements in the immune system and endocrine activity (Trijsburg et al. 1992). More recently attention has been extended to spiritual and existential wellbeing of patients. Work with a wide range of cancer patients, particularly those in advanced stages of cancer and staying in hospices, has shown that cancer patients can benefit a great deal from what may be termed 'spiritual therapy' concentrating on aspects such as philosophy of life and death, belief in religion, ability to forgive and improvement of dignity (Chochinov 2007). However, the exact mechanisms and methods of spiritual therapy have not yet been determined and therapists seem to have tried to incorporate spiritual issues within existing theoretical perspectives such as psychoanalysis (Rubin 2006), humanistic psychology (Elkins et al. 1988) and cognitive behavioural frameworks (Miller 2003).

An important lesson learned by researchers seems to be that even a disease like cancer is much more easily overcome when the mind is strengthened and conditioned. Hence not only quality of life may be optimized but chances of recovery may be increased. The techniques and perspectives of psychotherapy will be discussed in Chap. 2.

1.9 Conclusions

Psycho-oncology is a growing scientific field dealing with psychosocial aspects of prevention, etiology, diagnostics, treatment, and rehabilitation of cancer. There has been a controversial claim that certain personality types and traits may cause cancer. The popularity of these caner prone personality theories has gradually declined since the beginning of the 21st century. However, there seems to be certain patterns of behaviour such as adverse reactions to life events or negative and pessimistic attitudes which may play a role in the treatment of cancer if not in its etiology.

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Chapter 2
Psychotherapy in Cancer

Adrian Furnham, Kelly Petropoulou and Shahriar Shahidi

Abstract The present chapter reviews the current and popular therapies used to alleviate psychological trauma of cancer resulting in adverse and disturbing behavioural and psychological reactions. These therapies, including alternative medicine, may also be used to promote psychological well-being in cancer patients. We discuss the most common cancers and mention major charity and NGO organizations in the Middle East and Europe. We also discuss why cancer patients seek such therapies and some of the challenges and obstacles facing successful therapy for cancer.

Keywords Type of cancer · Choice of therapy · Therapy for cancer patients · Alternative medicine · Cancer · Psychotherapy

2.1 Introduction

It is said that most people will die with cancer, though not necessarily from it. Cancer medicine and research has progressed considerably over the past twenty years at least in the developed world. Both the detection and the cure of cancer have advanced considerably. It appears to be the case that the incidence of cancer is growing though it is not clear whether this is true or else detection is becoming more efficient and/or people are living longer.

There are various common types of cancer all of which can influence the type of medical and psychological cure that is sought. Some cancers are more observable (i.e. melanoma) than others; some lead to immediate and potentially embarrassing

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issues around daily life (i.e. bladder and colon cancer), while other may require surgery which affects cosmetic issues that can profoundly influence social self-confidence and sexual behaviour (i.e. breast, prostate).

Most common types of cancer (according to American Cancer Society: Cancer Facts and Figures 2015. Atlanta, Ga: American Cancer Society, 2015).

Type of cancer	Estimated new cases
Bladder	74,000
Breast (female-male)	231,840–2350
Colon and rectal (combined)	132,700
Endometrial	54,870
Kidney (renal cell and renal pelvis)	61,560
Leukemia (all types)	54,270
Lung (including bronchus)	221,200
Melanoma	73,870
Non-hodgkin lymphoma	71,859
Pancreatic	48,960
Prostate	220,800
Thyroid	62,450

In most countries there are large cancer charities. Some support research, others patients and still others relatives. Some specialise on particular illnesses. For instance in Great Britain Macmillan Cancer Support is one of the largest British charities and provides specialist health care, information and financial support to people affected by cancer.

The charity was founded, as the *Society for the Prevention and Relief of Cancer*, in 1911 by Douglas Macmillan following the death of his father from the disease. In 1924 the name was changed to the *National Society for Cancer Relief*, which it retained until 1989 when it was changed to *Cancer Relief Macmillan Fund*, later changed again to *Macmillan Cancer Relief*. From 5 April 2006 Macmillan Cancer Relief became known as *Macmillan Cancer Support* as this more accurately reflects its role in supporting people living with cancer. It has adopted the principles of being a "source of support" and a "force for change". This chapter is about the choice and use of psychotherapy in cancer patients. For most patients the initial diagnosis of cancer in acutely traumatic. It triggers off a range of emotions in themselves and those to whom they disclose the information including acute depression and suicidal thoughts. This in turn leads immediately to thoughts of cure and anxiety alleviation. The choice of therapy is for many considerable. The internet is full of ideas to both prevent and cure cancer.

It is interesting to note that such cancer charities are growing very fast in developing countries too. This is of great importance since cancer care is more difficult in developing countries. Drugs are inevitably more scarce and expensive and access to treatment, particularly psychological and social care by people living in more remote areas is more difficult.

Two charity organizations in Iran, for example have gained international status and are worth mentioning here. The Society to Support Children Suffering from Cancer widely known by its acronym, MAHAK, was set up in 1991 as a non-governmental and non-profit organization with the Department of Social Affairs for NGO activities at the Ministry of Interior of the Islamic Republic of Iran. Behnam Daheshpour Charity (BDC) is a non-governmental and non-profitable organization run by public donations and has one of the largest group of unpaid volunteers to care for cancer patients across Iran. The main duty of this organization is to support and help cancer patients. This charity was established in 1997 and named after the founder Behnam Daheshpour who himself suffered from cancer and passed away in the age of 21. BDC has looked after over 7000 cancer patients covered since 1997.

MAHAK strongly supports the parents of the children suffering from cancer. With such support, parents can concentrate on the treatment of their children. The motto of the charity is that no child with cancer will ever be refused treatment because of poor financial status.

Both charities provide supportive psycho-social and welfare services to deprived children and their families. Support services include social work, psychology and welfare services in addition to collection of donations and humanitarian assistance from people, institutions and organizations. Fundraising activities include donation boxes, membership schemes, advertising and special projects including art exhibitions as well as Public and International Relations that keep rapport with volunteers, donors at national and international level (Fig. 2.1).

The highly specialized paediatric hospitals working and liaising with these charities offer the latest methods and technology in detecting and treating cancer such as Leukemia, Brain tumors, Bone tumors, etc. (Fig. 2.2).

There are essentially two issues for most patients.

The first is how to cure the cancer in the sense of preventing its growth and eradicating it. For most this will involve some form of medical treatment such as chemo-therapy or surgery or radio-therapy but could also involve many other treatments.

Fig. 2.1 Behnam Charity Organization in Iran

Fig. 2.2 Cancer care has grown significantly in the Middle East through charity organizations and NGO's

The second is dealing with the anxiety and life-style changes that result from the diagnosis. This may involve a wide range of orthodox and novel "talking cures" or psychotherapies, as well as dietary and exercise changes as well as spiritual exercises.

Below are the sites that first come up when Googling (In 2015) what to do after one has received a cancer diagnosis:

1. Cancer Support Community (http://www.cancersupportcommunity.org/MainMenu/About-Cancer/Newly-Diagnosed/Emotional-Distress.html?gclid=CjwKEAjw2cOsBRD3xNbRp5eQxzYSJADZGYbz1HkNcy4_cnQfn_AwvK8XmZPJoUPI7n7hsBwhIhsPNRoC3kzw_wcB): newly diagnosed individuals can follow 10 links that include information regarding: Coping with Diagnosis, Coping with the Cost of Care, Communicating with your Healthcare Team, Treatment, Open to options, Managing Emotions, Emotional Wellbeing, Support, Keys to Being Patient Empowered and Relaxation and Visualisation.
2. Mayo Clinic (http://www.mayoclinic.org/diseases-conditions/cancer/in-depth/cancer-diagnosis/art-20046527): cancer specialist's advice on what to do after the diagnosis including finding out the details of the diagnosis and having someone with you who will help in retaining this information. Moreover, second opinion is recommended as well as what to look for in a doctor (listening, explaining, understanding). The specialist also advises that one should consider all types of treatment and choose the one more appropriate for their case. Finally, he suggests that one should value their personal opinion on treatments more than those of their family and friends as they can be overwhelming.

3. WebMD (http://www.webmd.com/cancer/features/cancer-diagnosis-what-to-do-next): STEPS: Find a partner, Get organized, Get informed, Be sure to consult only unbiased, trustworthy sources when you do your research, Consider a second opinion, Understand treatment options, Communicate with Friends and Family Members, Financial Self-Care.
4. Cancer.net (http://www.cancer.net/navigating-cancer-care/diagnosing-cancer/when-doctor-says-cancer): Learn about your diagnosis, Find medical care, Get organized, Find support to cope with challenges, Emotional support, Financial support.

In this chapter we will concentrate on three things. The first is the choice of therapy offered to patients. The second is the evidence of the efficacy of the therapy in general, but also specifically for cancer patients. The third is the use of alternative medicine in the treatment of cancer.

2.2 Choice of Therapy

The general public (as potential clients) is increasingly faced with a bewildering array of psychotherapy interventions available, although some are clearly similar in theory and practice. These include seeing a therapist, and/or taking medication or getting hypnosis. Deciding whether or not to seek help is associated with a range of factors including the availability of services, financial costs and individual socio-demographic and psychological variables. It is also crucially associated with the perceived effort required in, and possible psychological pain associated with, treatment which is the focus of this paper. The term psychological pain refers here to the distress associated with the treatment process.

Generally most members of the public believe that mental disorders, like the anxiety and depression that results from a diagnosis of cancer are treatable, psychiatric treatments are considered generally rather unhelpful whereas counselling is considered most helpful. Studies have also shown that people have set ideas about counselling before taking up therapy. Expectations have been found to be important determinants of where people turn to for help and effectiveness of counselling.

People have very different beliefs about what occurs during psychotherapy (Furnham and Telford 2012). Furnham and Wardley (1990) found respondents tended to believe that clients of psychotherapy did feel better in therapy, and were more confident and hopeful. Furnham and Wardley (1991) investigated lay theories of efficacy of therapies and prognosis for different problems. The more knowledgeable people were about psychology, the more sceptical they tended to be. Knowledge about psychological cures led to a greater awareness of the limited benefits of therapy. This was confirmed when Furnham et al. (1992) compared responses of lay adults, students and clinical psychologists and found the latter tended to be more cynical about the efficacy of therapy and prognosis of many disorders.

Lay theories about the *treatment*, as opposed to the cause, of mental disorders show marked differences from current practices in the mental health service. It has been found that lay people generally prefer psychotherapy to drug treatment, due to the perceived side effects. There is also a belief that 'will power' can effectively facilitate recovery from mental disorders such as agoraphobia and anorexia nervosa. However, medication is believed to be the most effective treatment for disorders with a higher perceived severity thus showing that lay and academic theories of treatment overlap to an extent.

However it is common for cancer patients both to be offered and to seek out some form of psychological treatment to help them adjust to their medical condition:

1. Chemotherapy—prescribing specific drugs to achieve a therapeutic purpose
2. Electroconvulsive therapy—electric shock treatment to cause convulsion
3. Psychosurgery—destruction of specific brain tissue to control behaviour and emotions
4. Megavitamin therapy—administering large dosages of specific vitamins
5. Psychotherapy—a talking cure aimed at changing feelings, attitudes and behaviour
6. Psychodynamic therapy—often based on Freudian ideas and stressing unconscious processes and early relationships
7. Systematic desensitization—people are helped to relax in situations that cause them great anxiety
8. Implosion therapy—exposing people to situations and things that cause them most fear
9. Aversion therapy—pairing an unpleasant event (shock) with an undesirable habit (drinking)
10. Token economies—rewarding and fining people for desirable and undesirable behaviour
11. Behaviour contracting—establishing a written contract/promise of appropriate behaviour pattern
12. Modelling/role playing—watching and then imitating a therapist showing an appropriate behaviour pattern
13. Assertiveness training—helping clients to express in various social contexts more effectively their needs and emotions
14. Rational-emotive therapy—helping people to think more rationally and be less magic-orientated or superstitious
15. Thought stopping therapy—helping people stop obsessive or compulsive thoughts
16. Non-directive therapy—therapist encourages talking but does not give advice, reassure or ask direct questions but does clarify, reflect and emphasise the positive
17. Existential therapy—helping people to be more aware and responsible for the choices in all areas of life experience

18. Gestalt therapy—helping people who intellectualize their problems by forcing them to confront conflicts and express emotion
19. Hypnosis—getting people into an altered state of consciousness and suggesting behavioural or attitudinal changes and helping them recall experiences
20. Biofeedback—helping people to relax and reduce anxiety by monitoring their physiological responses (heart rate)
21. Group therapy—getting groups of fellow sufferers to provide support and feedback
22. Primary scream (rebirth) therapy—attempting to get people to relive the trauma of their birth.

Many would object to this list because it is not inclusive and tends to group different therapies together. Thus there is Adlerian, Freudian, Jungian, Kleinian and various other psychotherapies that may be grouped under the Psychodynamic tradition. Others would talk about general counselling done by specialists.

Inevitably there has grown up specialist therapies for those who have cancer. According to the American Cancer Society some of the most common medical/biological and physiological cancer treatments include

- Surgery
- Chemotherapy
- Radiation therapy
- Targeted therapy
- Immunotherapy
- Hyperthermia
- Stem cell transplant (peripheral blood, bone marrow and cord blood transplants)
- Photodynamic therapy
- Lasers in cancer treatment
- Blood product donation and transfusion.

In their *Handbook of Psychotherapy in Cancer Care* Watson and Kissane (2011) reviewed a whole range of therapies which are summarised below

Type	Definition	Evidence
Supportive psychotherapy	A therapeutic intervention utilised intermittently or continuously that seeks to help patients deal with distressing emotions, reinforce pre-existing strengths and promote adaptive coping with illness. It explores the patient's self, body image and role changes within a relationship of mutual respect and trust	In 2007 the IOM (Institute of Medicine), National Academies of Science and the Committee concluded that a sound evidence base exists to recommend supportive psychotherapy as a valid therapeutic intervention

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Type	Definition	Evidence
Cognitive-behavioural therapies (CT and CBT)	The aim is to understand how a person's cognitive distortions, and subsequent irrational thinking, adversely affect their ability to cope optimally with stressful life events and then to help them to both identify their own distorted beliefs and negative automatic thoughts (NATs), and to challenge these in the light of evidence from actual behaviours of both themselves and others	Greer et al. showed that CBT adapted as an individualised therapy for cancer patients (adjuvant psychological therapy) could significantly reduce anxiety and helplessness compared to a no treatment control group. An advantage of CBT, demonstrated clearly by a number of studies, is its utility to bring positive benefits over a relatively short number of sessions
Cognitive analytic therapy	CAT is a recently developed integrative model of psychotherapy with a major focus on relational aspects of development and psychological distress. It is especially helpful with psychologically distressed, complex or "hard to help" patients. CAT can also be a consultancy tool to assist stressed staff teams and services as well as patients	CAT conforms to recognised general criteria for effective therapies and, in particular, for those more "sever and complex" and "difficult" personality type disorders. An increasing "formal" base, both naturalistic and controlled, has been accruing over recent years, despite its relative youth as a model
Mindfulness interventions	Due in part to the growing impact of Buddhist thought in our increasingly small global village, the concepts of Buddhism in general and mindfulness specifically have entered into the Western vernacular and experience. From this cultural introduction have flowed a variety of approaches designed to enhance quality of life	The review of literature documenting the impact of MBSR on cancer patients looks at psychological and physical symptoms. Significant support exists for the role of MBSR in the amelioration of psychological distress or mood disturbance with lower scores on measures of depression, anxiety, stress, fatigue and fear of recurrence of cancer
Relaxation and image based therapy	Consists of learning different ways in which to reduce the body's stress response in order to induce the "relaxation response". This is characterised by feelings of both physical and psychological relaxation	Relaxation and imagery are amongst the most popular complementary therapies used by patients with cancer. Luebbert et al. conducted a meta-analysis of randomised controlled trials employing relaxation based interventions and found considerable efficacy for the technique

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Type	Definition	Evidence
Motivational counselling in substance dependence	Receiving a cancer diagnosis has been framed as a "teachable moment" when individuals may be particularly receptive to changing their lifestyle or health	Meta-analyses showed a robust effect of this intervention particularly in alcohol-related problems. Effect sizes vary depending upon the comparison group and substance use to outcome, but when compared to wait list or no-treatment group, MI's average effect sizes were in the small to medium range. Compared to active treatment groups, for example CBT it is equally effective or more so
Narrative therapy	Denotes a number of psychosocial forms of intervention with individuals, couples, families, groups and organisations with a basis in narrative theories. The focus is on the narratives and the outlook and the vocabulary of the client which they bring to the therapy sessions	As of yet there are only a limited number of studies of the effect of narrative therapy
Dignity therapy	A patient-affirming psychotherapeutic intervention designed to address existential and psychosocial distress in people who have only a short time left to live	In a clinical trial of 100 end-stage patients, measures of suffering and depression showed significant improvement. Measures of dignity, hopelessness, desire for death, anxiety, will to live and suicide all showed favourable changes and patients who reported higher initial levels of despair were more likely to benefit
Written emotional disclosure	Based on the common assumption in theories of adjustment to traumatic events, that healthy adjustment occurs through repeated confrontation with the thoughts and memories of the trauma, which will assist the individual interpret the event, in this case the cancer, in a meaningful coherent framework	There is considerable evidence to suggest that a general tendency to cope through emotional non-expression will reduce the chance of adjustment to traumatic events such as cancer

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Type	Definition	Evidence
Supportive-expressive group psychotherapy	It is an intensive, weekly group psychotherapy that addresses fundamental existential, emotional, and interpersonal problems facing cancer patients	There is clear evidence that SEGT has psychosocial benefits. It reduces mood disturbance, depression, traumatic stress symptoms, emotional control, and maladaptive coping, and improves quality of life.
A short term, structured, psychoeducational intervention for newly diagnosed cancer patients	The goals of these interventions ought to focus on decreasing feelings of alienation and isolation by talking with others in a similar situation, reducing anxiety and helplessness about treatments and assisting in clarifying misperceptions and misinformation. The added benefit of such interventions is that they encourage more responsibility to get well and compliance with medical regimens	Following the six-week structured intervention, the experimental subjects showed significantly greater use of active-behavioural coping methods than the control subjects. In addition, the experimental subjects used significantly more active-positive and distraction coping strategies
Meaning-centered group psychotherapy	The goal of the intervention is to diminish despair, demoralisation, hopelessness and desire for hastened death by sustaining or enhancing a sense of meaning, even in the face of death	Early research demonstrated that a one-year supportive-expressive group psychotherapy, which included a focus on existential issues, decreased psychological distress and improved quality of life. However, results are inconsistent in their effects on depression, anxiety and desire for death. Results demonstrated significantly greater benefits from MCGP compared to SGP (supportive group psychotherapy) particularly in enhancing spiritual well-being and a sense of meaning
Couple-focused group intervention for women with early breast cancer and their partners	In order to deal with the many challenges and stresses of breast cancer, couples are likely to depend on one another as a key resource for both emotional and practical support	Couples report enjoying the groups and benefiting from the communication and stress management skills they learn

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Type	Definition	Evidence
Couples therapy in advanced cancer: using intimacy and meaning to reduce existential distress	Strives to optimise communication about several concerns, while helping the couple identify, affirm and "keep in circulation" sources of relational meaning that counter the distress which these dilemmas bring	Evidence supports the utility of couple-based interventions in the setting of advanced illness by improving relationship quality and reducing perceived relationship skew
Therapies of sexual dysfunction	All types of cancer can impact sexuality and intimacy. All patients regardless of age, sexual orientation, marital status or life circumstances should have the opportunity to discuss sexual matters with their health care professional	Evidence shows that a stronger bond is created between the health care provider and the patient and his/her partner after sexuality issues are addressed. If the intervention is ineffective, they are grateful that someone tried to help them
Focused family therapy in palliative care and bereavement	A focus on family-centred care seems imperative once it becomes clear that cure or disease containment is no longer achievable	Modest initial evidence exists

Clearly there is a great need for psycho-therapy of one sort or another to help cancer patients (Breitbart and Poppito 2014). A number of (as yet unanswered) questions arise from this literature. The first is why certain patients chose one therapy over another. The second, perhaps even more important, is the proven efficacy of that therapy.

2.3 Complementary and Alternative Medicine

Complementary and alternative medicine (CAM) has gained wide recognition and CAM is now big business (Ernst and Furnham 2000). Since the turn of the century there has been considerable and sour debate as to the efficacy of alternative medicine. Complementary medicine has been a wildly controversial topic over recent years, as techniques have been rigorously tested and fraudsters revealed. Singh and Ernst (2008) in the book *Trick or Treatment* evaluated 40 complementary techniques, and were scathing in their findings. They found that scientific evidence for the efficacy of these methods was lacking, and that the methods were not just ineffective, but positively dangerous in some cases. For example, methods such as homeopathy are labelled merely as placebos lacking in credible, repeatable effects. Singh was later sued by the British Chiropractic Association for libel for comments associated to the book, although they later dropped the case. Other widely available

books such as *Suckers*: *How Alternative Medicine Makes Fools of Us All* by Shapiro (2008) follow a similar line.

The great range of CAM inevitably means there is a considerable diversity of therapies and their attendant theories and philosophies. Yet there are common themes in the philosophies of CAM. Aakster (1986) believes that they differ from orthodox medicine in five ways.

Health: Whereas conventional medicine sees health as an absence of disease, alternative medicine frequently mentions a balance of opposing forces (both external and internal).

Disease: Conventional medical professionals see disease as a specific, locally defined deviation in organ or tissue structure. CAM practitioners stress body-wide signs, such as body language indicating disruptive forces and/or restorative processes.

Diagnosis: Regular medicine stresses morphological classification based on location and aetiology, while alternative interpretations often consider problems of functionality to be diagnostically useful.

Therapy: Conventional medicine often claims to destroy, demolish or suppress the sickening forces, while alternative therapies often aim to strengthen the vitalising, health-promoting forces. CAM therapists seem particularly hostile to chemical therapies and surgery.

The Patient: In much conventional medicine the patient is the passive recipient of external solutions, while in CAM the patient is an active participant in regaining health.

Aakster (1986) described three main models of medical thinking: The *pharmaceutical* model is a demonstrable deviation of function or structure than can be diagnosed by careful observation. The causes of disease are mainly germ-like and the application of therapeutic technology is all-important. The *integrational* model resulted from technicians attempting to reintegrate the body. This approach is not afraid of allowing for psychological and social causes to be specified in the aetiology of illness. The third model has been labelled *holistic* and does not distinguish between soma, psyche and social. It stresses total therapy and holds up the idea of a natural way of living.

Gray (1998) argued there are currently four quite different perspectives on CAM:

(1) *The biomedical perspective*: This is concerned with curing of disease and control of symptoms where the physician-scientist is a technician applying high level skills to his patient. This perspective asserts: (i) that the natural order is autonomous from human consciousness, culture, morality, psychology and the supernatural; (ii) that truth or reality resides in the accurate explanation of material (as opposed to spiritual, psychological or political) reality; (iii) that the individual is the social unit of primary importance (as opposed to society); and (iv) that a dualistic framework (e.g. mind/body) is most appropriate for describing reality. This approach is antagonistic toward and sceptical of CAM, believing many claims to be fraudulent and many practitioners unscrupulous.
(2) *The complementary perspective*: Though extremely varied, those with this perspective do share certain fundamental assumptions: (i) believing in the

importance of domains other than 'the physical' for understanding health, (ii) viewing diseases as symptomatic of underlying systematic problems, (iii) a reliance on clinical experience to guide practice and (iv) a cogent critique of the limits of the biomedical approach. Interventions at the psychological, social and spiritual level are all thought to be relevant and important, supporting the idea of a biopsychosocial (BPS) model. Many advocates are critical of biomedicine's harsh and often unsuccessful treatments, and point out the paradox of biomedicine often not being based on 'solid scientific evidence'.

(3) *The progressive perspective*: Proponents of this perspective are prepared to support either of the above, depending entirely on the scientific evidence. They are hardened empiricists who believe it is possible to integrate the best of biomedicine and unconventional approaches. Like all other health-care professionals, their approach is not value free—the advocates of this approach welcome the scientific testing of all sorts of unconventional therapies.

(4) *The post-modern perspective*: This approach enjoys challenging those with absolute faith in science, reason and technology, and deconstructing traditional ideas of progress. Followers are distrustful of, and cynical toward, science, medicine, the legal system and institutionalised religion and even parliamentary democracy. Post-modernists see truth as a socially and politically constructed idea and believe orthodox practitioners to be totalitarian persecutors of unconventional medicine. Proponents of this position argue (i) to have a complementary perspective in any debate is healthy, (ii) that CAM practitioners are also connected to particular economic and theoretical interests, (iii) that a variety of values and criteria for assessing success is beneficial and (iv) that the ill people themselves should be the final arbiters of the success of the therapy.

There is more diversity than unity within CAM. Whilst there have been calls to find regulatory bodies to oversee all CAM practices, this has proved very difficult because of the theoretical, historical and political differences between the various CAM specialities.

The popular interest in CAM has been matched by a relatively sudden and dramatic increase in research into the two central questions in this area:

1. *Does it work?*

Is there good evidence from double-blind, placebo-controlled, randomised studies that the therapy "cures illness" as it says it does? That is, is there any indisputable scientific evidence that documented findings of success are due to anything more than a placebo effect? Properly designed and executed studies are complex, very expensive and similar to the research effort to determine the efficacy of psychotherapy. Indeed it is the extensive research into the placebo effect that makes psychological input particularly valuable. The answer to the question is that either very little or no good evidence is available for the efficacy of most CAM, with the possible exception of herablism. However, as more and more sophisticated meta-analyses are published, there does seem to be clear evidence for small but robust positive effects of specific CAM treatments (Ernst and Pittler 1998).

2. *Why choose it?*

If the evidence is limited, equivocal and indeed often points to lack of efficacy, the central question must be why do patients choose at their own expense to visit a CAM practitioner? What do they get from the treatment? Why do they persist? This is where there have been many psychological studies. They concern the often mixed motives that patients have in shopping for health treatments.

The principal reason for individuals beginning any CAM treatment appears to be that they regard it as more natural and effective, and it allows a more active role for them. The second reason is the failure of orthodox medicine to provide relief for specific (usually chronic) complaints. The adverse effects of orthodox medicine, and a more positive patient-practitioner relationship are also important for many patients. There is little to support the widely held view that CAM-seeking patients are especially gullible or naïve, or have unusual (neurotic) personalities or (bizarre) value or belief systems. However, comparisons of users and non-users of CAM have shown evidence of different beliefs about health and disease in general.

2.4 Differences in the Consultation Between CAM and Orthodox Medicine

Are the popularity of CAM and its powerful placebo effect due to the often fundamental and dramatic differences between the stereotypic CAM and orthodox medicine (OM) consultation? There is only a very limited amount of research in this area.

Is it possible to generalise? Is there indeed such a thing as a typical consultation? Is the variation within each group (i.e. CAM vs. OM) different from/greater than the variation between each group? The differences between the consultations of an aromatherapist compared to an osteopath, or a psychiatrist compared with an orthopaedic surgeon consults are considerable. Indeed there may be different "schools of thought" which results in different types/styles of consultations within each CAM or OM speciality. Then, there may be differences depending on the biography, demographics and training of the individual practitioner. A typical consultation may be hard to define.

Consultations are so varied that any generalizations about the difference are only stereotypical, possibly misleading or meaningless.

And yet patients and practitioners acknowledge, even celebrate, the particular and peculiar approach to the consultation. Table 2.1 shows sixteen different criteria on which these two may differ and may in part explain the popularity of CAM. GPs all too often have too little time, may be perceived as patronising, and may not examine (touch) the patient. Further, patients are often not asked the full set of questions they expect to be asked for a "full" diagnosis. In short they are not treated like a modern adult consumer. CAM practitioners have longer consultations, appreciate patient's need to talk, to be examined/touched etc. The question is how the traditional/average CAM consultantation is different from (better than) the

Table 2.1 The prototypic CAM and OM Consultation

	CAM	OM
Time	More	Less
Touch	More	Less
Money	More	Less
History taking	Holistic	Specific
	Affective	Behavioural
Language	Healing	Cure
	Holistic	Dualistic
	Subjective	Objective
	Personal story	Case history
	Wellness	Illness
Patient role	Consumer	Sick role
Decision making	Shared/consumer	Doctor/paternalistic
Bedside manner	Charismatic	Cool
	Empathic	Professional
Sex ratio/role	F = M Feminine	M > F Masculine
Time spent talking	Patient > or = to practitioner	Practitioner > patient
Style	Authoritative	Authoritarian
	Supportive	Information
	Counselling	Advice giving
Confidence in methodology/outcome	Very high	High
Client relationship	Long term	Short term
Consulting rooms	Counselling	Clinical
Practitioner history	Second Profession	First Profession
Ideology	Strong Left wing	Moderate Middle way

traditional orthodox consultation. It is possible to compare and contrast the typical GP and CAM consultation across a number of variables (history taking approach, language used, patient role, decision making process, bedside manner etc.) to show how different they are, which may account for the popularity of the latter. Research is needed to confirm the view that it is very much the nature of the consultation which both differentiates CAM from OM and make it attractive. Current evidence supports this view.

There is some evidence that frequent CAM users are more health conscious and believe more strongly that they can influence their own state of health, both by lifestyle and through maintaining a psychological equilibrium. CAM patients appear to have less faith in 'provider control', that is in the ability of medicine (specifically orthodox doctors) to resolve problems of ill health.[5] Patients with

cancer using CAM were more likely to believe cancer was preventable through diet, stress reduction and environmental changes and to believe that patients should take an active role in their own health.

(a) *People shop for health*. They want to use all possible (and affordable) options in health care. People are not loyal to a brand, to orthodox medicine or any particular therapy. They shop, try-out, and experiment. CAM is therefore to many just another product/service. The question is how the particular brand offers something quite different that no other product or service offers. This raises the question as to what makes an individual "brand-loyal"; that is loyal to a therapy, a therapist or indeed a place of treatment.
(b) *People want a cure without side effects or pain*. This may offer a very strong, unique selling point for homeopathy over herbalism, acupuncture etc. because of the scare stories about poisoning with herbs or minerals, and pain/infection with acupuncture. It is for instance the "gentleness" of homeopathy and its dilutions that may be particularly attractive to people. The possible contradiction between being harmless *and* effective is often not confronted.
(c) *Because they have chronic illnesses or conditions they have difficulty living with*. Many patients with chronic painful conditions or addictions have tried many other cures. They turn to CAM sometimes as a last hope. Some therapies have a powerful psychological component, particularly those associated with touch (i.e. massage, reflexology).
(d) *Because they are disappointed by the traditional orthodox consultation*. As shown in Table 2.1, there are many reasons for patient's disappointment with orthodox medicine, but it seems that the nature and style of the consultation is the primary explanation of this.
(e) *Because patients want to learn more about self-care* (*fitness, wellness and prevention*). Orthodox medicine is seen as narrow and disease (complaint) orientated, which aims to destroy, demolish or suppress "sickening forces", through such things as chemical therapies and surgery. But many people prefer an emphasis on natural restorative processes on how to strengthen the vitalising health-promoting forces. The emphasis is quite different—illness versus wellness. Psychologists have long recognised this. CAM is often seen as restorative, balancing, natural and preventative, which fits in with the particular *zeitgeist*.
(f) *Patients believe in the "holistic" message*. It seems obvious to most patients that life-style, personal relationships and work operate together and simultaneously to have an impact on health. Equally they believe that there are many and manifold signs of wellness and illness from digestion, sleep patterns and body appearance, to more subtle non-verbal signs associated with gait, balance, body odour, etc. The implication is that the diagnostic interview needs to have questions about all aspects of the person's life not only their physical symptoms.

2.5 Conclusion

The diagnosis of cancer for any individual, as well as his/her family and friends, can be devastating. It can lead after shock and surprise to acute anxiety and depression. This can make the medical situation worse as the patient may make poor health decisions, not follow advice or increase the possibility of psychosomatic disorders.

This, in turn, may lead medical experts to advise a patient to seek psycho-therapy of one sort or another. Some patients may be directed to a particular type of therapy, while others may seek it out of their own accord. The number of people seeking out "cures" on the web has grown exponentially and doctors are increasing wary of patients coming to see them with computer sticks or bulging files of "information and advice" they have gleaned from different websites. It is often a testament to their fear and desperation.

There are many factors determining when, why and what sort of psychotherapy they seek. Firstly choices may be severely limited depending on where the patient lives, as well as his/her resources. Psychotherapy of any sort may be unavailable or only at a cost that the patient may not be able to afford. Secondly, some are very strongly directed to one sort of therapy or another. There may for instance.

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Chapter 3
New Developments in Psycho-oncology

Winfried Rief, Carola Seifart and Yvonne Nestoriuc

Abstract Despite various ups and downs in the history of psycho-oncology, it's more and more developing to an evidence-based intervention that is a necessary prerequisite of a competent cancer treatment center. Various psychosocial factors have been identified that contribute to the development, course, and prognosis of cancer, such as life stress, social network, or comorbid depression. Psycho-oncology offers training programs for physicians how to minimize distress in the "breaking bad news" situation, but full intervention programs how to cope with life stress, how to cope with comorbid pain and fatigue are also available. Latest developments offer interventions to reduce the burden that is associated with medical side effects of oncologic treatments. In addition to the medical treatments, psycho-oncologic interventions are crucial to optimize quality of life of cancer patients. Finally, psychosocial interventions how to deal with end-of-life issues are also developing, but need further evaluation.

Keywords Psycho-oncology · Stress · Depression · Breaking bad news · End of life issues

3.1 Psycho-oncology: From Past to Present

Cancer is considered to be mainly the result of genetic and biomedical causes, although additional environmental influences have been reported to be of relevance (e.g., nutrition; radiation; external mechanical irritation). In contrast to the

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biomedical illness model of cancer, former psychoanalysts [e.g., Franz Alexander and others (Alexander 1951)] hypothesized that cancer can be the result of some specific unconscious conflicts. These and other assumptions led to an over-psychologization of cancer, although empirical evidence for these psychological theories was low. Therefore the biomedical conceptualization of cancer was re-installed and further strengthened. Subsequently, the dualism between "cancer is a biomedical condition", or "cancer is a consequence of psychological conflicts" was abandoned, and replaced by the concept that cancer is always a biomedical condition, interacting with psychological and behavioral features of the patient. Patient's concerns, behaviors, fears, and other psychological factors such as familial or work related circumstances are expected to be able to influence the disease course, treatment adherence, and outcome.

3.2 Psycho-oncology: Some General Approaches

Stress has been one of the major psychological suspects to influence cancer. Such an assumption was further supported by the findings of psychoneuroimmunology, indicating that the activity of the immune system can be influenced by psychological and environmental factors (Reiche et al. 2004). However, studies on the influence of stress on the cause of cancer did not reveal robust results, until some meta-analyses [e.g., (Chida et al. 2008)] confirmed that stress related psychosocial factors are associated with higher cancer incidence in initially healthy populations ($p = 0.005$; 548 studies). The relation between stress and cancer occurrence is further supported by findings that people who develop cancer in adulthood report more early childhood adversities than healthy people (Holman et al. 2016). Moreover, after cancer has been developed, subsequent stressful life events are related to poorer cancer survival and higher mortality. While these influences of stress on cancer might not be of relevance for every single patient, the overall association seems to be well-established. Stress levels are different between types of cancer, with high scores for people with lung cancer, Hodgkin's, and pancreas cancer, and somewhat lower scores for gynecological, prostate, and colon cancer (Zabora et al. 2001). Consequently, stress management programs for patients with oncologic diseases have been developed, and shown that they are able to improve quality of life [e.g., cognitive-behavioral stress management for women suffering from breast cancer; (Antoni et al. 2006)].

Another relevant influence on cancer course is the social network of the patient. Again, several studies investigated this issue, and some large longitudinal studies confirmed the role of the social network, e.g. in women suffering from breast cancer (Kroenke et al. 2006). The risk of dying was increased by 66% in socially isolated women compared to women living in a good social network. Some comparisons even revealed higher effects: if women did not report of living children, the hazard ratio of dying was increased to HR = 5.6 compared to women who were mothers of children, and women reporting no friends had an increased risk of dying of HR = 4.1. Similar results were confirmed by Weihs et al. (2008).

A third general psychological factor contributing to cancer can be comorbid depression. Comorbid depression has been shown to predict development of cardiovascular diseases, but also to be associated with an increased risk of re-infarction after an initial myocardial infarction, and with an increased risk of lethal infarction (Barth et al. 2004). Therefore this type of analysis was also used for a potential association between comorbid depression and long-term survival in cancer. Pinquart and Duberstein (2010) confirmed that the relative risk of mortality is increased in cancer patients if they suffer from comorbid depression. Although the relative risk is only moderately increased (RR = 1.2), this effect is evident for all stages of cancer severity, and it is also of relevance if depression occurs before cancer. The type of cancer (leukemia, breast cancer, lung cancer, brain cancer) did not determine the association, but comorbid depression played a significant for all of these subtypes. Treatment of comorbid depression improves quality of life in cancer patients, and some studies even indicate an improvement of long-term survival after depression treatments in oncologic patients (Spiegel and Giese-Davis 2003).

Thus, these general psychological concepts were able to confirm a significant impact for the development, and especially the course and prognosis of cancer. In the following, we will highlight some new approaches and results, and we will structure the report according to the timeline: receiving the cancer diagnosis for the first time ("breaking bad news"), coping with cancer and cancer associated stress, pain and fatigue, managing side-effects of cancer treatments, managing fear of recurrence, and finally end of life decisions and palliative care.

3.3 Breaking Bad News: Informing Patients About Cancer Diagnosis

The situation of receiving a cancer diagnosis is extremely sensible. Depending on the circumstances of this situation, consequences such as serious anxieties, desperation, maladaptive behaviors, and non-adherence to medical recommendations can occur. For patients, this is typically a situation of extreme stress, with all the risk of cognitive misunderstandings, lack of comprehension of medical information, and drawing wrong conclusion.

To better prepare and structure the "breaking bad news" situation, medical societies have recommended the so-called Spikes Protocol (see Table 3.1). The Spikes Protocol recommends to plan and define the right setting for this difficult communication (setting). During the situation, doctors should check patients' perception of the setting and of the received information continuously (perception). The situation should be prepared and announced to the patient, and before starting, patients should be asked whether they are ready to receive information now and about their preferences for the level of details (invitation). During and at the end of the session, the comprehension of the information of the patient should be re-checked (knowledge). Patients' emotions should be observed during and after the

Table 3.1 The SPIKES protocol for physicians how to prepare and structure "breaking bad news" situations (McFarlane et al. 2008)

• **S**etting: Prepare an adequate setting (sitting on chairs), with sufficient privacy (room with closed doors, not the hallway), probably with relatives
• **P**erception: Ask how the patient perceives the medical situation
• **I**nvitation: Prepare patient for discussion; check patient's need about information; use participative discussion style
• **K**nowledge: Giving medical facts; cross-check patient's comprehension of the information you provided to him/her
• **E**motion: Assess/ask for patient's emotions during and at the end of the session; try to understand the individual reasons for these emotions
• **S**ummary, Strategy: Summarize major facts and the strategy how to proceed

session (emotion). And finally, patients should summarize the major content and the plan strategy together with the doctor, possibly set a date for a follow-up consultation (summary).

It is very obvious that patients can differ substantially in their preferences for the breaking bad news situation. Therefore we developed an assessment tool (Marburg Breaking Bad News Scale; MABBAN; Hofmann et al., submitted) and validated this scale with 344 German cancer patients. We included all aspects of the Spikes Protocol, and added further cognitions and preferences that were collected during pilot studies using unstructured interviews. Afterwards, patients' answers were factorized, and we found 5 major factors indicating patients' preferences:

1. Getting information
2. Comprehension
3. Emotional support
4. Clarity of delivered information
5. Pre-arrangement of disclosure

This scale was further used to analyze how patients' preferences fitted to the reality of their experienced breaking bad news situation. Table 3.2 shows that for some preferences the overlap was quite good (e.g., "having clarity about suffering and progress"), while other requirements were serious needs of the patients, but not fulfilled in the reality of the breaking bad news situation (e.g., possibility to ask questions, having enough time, undisturbed atmosphere, not talking over the phone). Most pronounced discrepancies between patients' preferences and the reality were for the items: "involving the patient in further planning", and "definite explanation of the course of disease", with more serious needs from the patients than covered by the physicians. While the latter could be a consequence of unclear medical status, the lack of patients' involvement in further planning is a crucial weakness that could be easily changed in doctors' behavior (Seifart et al. 2014).

Table 3.2 What patient's want and what they get: breaking bad news situations

Item (SPIKES number)	Patients' preference		Reality	
	"Entirely" (%)	*M* (*SD*)	"Entirely" (%)	*M* (*SD*)
Having clarity about suffering and progress after BBN (4)	96.8	1.03 (.18)	77.9	1.35 (1.22)
Reinsurance about patients' comprehension (4)	94.7	1.05 (.22)	–	–
Having enough time (1)	94.5	1.06 (.23)	64.4	1.62 (.96)
Possibility to ask questions (6)	93.8	1.06 (.24)	60.9	1.67 (.97)
Having the feeling that planned treatment is the best (4)	93.8	1.07 (.28)	60.9	1.77 (1.11)
Elaborate and coherent explanation of the disease (4)	93.2	1.07 (.27)	61.3	1.58 (.87)
Definite explanation of the diagnosis (4?)	89.8	1.11 (.36)	–	–
Undisturbed atmosphere (1)	86.9	1.17 (.51)	63.3	1.87 (1.25)
Involving the patient in further planning (6)	84.2	1.19 (.47)	48.1	2.14 (1.27)
Definite explanation of the course of disease (4)	82.9	1.21 (.53)	40.7	2.18 (1.17)

Highest ratings of patients preferences regarding different aspects of breaking bad news compared to experienced reality

3.4 Coping with Cancer-Associated Stress, Pain and Fatigue

While acute medical cancer therapies such as surgery, chemotherapy or radiation are able to reduce the health risk of cancer substantially, they typically do not address issues such as cancer-associated stress, pain and fatigue. However, these symptoms are the major determinants of disability associated with cancer and of special relevance to cancer survivorship. Therefore an adequate recognition and treatment of these syndromes is crucial to reduce cancer associated disability.

Cancer and cancer treatments often lead to the development and maintenance of chronic pain syndromes. In a large study including nearly 4000 women, we were able to show that pain conditions tend to persist even four years after acute cancer interventions (Rief et al. 2011). Both medical and psychological variables predict persistence of pain in cancer. Drug treatments with tamoxifen and aromatase inhibitors are associated with pain development such as arthralgia and often persist over years. Additionally, they increase the risk of intensifying preexisting conditions of osteoporosis. Moreover, psychological and behavioral variables such as depression and low physical activity also predict pain chronicity. Further, life events during the first 12 months after medical breast cancer treatment also influence pain maintenance. This highlights the association between life stress and the course of cancer which was already mentioned in the introduction section.

If comorbid pain syndromes are crucial determinants of disability in cancer patients, their detection is of central relevance. However, current classification systems such as ICD-10 do not allow to classify pain conditions adequately that are associated with cancer and cancer treatments. This hinders the development of adequate medical care plans and their financial compensation in health care systems. Therefore, the International Association for the study of pain IASP started a working group developing a pain classification system that also allows the classification of cancer associated pain (Rief et al. 2010; Treede et al. 2015). This classification system of chronic pain conditions became part of the ICD-11 beta draft, and has the potential to improve the recognition of pain conditions in cancer patients in the future.

Psycho-oncology has developed further interventions to cope with pain, to increase physical activity despite of fatigue symptoms, and to reduce stress. Special interest received the cognitive-behavioral stress reduction program (Antoni 2003), because investigators were able to show that participation in this program had beneficial effects not only on well-being, but also on immune functioning of affected women (Antoni et al. 2009). Details of this program especially developed for women with breast cancer are shown in Table 3.3.

During the last 20 years, mindfulness-oriented interventions have been further developed and applied in patients with cancer, and they received substantial popularity in many countries. Mindfulness-based interventions have been shown to induce positive emotions in cancer patients, although some recent studies also brought more critical results, e.g. in prostate cancer (Chambers et al. 2017). Even in the case of positive results, effect sizes of MBCT or MBST are often only in a modest range [e.g., in a meta-analysis of breast cancer patients, (Cramer et al. 2012)], not only in cancer, but in terms of physical well-being in general (Gotink et al. 2015). However, mindfulness-oriented interventions can also have beneficial effects on cancer-associated pain conditions, although again, it is unclear whether mindfulness-oriented interventions can really compete with other, well-established pain management programs, e.g. deriving from a CBT rationale (Johannsen et al. 2016).

Meanwhile these interventions can be considered to be necessary components of a multidisciplinary cancer care center providing psycho-oncologic support.

Table 3.3 The cognitive-behavioral stress management program for women with early-stage breast cancer: ingredients (Antoni 2003)

• Closed, structured group format in weekly sessions for a period of 10 weeks
• Relaxation and guided imagery exercises
• Stress reduction techniques such as rational thought replacement
• Learning to reframe their appraisals of stressful situations, improve their coping strategies and better match their coping choices to the nature of these situations
• Interpersonal skills to optimize their communication skills and use of social resources
• Anxiety reduction techniques
• Conflict resolution and emotional expression

3.5 Managing Side Effects of Cancer Treatments: A Psychological Approach

Many medical cancer treatments are known for their associated side effects and burden for the patients. However, the development and perception of side effects is not only a direct consequence of biomedical processes, but psychological factors play an additional critical role. Placebo and nocebo research has impressively shown that patient's expectations are a powerful predictor of the development of side effects (Enck et al. 2013). In patients with rheumatoid arthritis, their expectation to develop side effects predicted their future occurrence (Nestoriuc et al. 2010). Repeated application of side effect inducing drugs leads to learned symptom maintenance, even if future applications are mere placebos (Rheker et al. 2017). Therefore, psychological mechanisms offer a window to better understand the development of side effects, and furthermore, to establish new side effect prevention programs.

It is appealing to apply these results from other specialties of medicine to oncology. Aromatase inhibitory (AI) are typically recommended because they reduce the risk of cancer recurrence in gynecological cancer types. Given that breast cancer is the most common type of cancer in women, antihormonal therapy is the most frequently prescribed oral anticancer agent worldwide (Burstein et al. 2014). However, these drugs also induce substantial adverse events, typically increasing in frequency and intensity during the first 12 weeks of treatment. This is the more problematic, because many affected patients discontinue drug intake, although their medical benefit is convincing. We were able to show that again womens' expectations to develop side effects after breast cancer interventions, but before AI onset, were a powerful predictor of adverse events later (Nestoriuc et al. 2016). In interaction with onset symptoms, these expectations predicted side effects 2 years later (Nestoriuc et al. 2016). Furthermore, results of a longitudinal study showed that early disruptions in treatment adherence are influenced by the type of treatment information and resulting treatment expectations given to patients with breast cancer (Heisig et al. 2015). Patients, especially older ones, can benefit from enhanced treatment information about the mode of action, efficacy and potential side effects of their treatment given additionally to routine care.

If patients' expectations are such a powerful predictor of side effects, the question arises whether optimizing patient's expectations leads to a reduction of symptom load (von Blanckenburg et al. 2013). The following patient case story taken from Zimmermann and Heinrichs (2008) exemplifies the prominent role of individual expectations for symptom perception and psychological adaptation.

> For my next checkup, I was to receive a contrast agent. I was anxious, knowing that my body reacts strongly to that kind of thing. The nurse hooked me up to the IV, through which the contrast agent would enter my body. She told me that the contrast agent would make me feel hot and that there might be a burning sensation. She then left me alone. The minute she left the room, I felt the heat washing over me, it streamed through my body and it burned. I knew this checkup was going to be awful. I felt extremely frightened. After a few minutes the doctor entered the room and she told me: Ok, let's inject the contrast agent, shall we?

Just recently, we are able to show that optimizing patient's expectations can lead to improved outcome in heart surgery patients (Rief et al. 2017). In this study, patients who were scheduled for coronary artery bypass graft surgery (CABG) received two in-person sessions and two phone calls to optimize expectations for the time of the recovery after the surgery. With this minimal intervention prior to surgery, we were able to show that outcome especially in terms of disability can be substantially improved.

Therefore we decided to use a similar approach for women who suffered from breast cancer, and were referred for AI treatment. Again, women received a few intervention sessions before treatment start just to optimize their expectations about outcome, about the development of side effects, and their expectations about how they could cope with symptoms if they occur. First results of this study confirmed that quality of life and side effects burden can be substantially improved if women participate in such a program.

3.6 Fear of Recurrence

Fear of recurrence is a crucial problem in patients who suffered cancer, and can seriously limit quality of life. Patients might experience extreme concerns and debilitating fear before they have to undergo the next checkups in oncology. They might be at risk to overly observe their body with its minor sensations. Therefore addressing fear of recurrence is an additional necessary part of a timely psycho-oncologic program. Fear of recurrence has also shown to determine quality of life after medical cancer interventions (Simard et al. 2013).

The German psychologist Herschbach et al. (2010) has addressed this topic for many years. He developed a four session intervention program to deal with fear of recurrence in cancer. The program includes the following topics:

(1) Self-monitoring and diagnostics:
Description of anxieties; identification of triggers; psychoeducation: role of anxiety
(2) Exposure, cognitive restructuring:
"Thinking through" (no stop at points of fear); facing the catastrophic cognitions; acceptance of fears
(3) Behavior change; solution
Focus on resources of patients; reflection of values; planning of next steps

This program includes well established approaches of psychoeducation, cognitive and behavioral interventions, and resources-oriented positive psychology. While first results are positive, further development and evaluation is needed. Interventions combining these approaches with mindfulness techniques also revealed positive results (Dawson et al. 2016).

3.7 End of Life Decisions and Palliative Care

During the last two decades, special interest developed in improving palliative care. However, there is still a strong focus on anesthesia, instead of addressing the broad variety of psychosocial issues that are of relevance in this sensible period of life. Therefore we started to assess personal needs during the end of life period. Without doubt, medical issues are of high relevance, in particular in combination with nursing care. However, it is not only the anesthesia issue, but of even more relevance is how to keep personal autonomy in as many fields and as long as possible. Many decisions have to be drawn, and require the special assessment of patients needs. Organizational topics (such as heritage regulations, ensuring social status of relatives), organizing last meetings with significant persons, clarifying current conflicts and preparation of solutions are just a few examples. Religious and spiritual questions can receive priority, and several (yet not all) patients' would prefer to talk about. Emotional aspects such as fear of dying, fear of death, dealing with the mourning reaction of relatives can be further relevant topics.

To improve our knowledge about end of life needs, we investigated 89 advanced stage cancer patients. Patients confirmed that end of life issues are very important for them to be discussed; however, only 46% of patients reported to have talked to anyone about end-of-life issues before (Seifart et al., in press). Therefore patients seem to have an urgent need discussing end-of-life issues, but they are reluctant to initiate these conversations themselves. Sensible engagement is needed to help patients addressing these issues.

To summarize, from a psycho-oncological perspective the goals of palliative care are as follows:

- Supporting the patient how to achieve the maximum of personal autonomy, and how to receive respect even in situations that can be evaluated to be embarrassing for the patient.
- Pain treatments should be characterized by a maximum of shared decision making. Maximal pain suppression is not the goal of every patient, in particular if it is associated with some numbness because of opioids. Physicians and patients have to find the balance that fits the best to patient's needs.
- Evaluation and clarification of patient's needs and conflicts. Typically, this also requires to consider patient's biography, significant persons, needs to regulate things for the time after dying.
- Providing emotional support to cope with all the emotional challenges that can occur during end of life periods. It must be considered that patients make a significant differentiation between fear of dying (e.g., the physical suffering, the loss of autonomy) and fear of death (how will others continue their life? Where am I when I am dead?).
- Providing spiritual and religious consultation if requested.

- Supporting relatives how to deal with the dying person, how to deal with their own feelings of sadness, how to support the patient to keep as much autonomy and dignity as possible.
- If possible, provide dignity therapy or similar approaches (see below).

Dignity therapy has been developed by Chochinov and colleagues (Chochinov 2007; Chochinov et al. 2005). During a one hour session, the therapist asks a series of open-ended questions that encourage patients to talk about the following issues:

– Please tell me about parts of your life that you consider of most relevance of your life?
– What are the most important roles you have played in your life?
– What are your most important accomplishments, and what do you feel most proud of?
– Are there particular things you want your family to remember, and/or to remember about you?
– What are your hopes and dreams for your loved ones?
– What kind of advice or guidance do you want to give to some significant persons of your life?

The conversation is recorded, transcribed, edited and then returned within a few days to the patient, who is given the opportunity to read the transcript and make changes before a final version is produced. Patients are invited to share the document with family and friends.

Dignity therapy has been shown to result in high satisfaction scores of participating patients who are in the life period close to the end of it, and dignity therapy seems to have advantages compared to other psychological interventions (Chochinov et al. 2011). While these results are highly encouraging, we should be aware that this is just one of the first systematic psychological approaches of dealing with end of life issues, and preparing for the last goodbye. Thus these results should also encourage not only disseminating this approach, but also to further improve it, extend it, or develop more intense alternatives. Moreover, these first evaluations cannot be considered to be overly comprehensive, and therefore this and similar approaches need further scientific evaluation.

3.8 Conclusions

Psychological factors are of relevance for the development, course, and palliative periods of cancer diseases. General life stressors, social support, comorbid depression are just a few examples of factors that can influence and predict the course of oncologic diseases. Moreover, from a more general perspective, every patient develops his/her own subjective illness theory and illness beliefs. While the one patient can react to the onset of cancer with an extreme emotional crisis, the same medical condition can lead to optimism and belief in a positive prognosis in another

patient. Therefore the breaking bad news situation is of crucial relevance for initiating a helpful coping process of the patient, and physicians should be trained to establish optimal breaking bad news conversations. Physicians should be aware of the huge discrepancy that can occur between medical evaluations and patient's subjective beliefs. To predict patient's behavior, their subjective beliefs are of more relevance than physicians opinions. Therefore these subjective beliefs determine whether patients will be compliant, whether patients will be able to cope with side effects of oncologic interventions, or whether patients show dysfunctional withdrawal behavior. Side effects are strongly determined by patient's expectations. Hence, it is important to direct more careful efforts into patient-doctor encounters and especially, into information that cancer patients receive about their care. These encounters and information are likely to shape patient's expectations.

Psycho-oncology offers approaches for all phases of cancer diseases, starting from behavioral prevention programs, training physicians how to create non-damaging breaking bad news situations, supporting functional coping processes of patients, supporting patients to cope with symptoms that are of crucial relevance for quality of life (such as pain, fatigue, cancer-related stress) , supporting patients to develop helpful expectations about medical interventions, and providing special support during the sensible end of life period and during palliative care. However, research efforts in psycho-oncology have to be increased to better understand the crucial psycho-oncologic mechanisms, to develop better intervention programs that fit to the needs of the patients, and to provide better clinical trials to evaluate these interventions.

3.9 Summary

Psycho-oncology has developed to an evidence-based intervention that should be a prerequisite of any competent cancer treatment center. It offers intervention approaches for all phases of cancer, from prevention to dealing with end of life issues. Latest psychological innovations offer help to reduce the burden of side effects of oncologic treatments. We recommend developing screening tools to analyze patient's needs during different illness periods, and to offer psychological interventions that target the improvement of these problems.

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Chapter 4
Psychiatric and Psychosocial Aspects of Breast Cancer Diagnoses and Treatments

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Abstract This chapter explains some aspects of breast cancer. The main emphases are on some common psychiatric disorders like depression and psychosocial problems and their psychiatric treatments and psychological supports. Additionally, we have tried to take note of most psychosocial problems that a patient with breast cancer and her family are facing. We asserted that psychiatrists should take more active roles in their treatment plans as a member of the team treating patients with breast cancer. The main emphasis has been on the role of a consultation-liaison psychiatrist and some other members of the mental health providers to help patients better, and to present an integrative medical service with success. Depression is the most common psychiatric problem that breast cancer patients are affected by. Depression and other psychiatric disorders in cancer patients, especially in patients with breast cancer, are less diagnosed than the real rate. We also noted the anxiety disorder and sleep disorder at a glance. A psychiatrist accompanied by other treating physicians from other specialties and other departments help together in properly detecting and managing the psychiatric and psychosocial problems in cancer patients. The use of psychiatric medication in most patients is necessary. Psychiatric drug treatments in patients with breast cancer and their interactions with other medications and hormones used in breast cancer patients are explained briefly. Non pharmacological treatments especially Cognitive Behavioral Therapy (CBT), which have been effective in clinical trials for cancer patients are mentioned in this chapter and available by the mental health providers in most centers. Also noted, are the surgical treatments of the breast cancer. Varieties of surgical procedures have been described with simple words for clearer understanding. Patients and physicians can find most answers to their questions about the appropriate type of surgery and its potential complications in this chapter, followed by a brief note on breast cancer surgery in Iran.

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Abbreviations

ALND	Axillary lymph node dissection
BCQ	Breast cancer chemotherapy questionnaire
BCS	Breast-conserving surgery
BCT	Breast conserving therapy
CBT	Cognitive behavioral therapy
CT	Cognitive therapy
CRH	Corticotrophin-releasing hormone
DSM	Diagnostic statistical manual
DLPFC	Dorsolateral prefrontal cortex
ECA	Early childhood adversity
EORTC QLQ-BR 23	European organization for research and treatment of cancer breast cancer quality of life questionnaire
FCIT-B	Functional assessment of chronic illness therapy-breast
HRT	Hormonal replacement therapy
HADS	Hospital anxiety and depression scale
HPA axis	Hypothalamic–pituitary–adrenal axis
MDD	Major depressive disorder
MT	Mastectomy
QOL	Quality of life
QLQ-BR	Quality of life questionnaire breast cancer
RSCL	Rotterdam symptom checklist
SLDS-BC	Satisfaction with life domains scale for breast cancer
SLNB	Sentinel lymph node biopsy
5-TTLPR	Short allele serotonin transporter genotype
SEGT	Supportive expression group therapy

4.1 Introduction

Cancer among patients and in all societies is still a disease of fear. Cancer often suddenly appears in the body and jumps to various organs; and the macroscopic stage of the cancer usually progresses rapidly, and without treatment in most cases, the disease will be virulent. Despite medical advances in the twenty-first century, the frightening face of cancer still pervades the people and is known as one of the incurable diseases. Avicenna, the most famous ancient Iranian physician who lived about a thousand years ago, knew of some of the types of cancer and considered them as an incurable disease. However, Avicenna emphasized a general principle in Iranian traditional medicine; that treatments should be reviewed and updated based

on the circumstances of time, place and climate. Therefore today, Iranian traditional medicines, along with modern evidence based medicine, have numerous remedies and treatment plans, that help the cancer patients to improve their lives. Nowadays, psychiatric drugs with minimal side effects and abundant clinical benefits are available in the market and their efficacy has been proven in clinical trials (Derakhshan et al. 2008a). Because of evidence based clinical trials, most physicians are eager to prescribe medications to treat disorders or to reduce adverse effects of other therapeutic measures (Derakhshan et al. 2008b).

A woman who is diagnosed with breast cancer, due to the risk of imminent death, may give up her hope and feel despair. Her life expectancy is somewhat lost. Family and relatives at first cannot believe that their loved one is suffering from a difficult to cure sickness.

After hearing this bad news, the patient and her family are faced with serious mental stress and become shocked. The following examples are of recently diagnosed Iranian women with breast cancer:

> Something is behind the door, something which can make everything worse. I'm always waiting, but unfortunately not for a good thing.

> This is an intolerable feeling that you are always expecting unknown tragic events. You sleep with fear, wake up with fear, look at yourself with fear, touch yourself with fear and finally leave with fear. Constant fear is your life background.

Usually this state of shock does not last long and the patient and their family realizes that they must take action. At this stage, the patient needs calmness and support in order to prevent a feeling of loneliness. A psychiatrist should support the patient and her family and give them the opportunity to gradually progress from the stage of shock. The first paths of the patient and her family, if not with the help and supervision of a psychiatrist, may not be targeted at all. Thus the patient and those around her may be entering a phase of denial. They are often unconsciously busy with chores or even going on a vacation together. Sometimes the denial phase takes too long so the patient loses some opportunities and remedial measures that should be done within a certain time. A psychiatrist with reciprocal understanding and confidence with the patient can organize her thoughts and assist her in managing this crisis that has occurred. A psychiatrist helps the patient to manage her thoughts and feelings in order to make rational decisions and to demonstrate appropriate behaviors. Some people may quickly pass the first and second stages, and with a realistic approach, seek help from her physicians to manage their current situation. At this stage, the psychiatrist helps the patient feel a belonging to the large population of other breast cancer patients, acknowledging that she is not the only cancer patient and anyone may get cancer. Having cancer is a likely coincidence in medicine and does not relate to one's character or to her life history. Just like any disease in medicine, it depends on environmental and especially genetic factors in some persons.

Following this stage, some patients enter the acceptance stage. With the help of mental health providers and psychiatric services, these patients can cope with their disease. Based on the clinical evidence, these patients live longer and have a better

quality of life ahead of them. Some groups of patients who have a poor coping strategy may show aggressive behaviors after the denial phase. These patients fight with others and are accused to have childish behaviors. At this time the patient feels alone and believes that no one can understand them and she is a perishing victim.

A young Iranian woman explained her emotional dizziness with these words:

> I'm confused. I don't know their compassion is because of my illness or they really love me for myself. I don't like their pity. I can't understand their emotions.

Another woman with breast cancer said:

> My husband shows different emotions. He comes home so late but he also tries to show me compassion. I can't believe that he is really impacted by my suffering, rather I think he wants to flee and is looking for a proper opportunity.

She may refuse to accept help from any one. She may look for a culprit and quarrel with her family and friends. Sometimes a patient will demonstrate this anger toward herself thus hurting herself. In most studies the incidence rate of suicide in cancer patients and especially patients with breast cancer has been reported more than in the general population (Hjerl et al. 2002). At this stage, a consultation-liaison psychiatrist, who is the head of mental health service team, on the reciprocal trust and the therapeutic alliance with the patient, permits her to share problems with therapists. In this stage and other stages, the psychiatrist welcomes the contribution of family members, understanding their needs and patiently answering their questions, making decisions as an honest advisor, accompanying the patient and her family in all diagnostic and therapeutic stages.

In many cancers, including breast cancer, surgery is one of the requirements for treatment in the early stages. A trained and experienced surgeon, after necessary examination and testing, shares the diagnosis of breast cancer with the patient in a calm and intimate atmosphere. Optionally, every one of her family members can participate in this session and become involved later in the treatment steps. The treating physician should understand the initial reaction of the patient. Assure her, in a supportive and professional manner, that the disease has an appropriate treatment. The physician indicates that for cancer at any stage there are many appropriate and effective treatments available. The physician explains that the treatment of cancer is accomplished by teamwork and that mental health professionals are also important components of the team.

The head of the team is a psychiatrist who preferably is trained in the psychosomatic department as a consultation-liaison psychiatrist to manage an integrative mental health service with a dynamic interaction with physician of other departments in a general hospital. The attendance of a consultation-liaison psychiatrist provides an easy path to give patients an integrative medical service in a general hospital. A consultation-liaison psychiatrist has the role of supervision, along with the treating physician, in a comprehensive plan for the treatment and rehabilitation, especially psychosocial support for patients and families. Psychiatrists have been alongside with patients and families on the path to obtain their health blessedly. In every step, psychiatrists, in this manner, collaborate with other members of the

treatment team and also members of the mental health providers group that may be of clinical benefit for a comprehensive medical service. Some other members of mental health providers are as followings: clinical psychologists, occupational therapists and social workers. A clinical psychologist is trained to offer psychological support, psycho-education and psychotherapy. Occupational therapists and social workers help patients and families to manage the vocational and socioeconomic problems.

Almost all medical and psychiatric disorders can affect the cognitive function of the patients and therefore, some of their behaviors. Some disorders may have fewer effects on cognition and behavior, whereas, some other disease may have more influence on each. It depends on the nature of disorders and their consequences on the brain, cognitive function and behavior. (Derakhshan et al. 2011). The goal in the treatment of breast cancer is to manage progression of the disease; most patients desire a cancer-free outcome. In pursuit of these goals, many patients engage in multiple treatment modalities including surgery, chemotherapy, and radiation, each of which can have devastating physical, emotional, and spiritual effects, on the patients and their families. If the patient is a candidate for surgery, the surgeon is at the disposal on one or more occasions with the patient and her family, prior to the surgery, to answer their questions and to explain to them some probable complications. He will ensure them that the treatment team will utilize the utmost care for helping them and treatment of the disease. Before surgery, the patient may also need to have sessions with psychiatrist to have a talk about her worries and fears before the surgery and the consequences post-surgery, particularly regarding a mastectomy, the main surgical treatment of breast cancer, which has been worrisome for womenfolk. A mastectomy is a serious change in the body for a woman and could hurt her womanly character. Mastectomy could stir up a storm in her mind because of the damage to her femininity. To be expected most of patients need psychological assistance to cope with this phase. Psychiatrists, with accompaniment of other members of the mental health team, arrange one or more sessions in order to prevent less mental harm to patients and families. The aim is that she adapts well to the new circumstances as soon as possible and returns to her normal life. The patient naturally has various roles in her family and in her society; the role of being a good wife, a successful mother, a valuable person in the community. Psychiatrists, with accompaniment of other members of the mental health team try to help patients and families return to the individual and social roles as in the past or even better.

4.2 Epidemiology of Breast Cancer

Breast cancer is the most common malignancy in women worldwide, with 178,480 new cases in the United States each year (American Cancer Society 2007, 2009). About one in eight U.S. women (about 12%) will develop invasive breast cancer over the course of her lifetime. In 2017, an estimated 252,710 new cases of invasive breast cancer are expected to be diagnosed in women in the U.S., along with 63,410

new cases of non-invasive (in situ) breast cancer. About 2470 new cases of invasive breast cancer are expected to be diagnosed in men in 2017. A man's lifetime risk of breast cancer is about 1 in 1000 (American Cancer Society 2017).

Breast cancer accounts for 15% of deaths from cancer in women (Jemal et al. 2008). In the 1970s, the likelihood that a woman could be affected with breast cancer in the United States of America was one in thirteen people and this ratio in 1980 rose to one in eleven; and in 2004 rose to one in eight woman. Documents show that the incidence of new cases of breast cancer from the mid-1940s have gradually increased (Clarke et al. 2006) and it is estimated that 1.2 million new cases will be diagnosed annually worldwide (Jemal et al. 2008). As we mentioned, breast cancer incidence rates in the U.S. began decreasing in the year 2000, after increasing for the previous two decades. They dropped by 7% from 2002 to 2003 alone. One theory is that this decrease was partially due to the reduced use of hormone replacement therapy (HRT) by women after the results of a large study called the Women's Health Initiative were published in 2002. These results suggested a connection between HRT and increased breast cancer risk. About 40,610 women in the U.S. are expected to die in 2017 from breast cancer, though death rates have been decreasing since 1989. Women under 50 have experienced larger decreases. These decreases are thought to be the result of treatment advances, earlier detection through screening, and increased awareness. For women in the U.S., breast cancer death rates are higher than those for any other cancer, besides lung cancer.

Besides skin cancer, breast cancer is the most commonly diagnosed cancer among American women. In 2017, it's estimated that about 30% of newly diagnosed cancers in women will be breast cancer. In women under 45, breast cancer is more common in African-American women than white women. Overall, African-American women are more likely to die of breast cancer. For Asian, Hispanic, and Native-American women, the risk of developing and dying from breast cancer is lower. As of March 2017, there are more than 3.1 million women with a history of breast cancer in the U.S. This includes women currently being treated and women who have finished treatment (American Cancer Society 2017).

A woman's risk of breast cancer nearly doubles if she has a first-degree relative (mother, sister, or daughter) who has been diagnosed with breast cancer. Less than 15% of women who get breast cancer have a family member diagnosed with it.

About 5–10% of breast cancers can be linked to gene mutations (abnormal changes) inherited from one's mother or father. Mutations of the *BRCA1* and *BRCA2* genes are the most common. On average, women with a *BRCA1* mutation have a 55–65% lifetime risk of developing breast cancer. For women with a *BRCA2* mutation, the risk is 45%. Breast cancer that is positive for the *BRCA1* or *BRCA2* mutations tends to develop more often in younger women. An increased ovarian cancer risk is also associated with these genetic mutations.

In men, *BRCA2* mutations are associated with a lifetime breast cancer risk of about 6.8%; *BRCA1* mutations are a less frequent cause of breast cancer in men. About 85% of breast cancers occur in women who have no family history of breast cancer. These occur due to genetic mutations that happen as a result of the aging process and life in general, rather than inherited mutations.

The most significant risk factors for breast cancer are gender (being a woman) and age (growing older).

In Europe, breast cancer incidence has increased from 76 per 100,000 inhabitants in 1995 to 88 per 100,000 inhabitants in 2008. Mortality, however, decreased from 27.3 per 100,000 inhabitants in 1995 to 24.3 per 100,000 inhabitants in 2008 (Bray et al. 2002; Ferlay et al. 2010); resulting in a growing group of breast cancer survivors every year.

The prevalence of the disease in Western countries and North America is 8–10% and in Asian countries about one percent (Parkin et al. 2002). The highest rate of breast cancer is seen in the USA, Australia, New Zealand, South America, Eastern and Western Europe (Radice and Redaell 2003), the incidence is also rising in many developing countries. For example, breast cancer is the second leading cause of cancer and the leading cause of death for 35- to 59-year-old Taiwanese women (Chen et al. 2002). In Iran based on statistics, 6.7% per one thousand women are diagnosed with breast cancer (Hadi et al. 2002). About 21.4% of all cancers among women in Iran is breast cancer (Harirchi et al. 2004).

Although Iran has less incidence rates than other Asian countries, during the last four decades, increasing incidence rates has made breast cancer one of the most prevalent malignancies among Iranian women, which constitutes 25% of all cancers among Iranian women, with the highest rate occurring in those aged between 35 and 44 years according to the latest report of Iranian Cancer Institute in 2003 (Mohaghaghi et al. 2007).

4.3 Psychiatric Disorders in Cancer Patients

Psychiatric disorders such as depression and anxiety disorders are highly prevalent in the general population especially in the patients who have severe medical illness (Lecrubier 2001). Other medical illnesses like physical or mental trauma due to accidents or even after natural disasters also can increase psychological problems (Derakhshan et al. 2008c). A meta-analysis showed a prevalence of clinical depression of 16.3% and a prevalence of all types of depression in 20.7% of oncologic and hematologic patients (Lotje Van Esch et al. 2012). Anxiety prevalence was 10.3% (Mitchell et al. 2011). In this meta-analysis only studies that used a clinical interview to assess depression and anxiety were included.

In patients with breast cancer, depression is more prevalent from other psychiatric disorders and leads to worse health related quality of life and survival outcomes (Fann et al. 2008; Ng et al. 2010; Satin et al. 2009; Giese-Davis et al. 2011). The reported prevalence of depression in patient with breast cancer ranges from 4.5 to 37%. It is different in range because of socioeconomic and geographic and ethnical factors. According to some great studies the rate of depression in breast cancer all over was about 10 to 20%. Many studies estimate the rate of depression in patients with breast cancer based on screening instruments were from 15 to 30%, are generally higher in comparison with study estimates based on a structured

interviews range from 5 to 15% (Fann et al. 2008). Because of early detection and more appropriate and improved treatment, the five year survival rates of the patients with breast cancer have increased in the last two decades up to 89% after diagnosis the illness (Ganz et al. 1998; Spiegel 1996). One of the most important areas of psychological distress for women with breast cancer are related to fear of death, body image concerns, feeling the physically and emotionally side effects of treatment, and integration of new medical demands in the context of women's prior image of self and family.

Another major research line has revealed the correlation between coping skills and medical outcomes. The results shown that, patients with less ability to cope reported more severe physical symptoms, experienced more distress, and felt worse about their prognosis (KenneSarenmalm et al. 2007) each of these contributed to a decreased quality of life.

Understanding the normal processes of human coping and adaptation, and identifying those patients at risk because of poor internal (personality, cognitive style) or external (social) resources will assist the health providers to recognize what is going on with the cancer patients and how to best help them.

The 2015 Commission on Cancer Distress announced vulnerability factors for depression, anxiety and psychological distress among patients with breast cancer. Obviously the ability to identify patients who are prone to comorbid psychiatric symptoms at the beginning of their cancer diagnosis and during treatment helps to anticipate and deliver more appropriate and personalized cancer care for them (McFarland 2016).

The practitioner of medical and psychiatric settings focus toward maximizing quality of life (QOL) among survivors who often experienced persisting aversive symptoms such as fatigue, cognitive problems and menopausal symptoms (Ganz et al. 1998; Spiegel 1996).

4.3.1 Pathophysiology of Depression in Cancer Patients, How Important to Treat?

The experience of early childhood adversity (ECA), younger than 13 years of age, primes patients to be more vulnerable to depression later in their adult life by causing more reactivity to stress (Bower et al. 2014; Green et al. 2010; McLaughlin et al. 2010). ECA has been linked to neuronal dysregulation in the development of coronary artery disease, (Loucks et al. 2011), cancer related fatigue (Bower et al. 2011) and sleep difficulties even with mild stressors (Hanson and Chen 2010). Depressive symptoms that were found in conjunction with the homozygous short allele serotonin transporter genotype in parents (5-TTLPR) have also been associated with ECA (Taylor et al. 2006). Furthermore, the early family environment and ECA correlate with immune dysregulation (Danese and McEwen 2011), specifically in the breast cancer setting.

Therefore, ECA and neglect are associated with multiple psychological symptoms but most specifically depression in the setting of breast cancer. ECA contributes to psychological burden as a vulnerability factor that may help to explain individual patient trajectories and influence the provision of patient centered care for psychiatric symptoms in patients with breast cancer (McFarland 2016).

In the last decades, the increased prevalence of depression in cancer patients has been conceptualized as an emotional response to severe stress, including emotional loss, physical pain, and often social isolation. Investigations of cancer patients suffering from major depression revealed that neuroendocrine perturbations of these individuals (i.e. HPA axis hyperactivity) are likely due to CRH hyper secretion associated with depression and/or the potent stimulation of pro-inflammatory cytokines in response to infection, tumor progression, or anti-neoplastic interventions. During the past two decades, characterization of cytokine-induced mood/anxiety and neuro-vegetative sickness behavior symptoms in humans has preceded an increasing understanding of the associated alterations of immune-inflammatory pathways and monoamine neurotransmitters. According to some evidence (Dantzer et al. 2008) in individuals receiving immune-therapies, certain cytokine-induced symptoms, such as mood and anxiety symptoms, improve with antidepressant treatments.

One of the primary and the most important concerns faced by medical practitioners and psychiatric care givers and also their patients is the psychological "stigma". This problem comes with a diagnosis of cancer and other distress that many patients and their families are faced with after being confronted with this serious illness.

The aim for mental health care providers is identifying clinically significant levels of distress in patients with cancer, and once identified how to determine whether these symptoms indicate a need for further evaluation and psychiatric intervention.

Many researchers focus on how coping with this illness affects survival rates and quality of life in patients with breast cancer. Some major instruments that measure disease specific quality of life that proved to be valid and reliable in measuring how quality of life can affect patient outcomes include the European Organization for Research and Treatment of Cancer Breast Cancer Quality of Life Questionnaire (EORTC QLQ-BR 23), the Functional Assessment of Chronic Illness Therapy-Breast (FCIT-B), Breast Cancer Chemotherapy Questionnaire (BCQ), and the Satisfaction with Life Domains Scale for Breast Cancer (SLDS-BC) (Montazeri 2008).

Obviously symptoms of distress such as pain, fatigue, insomnia, and anxiety elicited in quality of life screenings are also symptoms that may indicate an underlying depression. The rate of diagnosis of depression in breast cancer is third after the prevalence of depression in pancreatic and or pharyngeal cancers (Massei 2004; Mcdaniel 1995).

This high rate of depression in patients with breast cancer highlights why it is important to identify it and then to provide appropriate resources and treatment.

The mechanisms, by which depression affects adherence to anti-cancer clinical interventions, can be explained within the three fields. Depression can interfere with the three categories of factors—cognitive, motivational and resource related—that are essential for adherence. Some important findings proven in clinical trials are listed below (Roth et al. 1998):

Subjects by which depression affects cancer treatments
• Inability to integrate cancer diagnosis and treatment information
• Reduced motivation towards self-care
• Difficulty planning
• Negative health beliefs and pessimism about treatment
• Avoidance of health-promoting behaviors
• Social isolation and withdrawal
• Reduced use of community resources
• Greater difficulty tolerating treatment side effects
• Lack of desire and difficulty cooperating with plans for treatment

4.3.2 Psychiatric Disorders in Cancer Patients, Dianosis and Treatments

First, to assist patients, they should be instructed to follow these three major components of adherence: information, motivation and strategy. Second, non-adherence should be viewed as a potential clinical marker for depression. Physicians need to be aware of the strong relationship between non-adherence and depression. Third, providers should foster a strong clinician-patient relationship, effective communication, and partnership. Building the therapeutic relationship will help providers to diagnose and effectively manage both depression and adherence (DiMatteo et al. 1994).

Depression is likely under diagnosed in many cancer patients (Maguire et al. 1978). Studies found that treating clinicians and health care professionals failed to identify patients with depression in those patients after undergoing mastectomy. They also learned that affected patients tended not to disclose psychological symptoms. Whether this failure to disclose was caused by shame, psychiatric stigma, or lack of confidence in the treatment team member's ability to assist them effectively, was unclear. Earlier consideration and diagnosis of psychological problems from clinicians and especially mental health providers in all departments may help patients get integrative medical service (Derakhshan et al. 2008c). Screening and referrals for treatment of depressive symptoms, even at subclinical levels, is important early in treatment. Nowadays, integrative medicine, a system that services the patient as a concentrated and coordinated service by the health care providers, suggests that one of the most important members of the team of cancer

treatment should be a psychiatrist. Especially those who are trained in the field of Consultant- Liaison Psychiatry, in psychosomatic medicine departments.

The diagnosis of major depressive disorder (MDD) by DSM-V criteria includes physical symptoms that may be indistinguishable from the symptoms that occur with the cancer itself or the symptoms that occur usually as side effects of treatments and medications. Symptoms such as, insomnia, loss of appetite, poor energy, and impaired concentration maybe act as confounding factors in the assessment of individual patients for depression. One of the best solutions to this problem is the elimination of somatic symptoms from measures of depression in patients with cancer in favor of an emphasis on psychological symptoms of distress. Therefore, important diagnostic symptoms in cancer patients include suicidal thoughts, guilt, helplessness, and hopelessness (Ibbotson et al. 1994).

Two individual studies examined this approach when they compared the Hospital Anxiety and Depression Scale (HADS), which was designed for general physical illness, with the Rotterdam Symptom Checklist (RSCL), which looks specifically at cancer. Both of these measures had high positive predictive values for accurately identifying symptoms of depression and anxiety in patients with advanced cancer (Ibbotson et al. 1994; Hopwood et al. 1991).

Factors can predispose patients with cancer to a higher risk of depression
• Previous history of psychiatric illness
• Strong family history of psychiatric illness and specifically mood disorders
• Poor social support
• Higher self-reported levels of distress related to the cancer diagnosis and issues surrounding treatment

There is also a growing evidence that some psychiatric disorders especially depression, anxiety and insomnia when they occur concomitantly with breast cancer are recognizable and treatable (Weinberger et al. 2010).

Demoralization, as a persistent inability to cope, together with feelings of helplessness, hopelessness, meaninglessness, subjective incompetence and diminished self-esteem, may be a precursor or even co-exist with depression.

Although several possible diagnostic criteria have been proposed for demoralization, it has not yet been defined in the DSM. De Figueiredo (2007) highlighted that, in a multi-axial system, demoralization includes symptoms of anxiety and depressive disorders (axis I), is affected by personality traits (axis II), is clearly to be associated with physical health problems (axis III) and demoralization could replace stressful life events in axis IV, and is related to the level of functioning (axis V). This confusion occurs because our current diagnostic systems fail to recognize four perspectives of every person: his/her disease, behavior, illness and life story. Demoralization is intimately related to a person's life story.

In the first year after diagnosis of breast cancer, patients are at highest risk for depression (Rowland et al. 1999) particularly among younger patients (Compas et al. 1999). Many clinicians believe that it is critical to initiate treatment of

depression during this year, when many of the most aggressive therapies are implemented. Many women treated for breast cancer report depressive symptoms that are serious but below the threshold for a diagnosis of Major Depressive Disorder (MDD) (Mitchell et al. 2011). It is valuable to know whether differences across the subclinical depressive symptom continuum influence health outcomes. All depressive symptoms should be considered carefully by health providers.

Patients who received chemotherapy compared to patients who do not receive adjuvant therapy have higher levels of depression (Fisch et al. 2003; Rihmer et al. 2005; Musselman et al. 2006). The effects of chemotherapy on fertility, sexuality and menopause associated health problems such as osteoporosis and cardiovascular disease can lead to high levels of distress (Thompson et al. 2000). The selective estrogen receptor modulator (SERM) tamoxifen has also been shown in some studies to affect mood, with some women needing to discontinue tamoxifen secondary to depression (Thompson et al. 1995; Cathcart et al. 1993; Duffy et al. 1997; Lee et al. 2007), although other data mostly from prevention studies have not found an association between tamoxifen and depression (Roth et al. 1998; Roscoe et al. 2005; Mathias et al. 2006; Kimmick et al. 2006).

More than one in four women, who later received a diagnosis breast cancer, had elevated levels of both state anxiety and depressive symptoms (CADS) just before diagnosis. This factor was also a major predictor of Quality Of Life, state anxiety, depressive symptoms, and fatigue 12 and 24 months after surgery. This implies that women with a higher score on both state anxiety and depressive symptoms should be identified as soon as possible in the process of diagnosis and treatment of breast cancer using validated questionnaires or screening instruments. Only by identifying this group of patients, tailored psychiatric care can be accomplished (Lotje Van Esch et al. 2012).

Breast cancer is the cancer most studied in terms of psychosocial effects. One of the larger studies (Christensen et al. 2009), examined 3321 early stage Danish breast cancer patients, found a 13.7% prevalence of major depression 12–16 weeks after surgery (17.9% in 18–35 year olds and 11.2% in 60–69 year olds).

Some other important results of that large study are the following:

Independent risk factors for the development of depression in patients with breast cancer
• Younger age
• Social status
• Ethnicity
• Comorbidity
• Psychiatric history
• Physical functioning
• Smoking
• Alcohol use
• Body mass index (BMI)

Another study (Kissane et al. 1999), found that in 303 early stage and 200 metastatic breast cancer patients, prevalence rates of major depression of 9.6 and 6.5% respectively. Fatigue, a past history of depression, and cognitive attitudes of helplessness, hopelessness or resignation were significantly associated with depression in both groups. Some research groups have assessed the duration of psychological distress in breast cancer patients. In a prospective study of 160 women awaiting breast surgery, they found a 22% prevalence of depression in women who had a mastectomy for breast cancer (Morris et al. 1977). This prevalence persisted for two years, compared to an 8% prevalence of depression in those with benign disease. One five-year observational cohort study of 222 early stage breast cancer patients (Burgess et al. 2005) revealed prevalence rates for depression and anxiety of 33% at diagnosis, 15% after one year and 45% after a recurrence was diagnosed.

Few researchers have correlated patients' history of depression with current depression and/or functioning. In a study of 303 relatively young (mean age 46) women with early (Stage I or II) breast cancer at 3 months after breast surgery, using a structured diagnostic interview, (Kissane et al. 1998), found that a past history of depression was associated with current depression.

Yet, another study, (Pasacreta et al. 1997), reported findings on a homogenous sample of 79 women evaluated with the Diagnostic Interview Schedule and the Center for Epidemiological Studies Depression Scale, three–seven months after their diagnosis of breast cancer. Women with elevated depressive symptoms had more physical symptom distress and more impaired functioning than subjects without depression.

4.3.3 Depression in Advanced Cancer and Palliative Care

Chronic diseases even physical or especially mental disorders which became advanced may influence some actions and behaviors of patients, like the executive function (Derakhshan et al. 2013). Executive function is a series of high performances of the brain that are extremely important for high level human actions. Conceptualization, abstraction and cognitive flexibility are important components of the executive function. Some parts of the brain may become injured because of chronic and advanced diseases. Dorsolateral-prefrontal cortex (DLPFC) is found in chronic mental disorders like schizophrenia and possibly chronic bipolar mood disorders as the area of damage tends to decrease the level of executive function in comparison with the general population (Weingerger et al. 1986). In patients with advanced cancer, depressive disorder is a common problem (Minagawa et al. 1996), which may affect different aspects of their lives.

Patients with advanced cancer often remain under diagnosed and undertreated (Breitbart et al. 1995). One of the barriers is the common misconception that it is normal for patients with advanced cancer to be sad. Although, despite such barriers, we must not forget the fact that depression is an independent predictor of poor survival in advanced cancer (Lloyd-Williams et al. 2009). Furthermore, it reduces quality of life and prolongs hospitalization (Pelletier et al. 2002). Most importantly, depression in advanced cancer is treatable, and validated assessment tools have been developed to facilitate diagnosis.

4.3.4 Psychosocial Aspects of Breast Cancer

Women with breast cancer encounter many psychosocial stresses as well as physical problems. This disease oftentimes challenges a woman's identity, self-esteem, body image and relationships. They have to change their lifestyle following a long period of treatment, and this may well influence their quality of life. Their everyday life is full of stress and worry about their family/sexual roles in addition to the feeling of uncertainty about their future life in terms of their general functionality status. Protective factors for distress include supportive care networks, such as family and support groups and professional resources provided by clinical staff, such as timely referrals to specialized services.

The concept of body image can be found as a focus of breast cancer literature which describes the level of investment women put into their body in order to help them determine their wellbeing. This disruption to body image in breast cancer is attributed to hair loss, as well as changes in the breast and weight. Studies show younger women do seek normality in their breasts following mastectomy, and seek breast reconstruction more often than older women.

Among what is known, younger women with breast cancer are at a heightened risk of anxiety and depression in comparison to older women and younger women experience more worries about their careers and finances than older women. There is also evidence that young women perceive their quality of life to be lower than older women as a result of breast cancer. This may be attributed to poorer emotional wellbeing, specific cancer-related concerns, depression and intrusive thoughts for this younger age group. On the other hand, older women with breast cancer experience more health problems than younger women in survival, independent of receiving chemotherapy. In general, older breast cancer survivors experience overall better quality of life and mental health than their younger counterparts, but they tend to have poorer physical health and health-related quality of life due to comorbid conditions (Campbell and Woodgare 2015).

As cancer treatment can increase premature menopause, fertility and pregnancy after breast cancer are important issues for many women. It is important that women

are made aware of the potential impact on their fertility and given information regarding their options after treatment to achieve a pregnancy. Decisions to conceive are challenging as women are weighing up their desire for children against fears of recurrence and potential ability to detect future cancers (Peate et al. 2017).

The spouse's reaction to the physical deficits caused by breast cancer may negatively affect women's self-esteem and confidence. These psycho-emotional problems can subsequently increase the physical problems. In contrast, when a spouse is knowledgeable and understanding, the situation goes toward improvement for both the woman and the family. Literature shows family relationships are improved for both younger and older breast cancer survivors. However, the intimate relationships of younger women are more likely to be strained in comparison to the intimate relationships of older women in the context of breast cancer survival. Additionally, younger adults with cancer experience increased loneliness and a greater sense of isolation from peers and support networks than older adults perhaps because they perceive themselves to be different from their peers as a result of cancer (Campbell and Woodgare 2015). These psychosocial problems of women with breast cancer across the lifespan, requires an urgent need for more consideration by health providers and research to facilitate a greater understanding of the psychosocial needs of these women.

Exploring the problems that women with breast cancer encounter is a realistic corner stone for planning and implementing medical and nursing interventions to help them live with their optimum level of functioning.

Education could be one of the most important modalities to help patients be more understood and managed. Sessions need to deliberate over the psychological, emotional and social distress experienced by the patients with an aim from living a longer life to living a better and more fulfilled life. In one recent study in India, they had favorable results with educational sessions. Their emphasis was educational (with recent updates on the surgical, medical and radiation therapy aspects of breast cancer treatment), practical (emphasized important issues like side-effects of treatment, patient advocacy, complementary therapies, spirituality, lifestyle changes, etc.), and entertainment (De Souza et al. 2017).

Breast cancer-related issues, both in the world and in Iran, have been mainly studied using quantitative approaches. These are not simply answering the complex questions regarding human nature, but exploring deep layers of human feelings needs a more holistic approach (Holloway and Wheeler 2009).

Qualitative research allows researchers to get to the inner experience of the participants to determine how meanings are formed through and in culture and to discover rather than simply test variables (Corbin and Strauss 2008).

A review study (Rustoen and Begnum 2000) shows that the research regarding quality of life in breast cancer has been mainly descriptive, through the use of standardized questionnaires, and there have been difficulties in implementing the results in cancer care. One reason for this could be that these quantitative tools have difficulties in capturing what is unique in patients' experiences and therefore, risk omitting important issues that patients may have expressed in a study with a qualitative approach (Luoma and Hakamies-Blomqvist 2004).

A qualitative research (Taleghani et al. 2006) in Iran found how women cope with breast cancer. The main themes emerging in their study that could demonstrate the process of coping with the disease included a religious approach (accepting the disease as God's will and spiritual fighting); thinking about the disease (positive thinking, positive suggestions, hope, international forgetfulness, negative thinking, hopelessness, fear and impaired body image); accepting the fact of the disease (active acceptance and passive acceptance); social and cultural factors; and finally finding support from significant others.

This could also be the voice of Muslim women with breast cancer worldwide and present their internal challenges with the issue, while their spiritual beliefs can help them as a supportive source despite of all negative aspects of the illness.

Because most Iranian breast cancer patients are less than 50 years old and the age of incidence in Iran is less than world's average, breast cancer should be considered more, and nurses should encourage women to attend in screening tests.

Promoting the patient-to-patient relationship and sharing the illness experiences is another way for softening what can be a terrible experience. This can be supported by nurses to suggest those women share with other women's challenges (Joulaee et al. 2012).

After treatment for breast cancer, most women receive an annual surveillance mammography to look for subsequent breast cancers. A supplemental breast MRI is sometimes used in addition to mammography despite the lack of clinical evidence for it.

A recent study found that many women experienced discomfort during breast imaging and anxiety related to the examination, primarily because they feared subsequent cancer detection (Brandzel et al. 2017). Furthermore, women reported trust in their providers and relied on providers for imaging decision-making. However, women wanted more information about the treatment surveillance transition to improve their care. Certainly there is significant opportunity in breast cancer survival care to improve women's understanding about breast cancer surveillance imaging by their treating physician or their psychiatrist in sessions of education; and to provide enhanced support to them at the time when their initial treatment ends and at the time of surveillance examinations and for a long time after that.

4.3.5 Sleep Problems in Patients with Breast Cancer

Insomnia as a symptom encompasses difficulty with initiating or maintaining sleep, early morning awakenings, or poor quality of sleep. In addition, reporting a symptom of insomnia carries an expectation of daytime impairment.

Patients with cancer commonly report sleep-related problems, such as insomnia, daytime sleepiness, and fatigue (Stepanski et al. 2007).

Poor sleep in patients with cancer was shown to predict decreased quality of life (Stepanski et al. 2007; Fortner et al. 2002). Patients with breast cancer frequently report dissatisfaction with their sleep and complain of symptoms of insomnia (Knobf et al. 1986). They experience frequent awakenings when undergoing radiation therapy or chemotherapy (Berger et al. 1999) and their insomnia may persist for many years after cancer therapy. Up to 44% of patients with breast cancer reported symptoms of insomnia 2–6 years after their diagnosis, (Kurtz et al. 1993; Couzi 1995) which indirectly implies that symptoms of insomnia may persist for years, even after successful management of breast cancer. As usual, insomnia in patients with cancer was viewed as a secondary condition, believed to be caused by, or associated with, pain, anxiety, depression, chemotherapy, or some other aspect of cancer; thus, treatment of the underlying causes would alleviate the insomnia. Current concepts suggest that the insomnia that accompanies any medical or psychiatric illness is a comorbid disorder. For example, a patient may develop insomnia at the time of diagnosis with cancer, so the insomnia may require independent management.

In addition to some medicines that may need to treat insomnia in the patient with breast cancer, by psychiatrists, much research suggests effective psychotherapeutic modalities such as cognitive behavioral therapy (CBT). Psychotherapy is effective for both primary and comorbid insomnia.

Below are some cognitive behavioral techniques to help breast cancer patients who have sleep problems (Yang et al. 2006; Morgenthaler et al. 2006).

Psychotherapy for sleep disorders
• Sleep hygiene education
• Sleep restriction therapy
• Cognitive therapy
• Stimulus-control therapy
• Relaxation therapy
• Paradoxical intention

Many studies have shown the benefits of antidepressant medication in patients with a diagnosis of breast cancer, excluding other malignancies. Reasons to evaluate this population independently of patients with other malignancies include the unique effect of this illness and its cancer treatments on body image, as well as the potential effect of hormonal therapy and chemotherapy on mood. Breast cancer affects women almost exclusively, and as women have twice the baseline rate of depression as men, (Rihmer et al. 2005) this might result in a higher rate of depression in patients with breast cancer compared with patients with other cancers.

4.3.6 Hormonal Therapy and Chemotherapy in Breast Cancer

There are several drugs or hormones available to prevent breast cancer. The following are those approved by the Food and Drug Administration (FDA) in the US.

Drugs approved to prevent breast cancer
• Evista (Raloxifene Hydrochloride)
• Keoxifene (Raloxifene Hydrochloride)
• Nolvadex (Tamoxifen Citrate)
• Raloxifene Hydrochloride
• Tamoxifen Citrate

There are many drugs that are approved for the treatment of breast cancer by the Food and Drug Administration (FDA) in the US.

Drugs approved to treat breast cancer		
• Abitrexate (Methotrexate)	• Abraxane (Paclitaxel Albumin-stabilized Nanoparticle Formulation)	• Ado-TrastuzumabEmtansine
• Afinitor (Everolimus)	• Anastrozole	• Aredia (Pamidronate Disodium)
• Arimidex (Anastrozole)	• Aromasin (Exemestane)	• Capecitabine
• Clafen (Cyclophosphamide)	• Cyclophosphamide	• Cytoxan (Cyclophosphamide)
• Docetaxel	• Doxorubicin Hydrochloride	• Ellence (Epirubicin Hydrochloride)
• Epirubicin Hydrochloride	• EribulinMesylate	• Everolimus
• Exemestane	• 5-FU (Fluorouracil Injection)	• Fareston (Toremifene)
• Faslodex (Fulvestrant)	• Femara (Letrozole)	• Fluorouracil Injection
• Folex (Methotrexate)	• Folex PFS (Methotrexate)	• Fulvestrant
• Gemcitabine	• Gemzar (Gemcitabine Hydrochloride)	• Goserelin Acetate
• Halaven (EribulinMesylate)	• Herceptin (Trastuzumab)	• Ibrance (Palbociclib)
• Ixabepilone	• Ixempra (Ixabepilone)	• Kadcyla (Ado-TrastuzumabEmtansine)
• Kisqali (Ribociclib)	• LapatinibDitosylate	• Letrozole
• Megestrol Acetate	• Methotrexate	• Methotrexate LPF (Methotrexate)
• Mexate (Methotrexate)	• Mexate-AQ (Methotrexate)	• Neosar (Cyclophosphamide)
• Nolvadex (Tamoxifen Citrate)	• Paclitaxel	• Paclitaxel Albumin-stabilized Nanoparticle Formulation
• Palbociclib	• Pamidronate Disodium	• Perjeta (Pertuzumab)
• Pertuzumab	• Ribociclib	• Tamoxifen Citrate
• Taxol (Paclitaxel)	• Taxotere (Docetaxel)	• Thiotepa

(continued)

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Drugs approved to treat breast cancer		
• Toremifene	• Trastuzumab	• Tykerb (LapatinibDitosylate)
• Velban (Vinblastine Sulfate)	• Velsar (Vinblastine Sulfate)	• Vinblastine Sulfate
• Xeloda (Capecitabine)	• Zoladex (Goserelin Acetate)	

4.3.7 The Effect of Hormonal Therapy or Chemotherapy on Mood

Breast cancer treatments may potentially increase the risk of some psychiatric disorders such as depression. Increased levels of depression are found in peri-menopausal patients, and in women taking antiestrogen treatments, such as tamoxifen, raloxifen, and letrozole. The antiestrogens may induce a menopausal state and may potentially contribute to increased levels of depression (Navari et al. 2008).

Breast cancer patients undergoing chemotherapy also report higher rates of depression (Thompson et al. 2000). Estrogen has been associated with increased serotonergic and noradrenergic activity and may therefore have antidepressant properties. The antiestrogen properties of tamoxifen may counteract the antide-pressant effects of estrogen and could produce depressive symptoms (Thompson et al. 1999). In one study, symptoms of acute estrogen deficiency correlated with the severity of psychiatric symptoms reported by 44% of 222 patients with breast cancer (Couzi 1995).

Tamoxifen may also cause subtle psychiatric symptoms that do not meet the criteria for a full major depressive episode (Thompson et al. 1999).

4.3.8 Pharmacological Treatments of Psychiatric Symptoms in Patients with Breast Cancer

Many different medications have been studied and found to be effective to treat psychiatric disorders in context of breast cancer. Some measures include depressive symptoms, quality of life, and compliance with oncological treatment has been improved in several studies in this population (Navari et al. 2008; Thompson et al. 2000; Roscoe et al. 2005).

Many medications have been shown to be effective in treating psychiatric dis-orders. Fluoxetine, Paroxetine, Sertraline, Citalopram, Bupropion, Venlafaxine, Mirtazapine, Amitriptyline and several other medicines have been studied and proved their effectiveness.

Another potential issue in concurrent treatment with antidepressants and tamoxifen is the potential for drug interactions. Tamoxifen is essentially a prodrug, and is converted to its more potent metabolite, endoxifen, by the cytochrome p450 enzyme CYP2D6. Therefore, medications that are inhibitors of CYP2D6 could potentially decrease the potency of tamoxifen and adversely affect breast cancer outcomes.

Women treated with tamoxifen were found to have low serum concentrations of endoxifen when concurrently treated with the strong 2D6 inhibitors, such as Fluoxetine or Paroxetine, and intermediate concentrations of endoxifen when concurrently treated with weak 2D6 inhibitors, such as Sertraline and Citalopram. Venlafaxine, which does not inhibit CYP2D6, had little effect on endoxifen concentrations (Henry et al. 2008).

4.4 Psychological Interventions

In a large multicenter trial, women with metastatic breast cancer were randomized to Supportive Expression Group Therapy (SEGT) or to a control group. Women assigned to the therapy group reported significantly less sadness, anxiety, anger, and confusion than women in the control group, and had significantly less worsening of pain than women in the control group (Goodwin et al. 2001). In another study, SEGT improved quality of life in women with metastatic breast cancer, helped treat depression, and prevented new onset of depressive symptoms. In both studies, survival time was not affected by the intervention (Goodwin et al. 2001; Kissane et al. 2007).

A major modality of psychotherapeutic intervention in these decacades is Cognitive Behavioral Therapy (CBT). Researchers also examined the effectiveness of CBT. In a randomized controlled trial with patients with any form of cancer (not exclusively breast cancer) , patients were randomized to a CBT group or a control group.

Techniques for CBT group therapy in cancer patients
• Identifying negative automatic thoughts and challenging them
• Using role playing to cope with anticipated stressful events
• Encouraging activities that fostered a sense of accomplishment and pleasure
• Identifying personal strengths and encouraging open communication
• Teaching progressive muscle relaxation to manage severe anxiety

The treatment group demonstrated improvement over the control group for several parameters, including anxiety, depression, and distress (Greer et al. 1992).

In another trial where women with metastatic breast cancer were randomized to individual cognitive therapy (CT) or wait-list control, the women in the CT group had significantly fewer depressive symptoms than the control group.

Psychological intervention and improvement in mood and anxiety symptoms did not have a significant effect on parameters of immunological function (Savard et al. 2006). In a meta-analysis of psychosocial interventions for patients with cancer (not exclusively breast cancer), different treatment modalities were separated into categories, including cognitive behavioral interventions, informational and educational treatments, non-behavioral psychotherapy, social support by nonprofessionals, and unusual treatments that combined different treatment approaches. No significant differences in efficacy were noted between the different treatment approaches (Sheard et al. 1999).

In a phenomenological approach study to explore the meaning of living with breast cancer for Iranian women (Joulaee et al. 2012), these experiences of participants indicated that they also could find a new kind of peace and comfort by the living with breast cancer through bowing to God's will. This was seen in previously religious and non-religious women. In a study of older women (Overcash 2004), it was found that believing in God was of paramount importance for these women, which could help them in not giving up. Other researchers also suggest that the religious beliefs can be a supportive resource for people with cancer or other incurable diseases (Taleghani et al. 2006, 2008), but in Muslims, this is more pronounced. Muslims believe that everything that happens in their life has come to them according to the God's discretion, and they should stoop to God's will. Relying on women's religious/spiritual beliefs might be an opportunity to help them in coping with their health problem. This is an increasingly important issue worldwide, so this opportunity could be used as a worthwhile source for raising women's coping mechanisms (Joulaee et al. 2012).

4.5 Surgery for Breast Cancer

Most women with breast cancer have some type of surgery as part of their treatment. Depending on the situation, surgery may be done for different reasons. For example, surgery may be done to:

- Remove as much of the cancer as possible (breast-conserving surgery or mastectomy)
- Find out whether the cancer has spread to the lymph nodes under the arm (sentinel lymph node biopsy or axillary lymph node dissection)
- Restore the breast's shape after the cancer is removed (breast reconstruction)
- Relieve symptoms of advanced cancer1

4.5.1 Surgery to Remove Breast Cancer

There are two main types of surgery to remove breast cancer:

4.5.1.1 Breast-Conserving Surgery (lumpectomy)

Breast-conserving surgery is sometimes called lumpectomy, quadrantectomy, partial mastectomy, or segmental mastectomy. In this surgery, only the part of the breast containing the cancer is removed. The goal is to remove the cancer as well as some surrounding normal tissue. How much of the breast is removed depends on the size and location of the tumor and other factors.

Who can get breast-conserving surgery?

Breast-conserving surgery (BCS) is a good option for many women with early-stage cancers. The main advantage is that a woman keeps most of her breast. However, she will in most cases also need radiation therapy. Women who have their entire breast removed (mastectomy) for early-stage cancers are less likely to need radiation, but they may be referred to a doctor who specializes in radiation, called a radiation oncologist, for evaluation because each patient's cancer is unique.

Most women and their doctors prefer BCS and radiation therapy when it's a reasonable option. BCS might be a good option if you:

- Are very concerned about losing your breast
- Are willing to have radiation therapy and able to get to the appointments
- Have not already had the breast treated with radiation therapy or BCS
- Have only one area of cancer on the breast, or multiple areas that are close enough together to be removed without changing the look of the breast too much
- Have a small tumor (5 cm [2 inches] or smaller), and a tumor that is small relative to your breast size
- Are not pregnant or, if pregnant, will not need radiation therapy immediately (to avoid risking harm to the fetus)
- Do not have a genetic factor such as a BRCA mutation, which might increase your chance of a second cancer
- Do not have certain serious connective tissue diseases such as scleroderma or lupus, which may make you especially sensitive to the side effects of radiation therapy
- Do not have inflammatory breast cancer

Some women might be worried that having a less extensive surgery might raise their risk of the cancer coming back. But the fact is that in most cases, mastectomy does not give you any better chance of long-term survival or a better outcome from treatment. Studies following thousands of women for more than 20 years show that when BCS can be done, having mastectomy instead does not provide any better chance of survival.

4.5.1.2 Mastectomy

Mastectomy is surgery to remove the entire breast. All of the breast tissue is removed, sometimes along with other nearby tissues.

Types of mastectomies

There are several different types of mastectomies, based on how the surgery is done and how much additional tissue is removed.

4.5.1.3 Simple (or total) Mastectomy

Simple mastectomy is the most common type of mastectomy used to treat breast cancer. In this procedure, the surgeon removes the entire breast, including the nipple, but does not remove underarm lymph nodes or muscle tissue from beneath the breast. (Sometimes lymph nodes are removed in a different procedure during the same surgery.) Most women, if they are hospitalized, can go home the next day.

4.5.1.4 Double Mastectomy

If a mastectomy is done on both breasts, it is called a double (or bilateral) mastectomy. When this is done, it is often as preventive surgery for women at very high risk for getting cancer in the other breast, such as those with a BRCA gene mutation.

4.5.1.5 Skin-Sparing Mastectomy

For some women considering immediate reconstruction, a skin-sparing mastectomy can be done. In this procedure, most of the skin over the breast (other than the nipple and areola) is left intact. This can work as well as a simple mastectomy. The amount of breast tissue removed is the same as with a simple mastectomy.

Implants or tissue from other parts of the body are used to reconstruct the breast.

Skin-sparing mastectomy may not be suitable for larger tumors or those that are close to the surface of the skin. This approach has not been used for as long as the more standard type of mastectomy, but many women prefer it because it offers the advantage of less scar tissue and a reconstructed breast that seems more natural.

4.5.1.6 Modified Radical Mastectomy

A modified radical mastectomy combines a simple mastectomy with the removal of the lymph nodes under the arm (called an axillary lymph node dissection).

4.5.1.7 Nipple-Sparing Mastectomy

Nipple-sparing mastectomy is a variation of the skin-sparing mastectomy. It is more often an option for women who have a small, early-stage cancer near the outer part of the breast, with no signs of cancer in the skin or near the nipple. (Cancer cells are more likely to be hidden in the nipple if the breast tumor is larger or close to the nipple, which means there is a higher risk the cancer will come back if the nipple is not removed.)

In this procedure, the breast tissue is removed, but the breast skin and nipple are left in place. This is followed by breast reconstruction. The surgeon often removes the breast tissue beneath the nipple (and areola) during the procedure to check for cancer cells. If cancer is found in this tissue, the nipple must be removed. Even when no cancer is found under the nipple, some doctors give the nipple tissue a dose of radiation during or after the surgery to try to reduce the risk of the cancer coming back.

There are still some problems with nipple-sparing surgeries. Afterward, the nipple does not have a good blood supply, so sometimes it can wither away or become deformed. Because the nerves are also cut, there is little or no feeling left in the nipple. For women with larger breasts, the nipple may look out of place after the breast is reconstructed. As a result, many doctors feel that this surgery is best done in women with small to medium sized breasts. This procedure leaves less visible scars, but if it isn't done properly, it can leave behind more breast tissue than other forms of mastectomy. This could result in a higher risk of cancer developing than for a skin-sparing or simple mastectomy. This was more of a problem in the past, but improvements in technique have helped make this surgery safer. Still, many experts do not yet consider nipple-sparing procedures a standard treatment for breast cancer.

4.5.1.8 Radical mastectomy

In this extensive operation, the surgeon removes the entire breast, axillary (underarm) lymph nodes, and the pectoral (chest wall) muscles under the breast. This surgery was once very common, but less extensive surgery (such as modified radical mastectomy) has been found to be just as effective and with fewer side effects, so this surgery is rarely done now. This operation may still be done for large tumors that are growing into the pectoral muscles.

Who should get a mastectomy?

Many women with early-stage cancers can choose between breast-conserving surgery (BCS) and mastectomy. You may have an initial gut preference for mastectomy as a way to "take it all out as quickly as possible." But the fact is that in most cases, mastectomy does not give you any better chance of long-term survival or a better outcome from treatment. Studies following thousands of women for more than 20 years show that when BCS can be done, doing mastectomy instead does not provide any better chance of survival (American Cancer Society 2016).

Although most women and their doctors prefer BCS (with radiation therapy) when it's a reasonable option, there are cases where mastectomy is likely to be the best choice. For example, mastectomy might be recommended if you:

- Are unable to have radiation therapy, or would prefer a more extensive surgery to having radiation therapy
- Have already had the breast treated with radiation therapy
- Have already had BCS along with re-excision(s) that have not completely removed the cancer
- Have two or more areas of cancer in the same breast that are not close enough together to be removed without changing the look of the breast too much
- Have a larger tumor (greater than 5 cm [2 inches] across), or a tumor that is large relative to your breast size
- Are pregnant and would need radiation therapy while still pregnant (risking harm to the fetus)
- Have a genetic factor such as a BRCA mutation, which might increase your chance of a second cancer
- Have certain serious connective tissue diseases such as scleroderma or lupus, which may make you especially sensitive to the side effects of radiation therapy
- Have inflammatory breast cancer

For women who are worried about breast cancer recurrence, it is important to understand that having a mastectomy instead of breast-conserving surgery plus radiation only lowers your risk of developing a second breast cancer in the same breast. It does not lower the chance of the cancer coming back in other parts of the body.

4.5.2 Lymph Node Surgery for Breast Cancer

If you have been diagnosed with breast cancer, it’s important to find out how far the cancer has spread. To help find out if the cancer has spread beyond the breast, one or more of the lymph nodes under the arm (axillary lymph nodes) are removed and checked under a microscope. This is an important part of staging. When the lymph nodes contain cancer cells, there is a higher chance that cancer cells have also spread to other parts of the body. Treatment decisions will often depend on whether cancer is found in the lymph nodes.

Lymph node removal can be done in different ways, depending on whether any lymph nodes are enlarged, how big the breast tumor is, and other factors.

4.5.2.1 Biopsy of an Enlarged Lymph Node

If any of the lymph nodes under the arm or around the collar bone are swollen, they may be checked for cancer spread directly with a needle biopsy (either a fine needle aspiration biopsy or a core needle biopsy). Less often, the enlarged node is removed with surgery. If cancer is found in the lymph node, more nodes will need to be removed during an axillary lymph node dissection (described below).

4.5.2.2 Types of Lymph Node Surgery

Even if the nearby lymph nodes are not enlarged, they will still need to be checked for cancer. This can be done in two different ways. Sentinel lymph node biopsy is the most common and least invasive way, but in some cases a more extensive axillary lymph node dissection might be needed.

4.5.2.3 Sentinel Lymph Node Biopsy (SLNB)

In a sentinel lymph node biopsy (SLNB), the surgeon finds and removes the first lymph node(s) to which a tumor is likely to spread (called the *sentinel nodes*). To do this, the surgeon injects a radioactive substance and/or a blue dye into the tumor, the area around it, or the area around the nipple. Lymphatic vessels will carry these substances along the same path that the cancer would be likely to take. The first lymph node(s) the dye or radioactive substance travels to will be the sentinel node (s).

After the substance has been injected, the sentinel node(s) can be found either by using a special device to detect radioactivity in the nodes that the radioactive substance flows into, or by looking for lymph nodes that have turned blue. To double check, both methods are often used. The surgeon cuts the skin over the area and removes the node(s) containing the dye or radiation.

The removed lymph nodes (often 2 or 3 nodes) are then checked closely for cancer cells by a doctor called a *pathologist*. This is sometimes done during the surgery. This way, if cancer is found in the sentinel lymph node(s), the surgeon may do a full axillary lymph node dissection (ALND) to remove more lymph nodes. If no cancer cells are seen in the node(s) at the time of the surgery, or if the sentinel node(s) are not checked by a pathologist at the time of the surgery, they will be examined more closely over the next several days.

If cancer is found in the sentinel node(s) later, the surgeon may recommend a full ALND at a later time to check more nodes for cancer. Recently, however, studies have shown that in some cases it may be just as safe to leave the rest of the lymph nodes behind. This is based on certain factors, such as the size of the breast tumor, what type of surgery is used to remove the tumor, and what treatment is planned after surgery. Based on the studies that have looked at this, skipping the ALND may be an option for women with tumors 5 cm (2 inches) or smaller who are having

breast-conserving surgery followed by radiation. Because this hasn't been studied well in women who have had mastectomy, it isn't clear that skipping the ALND would be safe for them.

If there is no cancer in the sentinel node(s), it's very unlikely that the cancer has spread to other lymph nodes, so no further lymph node surgery is needed. Although SLNB has become a common procedure, it requires a great deal of skill. It should be done only by a surgeon who has experience with this technique. If you are thinking about having this type of biopsy, ask your health care team if they do them regularly.

4.5.2.4 Axillary Lymph Node Dissection (ALND)

In this procedure, anywhere from about 10 to 40 (though usually less than 20) lymph nodes are removed from the area under the arm (axilla) and checked for cancer spread. ALND is usually done at the same time as a mastectomy or breast-conserving surgery(BCS), but it can be done in a second operation. This was once the most common way to check to see if breast cancer had spread to nearby lymph nodes and it is still sometimes needed. For example, an ALND may be done if a previous biopsy has shown one or more of the underarm lymph nodes have cancer cells.

Breast cancer treatment has evolved significantly over the past decades. Several randomized trials with long term follow-up provided evidence for equivalence of breast conserving therapy (BCT) and mastectomy (MT) in terms of overall survival.1–3

BCT is now considered the standard of care for early stage breast cancer. Additionally following conserving the breast, patients reported higher shorthand Long-term quality of life (QOL) at least in some subscales.

Several years after introduction of BCT mastectomy was still the treatment of choice in many countries with substantial geographical variations in the rates of performing BCT. This has led to 6-8mandating the surgeons to explain treatment options for all patients who could benefit from BCT in 20 states of the United States.

Various studies were conducted to assess the factors that might affect both surgeons' and patients' decisions in preferring mastectomy over BCT. The results elucidated that lack of experience of surgeons in BCT; patients' concerns about the survival after BCT, and socioeconomic status of patients were the main predictors of BCT underutilization in different countries. One of the important influential factors on patients' choice of surgery is the surgeons 'recommendation.

4.5.3 Reconstruction

4.5.3.1 Types of Breast Reconstruction Procedures

There are several types of reconstructive surgery available, and the reconstruction process sometimes means more than one operation. Give yourself plenty of time to make the best decision for you. You should make your decision about breast reconstruction only after you are fully informed.

Two main types of operations can be done to reconstruct the shape of your breast or breasts:

- Breast implants (using silicone or saline inserts)
- Tissue flap procedures (using your own body tissues)

Sometimes a combination of an implant and flap procedure is used to get the best result.

In addition, nipple and areola reconstruction procedures can be done to help make the reconstructed breast look more like the original breast.

4.5.4 Surgical Aspects of Breast Cancer in Iran

A cross-sectional study (Najafi et al. 2015) conducted among Iranian surgeons demonstrated that only 19 % of surgeons considered BCT as their preferred method of treatment for breast cancer. According to some reports, breast cancer awareness has increased recently in Iran due to improved general socioeconomic status and relevant educational programs in the media. Accordingly, a decreasing trend in breast cancer tumor size and down staging has been detected in the last few years. Thus, it is expected that surgeons' attitude and practice has also improved during the preceding years.Major medical universities in Iran have initiated surgical oncology fellowship programs in the past eight years which include breast cancer surgery as part of the curriculum. Two breast cancer fellowship programs are also initiated in the last 2 years. All of the above mentioned educational programs have contributed to the dramatic increase in the frequency of surgeons performing BCT for patients who could benefit from this less invasive surgical approach. Other studies have also mentioned surgeon's sub specialization as a factor contributing to increased use of BCT.

4.6 Conclusion

Today cancer patients need to be more concerned about the psychiatric and psychological aspects of disease. Increasing cooperation between different departments and between treating physicians with consultation-liaison psychiatrists could provide a better integrating medical service to the patients in all steps including diagnosis, treatments and rehabilitation phases.

Early diagnosis of psychiatric disorders in patients with all stages of breast cancer and its pharmacological and psychotherapeutic treatments improves quality of life, makes patients more compliant with other modalities of treatment and increases the chances of survival.

4.7 Summary

In this chapter, we have tried to take note of the most important psychosocial problems that a patient with breast cancer and her family are facing. Depression is the most common psychiatric problem that breast cancer patients are affected by. Depression and other psychiatric disorders in cancer patients, especially in patients with breast cancer, are less diagnosed than the real rate. Depression can interfere with the three categories of factors—cognitive, motivational and resource related—that are essential for adherence to treatment in cancer patients. Women of a younger age, social status and psychiatric history accompanied by smoking and alcohol usage are some independent risk factors for the development of depression in patients with breast cancer.

A woman who has received a diagnosis of breast cancer, facing the risk of imminent death, may feel despair. She may experience many stages a phase of shock at first, followed by denial, aggressiveness, haggling and acceptance. A psychiatrist with reciprocal understanding and confidence with the patient can organize her thoughts and assist her in managing this crisis that has occurred. He or she helps the patient and her family to manage their thoughts and feelings in order to make rational decisions and to demonstrate appropriate behaviors. In every step, psychiatrists, in this manner, collaborate with other members of the treatment team and also members of the mental health providers group that may be of clinical benefit for a comprehensive medical service.

Psychiatrists should take more active roles, as we mentioned, as a member of the team treating patients with breast cancer. The main emphasis has been on the role of a consultation-liaison psychiatrist and some other members of the mental health providers like a clinical psychologist, occupational therapist and a social worker, to help patients better and to present an integrative medical service with success.

Pharmacological treatments for psychiatric disorders such as depressive disorder sleep problems, and drugs to prevent and treat breast cancer as much as hormonal treatments and their possible interactions have been discussed appropriately. We

also have noted to psychotherapeutic interventions, especially cognitive behavioral therapy (CBT) in most disorders.

We have also explained surgical treatments of breast cancer in simple words.

Most women with breast cancer need some type of surgery as an important measure of their treatment. Depending on the patient's condition, surgery may be done to remove as much of the cancer as possible (breast-conserving surgery or mastectomy), to find out whether the cancer has spread to the lymph nodes under the arm (sentinel lymph node biopsy or axillary lymph node dissection), to restore the breast's shape after the cancer is removed (breast reconstruction) or to relieve symptoms of advanced cancer.

The physician indicates that for cancer at any stage, there are many appropriate and effective treatments available. Successful treatment of cancer is accomplished by teamwork and which of; mental health professionals are also important components of the team.

Psychiatrists, with accompaniment of other members of the mental health team, arrange many sessions in order to prevent less mental harm to patients and families. The aim is that she adapts well to the new circumstances as soon as possible and returns to her normal new life.

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Chapter 5
Cancer Genetics at a Glance: The Comprehensive Insights

Asaad Azarnezhad and Parvin Mehdipour

Abstract The concept, that cancerogenesis results from genetic alterations is dated to 1866, when Broca described a familial clustering of breast and liver cancers. Thus, Haaland in 1900 and Warthin in 1913, on the basis of pedigree analyses, proved that the susceptibility to cancer might be inherited as Mendelian autosomal trait. All cancers result from an accumulation of genetic alterations in a single cell. No matter which theory is taken into consideration, a first critical mutation may be either inherited (germ line mutation) or acquired (somatic mutation). Therefore, cancer could be either hereditary (5–10% of all cancers), familial (approximately 15% of all cancers) or sporadic (70–75% of all cancers). However, cancer is initiated by mutation in either tumor suppressor genes or oncogenes. In the exposing era of new generation of sequencing and molecular profiling, the role of genetic alterations including microdeletions, micro-insertions, cytogenetic abnormalities, DNA methylation, single nucleotide polymorphism (SNP), single nucleotide substitutions have been more identified. The tools of genetics have been used to systematically examine how cancers arise. Insight into the genetic and molecular horizon of cancer pathogenesis will provide a new opportunity for identification of potential prognostic, diagnostic and drug target biomarkers. In the current chapter we explored to present an overview on the genetic aspects including breast, hematopoietic, brain, bladder, hepatocellular, and alimentary system-related cancers. In each section, a brief introduction on cancer genetics is provided which is followed by comprehensive molecular and functional information through the tables. It was our aim to provide an outline on genetic and signaling networks involved in cancer susceptibility and tumor behavior at a glance. This chapter is reflective of the genetics/functional-based insights of the key genes involved in different types of cancer. The involved machinery is also provided through the alterations of mutation, expression profiles, miRNA deregulation, and epigenetic

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deregulation of genes in tumor pathogenesis. These events may lead to sub-classification in specific cancers.

Keywords Cancer · Cancerogenesis · Germ line mutation · Somatic mutation · Hereditary · Familial · Sporadic · Tumor suppressors · Oncogenes · DNA methylation · Single nucleotide polymorphism (SNP) · Breast cancer · CNS and brain tumors · Hematological cancer · Alimentary system-related cancer · Pedigree analysis

Abbreviations

3′UTR	3′untranslated
ABC	ATP-binding cassette
ACC	Adenoid cystic carcinoma
Ak1	Adenylate kinase
ALK7	Activin receptor-like kinase 7
ALL	Acute lymphoblastic leukemia
AML	Acute myelogenous leukemia
AMoL	Acute monocytic leukemia
APP	Amyloid precursor protein
APRS	Apert syndrome
AREG	Amphiregulin
AT	Ataxia telangiectasia
ATC	Anaplastic thyroid cancer
ATM	Ataxia telangiectasia-mutated gene
ATM	Ataxia telangiectasia-mutated gene
ATRX	Alpha-thalassemia mental retardation syndrome, X-linked
ATRX	X-linked alpha-thalassemia mental retardation syndrome
ATXN1	Ataxin 1
AUL	Acute undifferentiated leukemia
B-cell NHL	B-cell non-Hodgkin lymphomas
B-NHL	B-cell non Hodgkin lymphoma
BBDS	Bent bone dysplasia syndrome
BC	Breast cancer
BDNF	Brain-derived neurotrophic factor
BRCA1	Breast cancer 1 early onset protein
BRCA2	Breast cancer 2 early onset protein
BROVCA1	Breast-ovarian cancer, familial, 1
BSTVS	Beare-Stevenson cutis gyrata syndrome
CAPN1	Calpain 10
CARD	C-terminal caspase-recruitment domain
CCND1	Cyclin D1
CCND2	Cyclin D2
Cdk4	Cyclin Dependent Kinase 4

CEA	Carcino-embryonic antigen
CEACAM1	CEA-cell adhesion molecule
CGH	Comparative genomic hybridization
CHNG6	Congenital hpothyroidism non-goitrous 6
CIP2A	Cancerous inhibitor of PP2A
CLASP	Clathrin-associated sorting protein
CLL	Chronic lymphocytic leukemia
CLOVE	Congenital lipomatous overgrowth, vascular malformations, and epidermal nevi
CML	Chronoc myeloblastic leukemias
CNS	Central nervous system
CNV	Copy number variation
CPC	Chromosome passage protein complex
CPC	Chromosome passage protein complex
CS	Crouzon syndrome
CTD	C-terminal domain
CVID10	Immunodeficiency, common variable, 10
CWS1	Cowden syndrome 1
DAG	Diacylglycerol
DDR	Discoidin domain receptor
DNMT1	DNA (cytosine-5-)-methyltransferase 1
DSBs	Double strand breaks
DSBs	Double strand breaks
EG-VEGF	Endocrine gland-derived vascular endothelial growth factor
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EMT	Epithelial-mesenchymal transition
EMT	Epithelial to mesenchymal transition
ENDMC	Endometrial cancer
ENDMC	Endometrial cancer
ERK	Extracellular-regulated kinase
ESCC	Esophageal squamous cell carcinoma
EZH2	Enhancer of zeste homolog 2
FA	Fanconi anemia
FA	Fanconi anemia
FAB	French-American-British
FANCJ	Fanconi anemia complementation group J
FANCN	Fanconi anemia complementation group N
FAP	Familial adenomatous polyposis
FNMTC	Familial forms of non-medullary thyroid carcinoma
FSPC	Familial scaphocephaly syndrome
FTC	Follicular thyroid cancer
GABA	Gamma-aminobutyric acid

GAP	GTPase-activating protein
GC	Gastric cancer
GC	Germinal center
GEF	Guanine nucleotide exchange factor
GIST	Gastrointestinal stromal tumor
GNA12	Guanine nucleotide-binding alpha-12
GNA13	Guanine nucleotide-bindingalpha-13
GPIa	Glycoprotein Ia
HA	Hyaluronic acid
HCC	Hepatocellular carcinoma
HDD	Hereditary desmoid disease
HDGC	Hereditary diffuse gastric cancer
HDR	Homology-directed repair
HIF	Hypoxia-inducible-factor
HNSCC	Head and neck squamous cell carcinoma
HOKs	Human oral keratinocytes
HPV	Human papilomavirus
HRR	Homologous recombination repair
IDA	Inner dynein arms
IGF2	Insulin-like growth factor 2
IGFBP-5	Insulin-like growth factor binding protein-5
IGFBP7	Insulin-like growth factor-binding protein 7
IL-6	Of Interleukin 6
IL2	Interleukin-2
IL4	Interleukin-4
IL13	Interleukin-13
ILK	Integrin-linked kinase
JMML	Juvenile myelomonocytic Leukemia
JWS	Jackson-Weiss syndrome
LADDS	Lacrimo-auriculo-dento-digital syndrome
LMP1	Latent membrane protein 1
LOH	Loss of heterozygosity
LOH	Loss of heterozygosity
LSCC	Laryngeal squamous cell carcinoma
M6P	Mannose 6-phosphate
MALT	Mucosa-associated lymphoid tissue
MAP3K	MAP kinase kinase kinase
MAP	Mitogen-activated protein
MAPK	Mitogen-activated protein kinase
MCL	Mantle-cell lymphoma
MCM	Mini-chromosome maintenance proteins
MDB	Medulloblastoma
MicroRNAs	miRNAs
miRNA	MicroRNA

miRNAs	Micro RNAs
miRNAs	MicroRNAs
MLL	Mixed-lineage leukemia
MMP-9	Matrix metalloproteinase-9
MMP	Matrix metalloproteinase
MMP	Matrix metalloproteinase
MMPs	Matrix metalloproteases
MMPs	Matrix metalloproteinases
MMPs	Matrix metalloproteinases
MMR	Mismatch repair system
MMR	Mismatch repair system
MONA	Multicentric osteolysis, nodulosis, and arthropathy
MPL	Myeloproliferative leukemia
MPL	Myeloproliferative leukemia
MPL	Myeloproliferative leukemia
mRNAs	Messenger RNAs
NBSLD	Nijmegen breakage syndrome-like disorder
NER	Nucleotide excision repair
NER	Nucleotide excision repair
NHL	Non-Hodgkin lymphoma
NICD	Notch intracellular domain
NMI	N-myc interactor
NPC	Nasopharyngeal carcinoma
NPC	Nasopharyngeal carcinoma
NT	Normal thyroid
O6-MeG	O6-methylguanine
OC	Ovarian cancer
OC	Ovarian cancer
OC	Ovarian cancer
ODA	Outer dynein arms
OLP	Oral lichen planus
OPN	Osteopontin
OPSCC	Oropharyngeal squamous cell carcinoma
OS	Overall survival
OSCC	Oral squamous cell carcinoma
OTSCC	Oral tongue squamous cell carcinoma
OTSCC	Oral tongue squamous cell carcinoma
PAMPs	Pathogen-associated molecular patterns
PARP	Poly ADP-ribose polymerase
PBT	Piebald trait
PC	Prostate cancer
PC	Prostate cancer
PCH10	Pontocerebellar hypoplasia type 10
PDPN	Podoplanin

PI 3-Kinases	Phosphoinositide 3-kinases
PI 3-Kinases	Phosphoinositide 3-kinases, PI 3-Ks
PJS	Peutz-Jeghers syndrome
PML-NBs	PML-nuclear bodies
PMS	Preleukemic Myelodysplastic syndrome
PNCA4	Pancreatic cancer 4
PNCA	Pancreatic cancer
PPRE	PPAR response elements
PS	Pfeiffer syndrome
PTC	Papillary thyroid carcinoma
PTGS	Prostaglandin-endoperoxide synthase
PTP	Protein tyrosine phosphatase
RB	Retinoblastoma
SBE	Smad-binding element
SCA1	Spinocerebellar ataxia type 1
SCCA	Squamous cell carcinoma
SCCHN	Squamous cell carcinoma of the head and neck
SDSA	Synthesis dependent strand annealing
Skp2	S-phase kinase-associated protein 2
Skp2	S-phase kinase-associated protein 2
SNCLC	Somatic Nonsmall cell lung cancer
SOD	Superoxide dismutase
SSTR	Somatostatin receptors
T-ALL	T-cell acute lymphoblastic leukemia
T-CLL	T-cell leukemias
T-CLL	T-cell lymphomas
T-MDS	Therapy-related myelodysplastic syndrome
TAM	Tyro3-Axl-Mer
TAOS1	Tumor amplified and overexpressed sequence 1
TC	Thyroid cancer
TC	Tongue cancer
TCC	Transitional cell carcinoma
TGCT	Testicular germ cell tumor
TGCT	Testicular germ cell tumor
TGFB	Transforming growth factor beta
TGFBR1 and TGFBR2	Transmembrane type I and type II receptors
TLR	Toll-like receptor
TMNG	Thyroid multinodular goiter
TPLL	T-prolymphocytic leukemia
TRIM	Tripartite motif
TSH	Thyroid stimulating hormone
TTP	Tristetraprolin
UVA	Ultraviolet A light
VDR	Vitamin D receptor

VEGF	Vascular endothelial growth factor
VEGFR-2	Vascular endothelial growth factor receptor 2
VNTR	Variable number tandem repeats
WHO	World Health Organization
WNV	West Nile virus
XIAP	X-linked inhibitor of apoptosis protein

5.1 Genetic Aspects of Bladder Cancer

Bladder cancer is one of the neoplasms of epithelial cells. Bladder cancer is the second most common cancer of genitourinary system. The average age of individuals diagnosed with this cancer is revealed to be 65 years. The incidence of bladder cancer is higher in men than in women and has been observed in Caucasians more frequently than in blacks. In Iran, men are four times more likely than women to be diagnosed with bladder cancer. The most common type of bladder cancer is initiated in cells lining the inside of the bladder and is called transitional cell carcinoma (TCC) (Kirkali et al. 2005; Torre et al. 2015). Hematuria, pain during urination, frequent urination are some symptoms of bladder cancer. These signs and symptoms are not specific to bladder cancer, however they also can be caused by noncancerous conditions such as infections (Burger et al. 2013).

In the United States of America, bladder cancer is the fourth most common type of cancer in men and the ninth most common cancer in women. About 45,000 men and 17,000 women are diagnosed with bladder cancer each year (Torre et al. 2015). As like as of the most cancers, the exact causes of bladder cancer are not known; however, many risk factors are associated with this disease. Major risk factors include environmental, such as smoking and exposure to certain industrial chemicals. Studies suggest that chronic bladder inflammation, a parasitic infection known as Schistosomiasis. Nonetheless, the bladder cancer is a genetic disease of somatic cells in which gene and genome instability are the common events in development of the cancer (Kaufman et al. 2009).

Deletions of part or all of chromosome 9 are common cytogenetic defect in bladder tumors. Researchers believe that several genes that control cell growth and division are probably located on chromosome 9. Genetic factors are also likely to play an important role in determining bladder cancer risk. Researchers have studied the effects of mutations in several genes, including *FGFR3*, *RB1*, *HRAS*, *TP53*, and *TSC1*, on the formation and growth of bladder tumors (Vogelstein and Kinzler 2002). Each of these genes plays a critical role in regulating cell division by preventing cells from dividing too rapidly or in an uncontrolled manner. Alterations in these genes may clarify why some bladder cancers grow and spread more rapidly than others. Apparently, bladder cancer is typically not inherited.

Most often, tumors result from genetic mutations that occur in bladder cells during the individual's lifetime. These non-inherited genetic alterations are the somatic mutations. A list containing the most reported genetic factors involved in bladder cancer are shown in Table 5.1.

5.1.1 Genetic Etiology

See Table 5.1.

5.1.2 Micro RNA and Bladder Cancer

MicroRNAs (miRNAs), a class of small noncoding RNAs, regulate protein-coding gene expression by repressing translation or cleaving RNA transcripts in a sequence-specific manner. Role of this small biomolecule has been reported in development of many cancers. By advent of new methods, genome-wide miRNA expression profiling has opened a new window to miRNA discovery in many diseases including cancers (Vogelstein and Kinzler 2002). Here, we have summarized a list containing of the most reported miRNAs deregulated in bladder cancer (Table 5.2).

5.2 Genetic Aspects of Brain and CNS Cancers

An abnormal growth of nerve cells within the brain or central canal of the spinal cord leads to brain tumors that may be either benign or malignant. A group of complex and rare diseases including more than 50 distinct cancer comprises of tumors of the brain and central nervous system (CNS). Different type of brain tumors are varied in physiology, pathology, and molecular aspects (Louis et al. 2007). The outstanding is that morbidity of brain tumors is high (evaluated to be associate with 33% of all brain tumors) and regularly life-undermining (Hayat 2012; Ricard et al. 2012; Ferlay et al. 2013).

With approximately 256,000 new cases per year, brain and CNS tumors account for 3% of new cancer cases globally. Geographical incidence of these cancers is diverse across the world with the most frequency in Australia, North America, and northern European countries (Darefsky and Dubrow 2009; Ferlay et al. 2013). The lowest incidence of brain and CNS tumors is belonging to Africa. Distinctive distribution pattern of these cancer, according to the sex, has been shown that men have more risk for development of brain and CNS tumors. Also, ages between 60 years and older reflected highest incidence rate, followed by age of 0–4 years (children) and adolescents (Fisher et al. 2007).

Table 5.1 Most reported genetic factor involved in Bladder cancer either by mutation or by deregulation

Gene	Gene locus	Molecular pathology	Gene function	OMIM	References
TP53	17p13.1	Point mutations	– Acts as a tumor suppressor in many tumor types – Induces growth arrest or apoptosis depending on the physiological circumstances and cell type – Involved in cell cycle regulation as a trans-activator that acts to negatively regulate cell division by controlling a set of genes required for this process	191170	Bartkova et al. (2005)
FGFR3	4p16.3	Somatic mutations	– Acts as cell-surface receptor for fibroblast growth factors and – Plays an essential role in the regulation of cell proliferation, differentiation and apoptosis	134934	Neuzillet et al. (2014)
CDKN2A	9p21	Somatic mutations	– Capable of inducing cell cycle arrest in G1 and G2 phases – Acts as a tumor suppressor	600160	Scher et al. (2012)
RB1	13q14.2	Somatic mutations	– A negative regulator of the cell cycle and was the first tumor suppressor gene found – The encoded protein also stabilizes constitutive heterochromatin to maintain the overall chromatin structure – The active, hypophosphorylated form of the protein binds transcription factor E2F1	614041	Horowitz et al. (1990)

(continued)

Table 5.1 (continued)

Gene	Gene locus	Molecular pathology	Gene function	OMIM	References
HRAS	11p15.5	Somatic mutations	– Ras proteins bind GDP/GTP and possess intrinsic GTPase activity – The product encoded by this gene functions in signal transduction pathways	190020	Pinto-Leite et al. (2014)
KRAS	12p12.1	Somatic mutations	– Ras proteins bind GDP/GTP and possess intrinsic GTPase activity – Plays an important role in the regulation of cell proliferation.	190070	Kompier et al. (2010)
BIRC5	17q25	Somatic mutations Dis-regulation	– Multitasking protein that has dual roles in promoting cell proliferation and preventing apoptosis – Component of a chromosome passage protein complex (CPC) which is essential for chromosome alignment and segregation during mitosis and cytokinesis	603352	Jeon et al. (2013)
NAT2	8p22	Somatic mutations	– Participates in the detoxification of a plethora of hydrazine and arylamine drugs – Catalyzes the N- or O-acetylation of various arylamine and heterocyclic amine substrates and is able to bioactivate several known carcinogens	612182	Golka et al. (2013)
GSTM1	1p13.3	Somatic mutations	– Conjugation of reduced glutathione to a wide number of exogenous and endogenous hydrophobic electrophiles	138350	Reszka et al. (2014)

(continued)

Table 5.1 (continued)

Gene	Gene locus	Molecular pathology	Gene function	OMIM	References
TNF	6p21.3	Somatic mutations Dis-regulation	– This cytokine is involved in the regulation of a wide spectrum of biological processes including cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation	191160	Du et al. (2014)
GSTP1	11q13	Somatic mutations	– Conjugation of reduced glutathione to a wide number of exogenous and endogenous hydrophobic electrophiles – Regulates negatively CDK5 activity via p25/p35 translocation to prevent neurodegeneration	134660	García-Closas et al. (2005)
CDKN1A	6p21.2	Somatic mutations Dis-regulation	– May be the important intermediate by which p53/TP53 mediates its role as an inhibitor of cellular proliferation in response to DNA damage	116899	Schepeler et al. (2013)
PPARG	3p25	Somatic mutations CNV	– Nuclear receptor that binds peroxisome proliferators such as hypolipidemic drugs and fatty acids – Once activated by a ligand, the nuclear receptor binds to DNA specific PPAR response elements (PPRE) and modulates the transcription of its target genes, such as acyl-CoA oxidase – It therefore controls the peroxisomal beta-oxidation pathway of fatty acids	601487	Conconi et al. (2012)

(continued)

Table 5.1 (continued)

Gene	Gene locus	Molecular pathology	Gene function	OMIM	References
XRCC1	19q13.2	Somatic mutations polymorphism	– Corrects defective DNA strand-break repair and sister chromatid exchange following treatment with ionizing radiation and alkylating agents	194360	Chiang et al. (2014)
CASP3	4q34	Somatic mutations polymorphism	– Involved in the activation cascade of caspases responsible for apoptosis execution	600636	Mittal et al. (2011)
ERBB2	17q12	Somatic mutations CNV	– Enhancing kinase-mediated activation of downstream signalling pathways, such as those involving mitogen-activated protein kinase and phosphatidylinositol-3 kinase	164870	Toncheva and Zaharieva (2005)
CD44	11p13	Dis-regulation	– Receptor for hyaluronic acid (HA) – Mediates cell-cell and cell-matrix interactions through its affinity for HA, and possibly also through its affinity for other ligands such as osteopontin, collagens, and matrix metalloproteinases (MMPs) – Adhesion with HA plays an important role in cell migration, tumor growth and progression	107269	Yu et al. (2014)
KIT	4q12	Somatic mutations Dis-regulation	– Tyrosine-protein kinase that acts as cell-surface receptor for the cytokine KITLG/SCF – Plays an essential role in the regulation of cell survival and proliferation, hematopoiesis, stem cell maintenance,	164920	Terada (2009)

(continued)

Table 5.1 (continued)

Gene	Gene locus	Molecular pathology	Gene function	OMIM	References
			gametogenesis, mast cell development, migration and function, and in melanogenesis		
NAT1	8p22	Gene polymorphisms	– Participates in the detoxification of a plethora of hydrazine and arylamine drugs – Catalyzes the N- or O-acetylation of various arylamine and heterocyclic amine substrates and is able to bioactivate several known carcinogens	108345	Wu et al. (2013a, b)
AKT1	14q32.32	Mutation Methylation	– Akt (Protein kinase B, PKB) is a serine/threonine kinase that plays a key in regulating cell survival, insulin signaling, angiogenesis and tumor formation – Akt is a downstream mediator of the PI 3-K pathway, which results in the recruitment of Akt to the plasma membrane	164730	Sun et al. (2012a, b) Askham et al. (2010)
MTOR	1p36.2	Somatic mutations Dis-regulation	– Serine/threonine protein kinase which is a central regulator of cellular metabolism, growth and survival in response to hormones, growth factors, nutrients, energy and stress signals	601231	Chen et al. (2009a, b)
PIK3CA	3q26.3	Gene mutation	– PI 3-Kinases (phosphoinositide 3-kinases, PI 3-Ks) are a family of lipid kinases capable of phosphorylating the 3′OH of the inositol ring of phosphoinositides – They are responsible for coordinating a diverse range of cell functions including proliferation and survival	171834	Duenas et al. (2015)

(continued)

Table 5.1 (continued)

Gene	Gene locus	Molecular pathology	Gene function	OMIM	References
CDKN1B	12p13.1-p12	Somatic mutations Dis-regulation	– Important regulator of cell cycle progression. Involved in G1 arrest. Potent inhibitor of cyclin E- and cyclin A-CDK2 complexes – Forms a complex with cyclin type D-CDK4 complexes and is involved in the assembly, stability, and modulation of CCND1-CDK4 complex activation – Acts either as an inhibitor or an activator of cyclin type D-CDK4 complexes depending on its phosphorylation state and/or stoichometry	600778	Lee et al. (1999)
MUC1	1q21	Somatic mutations Dis-regulation	– Mucins are O-glycosylated proteins that play an essential role in forming protective mucous barriers on epithelial surfaces – These proteins also play a role in intracellular signaling	158340	Suzuki et al. (2012a, b)
AR	Xq12	Somatic mutations Dis-regulation	– Steroid hormone receptors are ligand-activated transcription factors that regulate eukaryotic gene expression and affect cellular proliferation and differentiation in target tissues	313700	Hsieh et al. (2013)
VEGFA	6p12	Somatic mutations Dis-regulation	– Growth factor active in angiogenesis, vasculogenesis and endothelial cell growth – Induces endothelial cell proliferation, promotes cell migration, inhibits apoptosis and induces permeabilization of blood vessels	192240	Zaravinos et al. (2012)

(continued)

Table 5.1 (continued)

Gene	Gene locus	Molecular pathology	Gene function	OMIM	References
NRAS	1p13.2	Somatic mutations	– Ras proteins bind GDP/GTP and possess intrinsic GTPase activity	164790	Ouerhani and Elgaaied (2011)
CDKN2B	9p21	Copy number variation (CNV)	– Interacts strongly with CDK4 and CDK6. Potent inhibitor – Potential effector of TGF-beta induced cell cycle arrest	600431	Aveyard and Knowles (2004)
FGFR2	10q26	Point mutations	– Tyrosine-protein kinase that acts as cell-surface receptor for fibroblast growth factors – Plays an essential role in the regulation of cell proliferation, differentiation, migration and apoptosis, and in the regulation of embryonic development	176943	Spiegelberg et al. (2014)
FGFR1	8p11.23	CNV & dis-regulation	– Tyrosine-protein kinase that acts as cell-surface receptor for fibroblast growth factors – Plays an essential role in the regulation of embryonic development, cell proliferation, differentiation and migration	136350	Tomlinson and Knowles (2010) Simon et al. (2001)
E2F3	6p22	Mutation & dis-regulation	– Transcription activator that binds DNA cooperatively with DP proteins through the E2 recognition site, 5-TTTC [CG] CGC-3 found in the promoter region of a number of genes whose products are involved in cell cycle regulation or in DNA replication	600427	Huang et al. (2011)

(continued)

Table 5.1 (continued)

Gene	Gene locus	Molecular pathology	Gene function	OMIM	References
FAS	10q24.1	Point alteration & methylation	– Receptor for TNFSF6/FASLG – Involved in extrinsic pathway of apoptosis – FAS-mediated apoptosis may have a role in the induction of peripheral tolerance, in the antigen-stimulated suicide of mature T-cells, or both	134637	Verim et al. (2014) Li et al. (2011a, b, c, d, e)
IL10	1q31-q32	Gene polymorphisms	– Inhibits the synthesis of a number of cytokines, including IFN-gamma, IL-2, IL-3, TNF and GM-CSF produced by activated macrophages and by helper T-cells	124092	Chen et al. (2013a, b)

Table 5.2 miRNA signature involved in bladder cancer

miRNAs	Targets	miRNA_function	Effect	References
miR-125b	MMP13	– Inhibit cell migration – Inhibit cell invasion	Tumor-suppressive	Wu et al. (2013a, b)
miR-145	SOCS7	– Induce caspase-dependent apoptosis	Tumor-suppressive	Noguchi et al. (2013a, b)
miR-133a	EGFR	– Inhibit cell proliferation – Inhibit cell migration – Inhibit cell invasion	Tumor-suppressive	Zhou et al. (2013a, b)
miR-133b	EGFR	– Inhibit cell proliferation – Inhibit cell migration – Inhibit cell invasion	Tumor-suppressive	Zhou et al. (2013a, b)
miR-143	AKT1	– Inhibit cell growth	Tumor-suppressive	Noguchi et al. (2013a, b)
miR-145	ILK	– Inhibit cell growth	Tumor-suppressive	Noguchi et al. (2013a, b)

(continued)

Table 5.2 (continued)

miRNAs	Targets	miRNA_function	Effect	References
miR-1280	ROCK1	– Inhibit cell proliferation – Inhibit colony formation – Induce cell cycle G2/M transition arrest – Induce apoptosis – Inhibit cell migration – Inhibit cell invasion	Tumor-suppressive	Majid et al. (2012)
miR-1	PTMA PNP	– Inhibit cell proliferation – Inhibit cell invasion – Induce apoptosis	Tumor-suppressive	Yamasaki et al. (2012)
miR-133a	PTMA PNP	– Inhibit cell proliferation – Inhibit cell invasion – Induce apoptosis	Tumor-suppressive	Yamasaki et al. (2012)
miR-195	CDK4	– Induce cell cycle G1 arrest – Inhibit cell growth	Tumor-suppressive	Lin et al. (2012)
miR-195-5p	SLC2A3	– Decrease cell glucose uptake – Inhibit cell growth – Promote apoptosis	Tumor-suppressive	Fei et al. (2012)
miR-574-3p	MESDC1	– Inhibit cell proliferation – Inhibit cell migration – Inhibit cell invasion – Induce apoptosis	Tumor-suppressive	Tatarano et al. (2012)
miR-1	SRSF9	– Induce apoptosis	Tumor-suppressive	Yoshino et al. (2012)
miR-493	FZD4 RHOC	– Inhibit cell growth – Inhibit cell migration	Tumor-suppressive	Ueno et al. (2012)
miR-1826	CTNNB1 VEGFC MEK1	– Inhibit cell viability – Inhibit cell invasion – Inhibit cell migration – Induce apoptosis – Induce cell cycle G1 arrest	Tumor-suppressive	Hirata et al. (2012)
miR-18a	DICER1	– suppress cell proliferation	Tumor-Suppressive	Tao et al. (2012)
miR-34a	CDK6 SIRT1	– Increase cisplatin sensitivity	NPA	Tao et al. (2012)
miR-143	MAPK7	– Inhibit cell growth – Induce apoptosis – Increase cisplatin sensitivity	Tumor-suppressive	Noguchi et al. (2011), Wang et al. (2017)

(continued)

Table 5.2 (continued)

miRNAs	Targets	miRNA_function	Effect	References
miR-133a	GSTP1	– Reduce cell viability – promote apoptosis	Tumor-suppressive	Yoshino et al. (2011)
miR-1	TAGLN2	– Inhibit cell proliferation – Induce apoptosis – Inhibit cell migration – Inhibit cell invasion	Tumor-suppressive	Yoshino et al. (2011)
miR-133a	TAGLN2	– Inhibit cell proliferation – Induce apoptosis – Inhibit cell migration – Inhibit cell invasion	Tumor-suppressive	Chiyomaru et al. (2012)
miR-203	BCL2L2	– Promote apoptosis – Inhibit cell proliferation	Tumor-suppressive	Bo et al. (2011)
miR-19a	PTEN	– Promote cell growth – Inhibit apoptosis	Oncogenic	Cao et al. (2011)
miR-1	LASP1	– Inhibit cell viability	Tumor-suppressive	Chiyomaru et al. (2012)
miR-133a	LASP1	– Inhibit cell viability	Tumor-suppressive	Chiyomaru et al. (2012)
miR-218	LASP1	– Inhibit cell viability	Tumor-suppressive	Chiyomaru et al. (2012)
miR-125b	E2F3	– Suppress colony formation – Suppress tumor development	Tumor-suppressive	Huang et al. (2011)
miR-133a	FSCN1	– Inhibit cell viability	Tumor-suppressive	Chiyomaru et al. (2012)
miR-145	FSCN1	– Inhibit cell viability	Tumor-suppressive	Chiyomaru et al. (2012)
miR-145	CBFB PPP3CA CLINT1	– Induce apoptosis	Tumor-suppressive	Chiyomaru et al. (2010)
miR-200b	ERRFI1	– Decrease cell migration – Increase sensitivity to EGFR-blocking agents	Tumor-suppressive	Adam et al. (2009)
miR-200c	ERRFI1	– Decrease cell migration – Increase sensitivity to EGFR-blocking agents	Tumor-suppressive	Adam et al. (2009)
miR-129	GALNT1 SOX4	– Inhibit cell growth – Induce cell death	Tumor-Suppressive	Dyrskjøt et al. (2009)

Table 5.3 List of genes reported to be involved in brain tumors pathogenesis

Gene	Location	Function	OMIM	References
PTEN	10q23.3	– This gene was identified as a tumor suppressor that is mutated in a large number of cancers at high frequency – The protein encoded by this gene is a phosphatidylinositol-3, 4,5-trisphosphate 3-phosphatase – It contains a tensin like domain as well as a catalytic domain similar to that of the dual specificity protein tyrosine phosphatases – It negatively regulates intracellular levels of phosphatidylinositol-3,4, 5-trisphosphate in cells and functions as a tumor suppressor by negatively regulating AKT/PKB signaling pathway – The longer isoform of protein may help regulate energy metabolism in the mitochondria	601728	Cohen and Colman (2015)
MMP2	16q12.2	– This gene is a member of the matrix metalloproteinase (MMP) gene family – is zinc-dependent enzyme capable of cleaving components of the extracellular matrix and molecules involved in signal transduction – The protein encoded by this gene is a gelatinase A, type IV collagenase – This protein is thought to be involved in multiple pathways including roles in the nervous system, endometrial menstrual breakdown, regulation of vascularization, and metastasis	120360	Gong et al. (2014)
MGMT	10q26	– Involved in the cellular defense against the biological effects of O6-methylguanine (O6-MeG) in DNA – Repairs alkylated guanine in DNA by stoichiometrically transferring the alkyl group at the O-6 position to a cysteine residue in the enzyme – This is a suicide reaction: the enzyme is irreversibly inactivated	156569	Taylor and Schiff (2015)
KRIT1	7q21.2	– This gene encodes a protein containing four ankyrin repeats and plays a critical role in beta1-integrin-mediated cell proliferation	604214	Kehrer-Sawatzki et al. (2002)

(continued)

Table 5.3 (continued)

Gene	Location	Function	OMIM	References
		– It associates with junction proteins and RAS-related protein 1A (Rap1A), which requires the encoded protein for maintaining the integrity of endothelial junctions – It is also a microtubule-associated protein and may play a role in microtubule targeting. Mutations in this gene result in cerebral cavernous malformations		
PDGFA	7p22	– A Growth factor that plays an essential role in the regulation of embryonic development, cell proliferation, cell migration, survival and chemotaxis – Potent mitogen for cells of mesenchymal origin – Required for normal lung alveolar septum formation during embryogenesis, normal development of the gastrointestinal tract, normal development of Leydig cells and spermatogenesis – Required for normal oligodendrocyte development and normal myelination in the spinal cord and cerebellum. Plays an important role in wound healing	173430	Collins (2004)
DMBT1	10q26.13	– May be considered as a candidate tumor suppressor gene for brain, lung, esophageal, gastric, and colorectal cancers – May play roles in mucosal defense system, cellular immune defense and epithelial differentiation – May play a role as an opsonin receptor for SFTPD and SPAR in macrophage tissues throughout the body, including epithelial cells lining the gastrointestinal tract – May play a role in liver regeneration – May be an important factor in fate decision and differentiation of transit-amplifying ductular (oval) cells within the hepatic lineage – Required for terminal differentiation of columnar epithelial cells during early embryogenesis	601969	Biegel (1999), Weber (2007)

(continued)

Table 5.3 (continued)

Gene	Location	Function	OMIM	References
		– May function as a binding protein in saliva for the regulation of taste sensation – Binds to HIV-1 envelope protein and has been shown to both inhibit and facilitate viral transmission – Displays a broad calcium-dependent binding spectrum against both Gram-positive and Gram-negative bacteria, suggesting a role in defense against bacterial pathogens – Binds to a range of poly-sulfated and poly-phosphorylated ligands which may explain its broad bacterial-binding specificity – Inhibits cytoinvasion of S.enterica. Associates with the actin cytoskeleton and is involved in its remodeling during regulated exocytosis – Interacts with pancreatic zymogens in a pH-dependent manner and may act as a Golgi cargo receptor in the regulated secretory pathway of the pancreatic acinar cell		
NOTCH2	1p13-p11	– Functions as a receptor for membrane-bound ligands Jagged1, Jagged2 and Delta1 to regulate cell-fate determination – Upon ligand activation through the released notch intracellular domain (NICD) and forms a transcriptional activator complex with RBPJ/RBPSUH and activates genes of the enhancer of split locus – Affects the implementation of differentiation, proliferation and apoptotic programs (By similarity) – Involved in bone remodeling and homeostasis – In collaboration with RELA/p65 enhances NFATc1 promoter activity and positively regulates RANKL-induced osteoclast differentiation – Positively regulates self-renewal of liver cancer cells	600275	Stockhausen et al. (2012)

(continued)

Table 5.3 (continued)

Gene	Location	Function	OMIM	References
GFAP	17q21	– This gene encodes one of the major intermediate filament proteins of mature astrocytes – It is used as a marker to distinguish astrocytes from other glial cells during development – Mutations in this gene cause Alexander disease, a rare disorder of astrocytes in the central nervous system	137780	Moeton et al. (2014)
RTEL1	20q13.3	– ATP-dependent DNA helicase implicated in telomere-length regulation, DNA repair and the maintenance of genomic stability – Acts as an anti-recombinase to counteract toxic recombination and limit crossover during meiosis – Regulates meiotic recombination and crossover homeostasis by physically dissociating strand invasion events and thereby promotes noncrossover repair by meiotic synthesis dependent strand annealing (SDSA) as well as disassembly of D loop recombination intermediates – Also disassembles T loops and prevents telomere fragility by counteracting telomeric G4-DNA structures, which together ensure the dynamics and stability of the telomere	608833	Ricard et al. (2012) Kheirollahi et al. (2013) Mehdipour (2013)
CCM2	7p13	– Component of the CCM signaling pathway which is a crucial regulator of heart and vessel formation and integrity – May act through the stabilization of endothelial cell junctions (By similarity) – May function as a scaffold protein for MAP2K3-MAP3K3 signaling – Seems to play a major role in the modulation of MAP3K3-dependent p38 activation induced by hyperosmotic shock	607929	Draheim et al. (2014)
OLIG2	21q22.11	– Required for oligodendrocyte and motor neuron specification in the spinal cord, as well as for the development of somatic motor neurons in the hindbrain	606386	Preusser et al. (2007)

(continued)

Table 5.3 (continued)

Gene	Location	Function	OMIM	References
		– Cooperates with OLIG1 to establish the pMN domain of the embryonic neural tube. Antagonist of V2 interneuron and of NKX2-2-induced V3 interneuron development (By similarity)		
PDCD10	3q26.1	– Promotes cell proliferation. Modulates apoptotic pathways – Increases mitogen-activated protein kinase activity and STK26 activity – Important for cell migration, and for normal structure and assembly of the Golgi complex. Important for KDR/VEGFR2 signaling – Increases the stability of KDR/VEGFR2 and prevents its breakdown – Required for normal cardiovascular development. Required for normal angiogenesis, vasculogenesis and hematopoiesis during embryonic development	609118	Tanriover et al. (2008), Lambertz et al. (2015)
CIC	19q13.2	– Transcriptional repressor which may play a role in development of the CNS	612082	Lee et al. (2005a, b), Yip et al. (2012)
S100A6	1q21	– May function as calcium sensor and modulator, contributing to cellular calcium signaling – May function by interacting with other proteins, such as TPR-containing proteins, and indirectly play a role in many physiological processes such as the reorganization of the actin cytoskeleton and in cell motility – Binds 2 calcium ions. Calcium binding is cooperative	114110	Heizmann et al. (2004)
RAF1	3p25	– This gene is the cellular homolog of viral raf gene (v-raf) – The encoded protein is a MAP kinase kinase kinase (MAP3 K), which functions downstream of the Ras family of membrane associated GTPases to which it binds directly – Once activated, the cellular RAF1 protein can phosphorylate to activate the dual specificity protein kinases MEK1 and MEK2, which in turn	164760	Jones et al. (2009)

(continued)

Table 5.3 (continued)

Gene	Location	Function	OMIM	References
		phosphorylate to activate the serine/threonine specific protein kinases, ERK1 and ERK2 – Activated ERKs are pleiotropic effectors of cell physiology and play an important role in the control of gene expression involved in the cell division cycle, apoptosis, cell differentiation and cell migration		
ATM	11q22.3	– Helps control the rate at which cells grow and divide – Plays an important role in the normal development and activity of several body systems, including the nervous system and the immune system – ATM protein assists cells in recognizing damaged or broken DNA strands and involved in double strand breaks (DSBs) repair	607585	Mehdipour et al. (2008), Kheirollahi et al. (2011), Mehdipour and Karami (2015)
ROS1	6q22	– This proto-oncogene, highly-expressed in a variety of tumor cell lines, belongs to the sevenless subfamily of tyrosine kinase insulin receptor genes – The protein encoded by this gene is a type I integral membrane protein with tyrosine kinase activity – The protein may function as a growth or differentiation factor receptor	165020	Karayan-Tapon et al. (2014)
TGFB2	1q41	– This gene encodes a member of the transforming growth factor beta (TGFB) family of cytokines – Regulates proliferation, differentiation, adhesion, migration, and other functions in many cell types by transducing their signal through combinations of transmembrane type I and type II receptors (TGFBR1 and TGFBR2) and their downstream effectors, the SMAD proteins – Disruption of the TGFB/SMAD pathway has been implicated in a variety of human cancers	190220	Han et al. (2015)
LRP5	11q13.4	– Component of the Wnt-Fzd-LRP5-LRP6 complex that triggers beta-catenin signaling	603506	Joiner et al. (2013)

(continued)

Table 5.3 (continued)

Gene	Location	Function	OMIM	References
		through inducing aggregation of receptor-ligand complexes into ribosome-sized signalsomes. Cell-surface coreceptor of Wnt/beta-catenin signaling, which plays a pivotal role in bone formation – Plays a role in norrin (NDP) signal transduction. The Wnt-induced Fzd/LRP6 coreceptor complex recruits DVL1 polymers to the plasma membrane which, in turn, recruits the AXIN1/GSK3B-complex to the cell surface promoting the formation of signalsomes and inhibiting AXIN1/GSK3-mediated phosphorylation and destruction of beta-catenin – Appears be required for postnatal control of vascular regression in the eye (By similarity). Required for posterior patterning of the epiblast during gastrulation		
NKX2-2	20p11.22	– Acts as a transcriptional activator – Required for the maintenance of NEUROD1 expression in the horomone-producing endocrine cells of the pancreas – May be involved in specifying diencephalic neuromeric boundaries, and in controlling the expression of genes that play a role in axonal guidance – Associates with chromatin at the NEUROD1 promoter region. Binds to a subset of consensus elements within the NEUROD1 promoter	604612	Muraguchi et al. (2011)
FAT1	4q35	– Plays an essential role for cellular polarization, directed cell migration and modulating cell-cell contact	600976	Madan et al. (2016)
IQGAP1	15q26.1	– Binds to activated CDC42 but does not stimulate its GTPase activity – It associates with calmodulin – Could serve as an assembly scaffold for the organization of a multimolecular complex that would interface incoming signals to the reorganization of the actin	603379	Rotoli et al. (2017)

(continued)

Table 5.3 (continued)

Gene	Location	Function	OMIM	References
		cytoskeleton at the plasma membrane – May promote neurite outgrowth		
GLTSCR2	19q13.3	– GLTSCR2 protein binds to PTEN tumor suppressor and regulates its stability in cells – Recent reports show the implication of GLTSCR2 in cell proliferation and apoptosis	605691	Kim et al. (2008)
LRRN2	1q32.1	– The protein encoded by this gene belongs to the leucine-rich repeat superfamily – This gene was found to be amplified and overexpressed in malignant gliomas – The encoded protein has homology with other proteins that function as cell-adhesion molecules or as signal transduction receptors and is a candidate for the target gene in the 1q32.1 amplicon in malignant gliomas	605492	Hamano et al. (2004)
CLP1	11q12	– This gene encodes a member of the Clp1 family – The encoded protein is a multifunctional kinase which is a component of the tRNA splicing endonuclease complex and a component of the pre-mRNA cleavage complex II – This protein is implicated in tRNA, mRNA, and siRNA maturation. Mutations in this gene are associated with pontocerebellar hypoplasia type 10 (PCH10)	608757	Karaca et al. (2014)

Gliomas account for 70–80% of all the brain and CNS tumors and are the most common form of brain tumors in adults, while pilocytic astrocytomas, medulloblastomas and germ-cell tumors are the most brain malignancies in children (Crocetti et al. 2012; Ostrom et al. 2014). Age, gender, home and work exposures, family history, exposure to infections, viruses, and allergens, electromagnetic fields, race and ethnicity, ionizing radiation, head injury and seizures, N-nitroso compounds, and exposure to nerve agents are main factors that may raise a person's risk of developing a brain tumor. About 5% of brain tumors are possibly linked to hereditary genetic factors or some conditions including Li-Fraumeni syndrome, neurofibromatosis, nevoid basal cell carcinoma syndrome, tuberous sclerosis,

Turcot syndrome, and von Hippel-Lindau disease (Michaud and Batchelor 2013). Hereditary or sporadically, brain tumors will arise from cells harboring genetic defects in crucial genes responsible for maintenance of cell growth and death. In Table 5.3, most reported cancer genes and non-coding miRNAs involved in pathogenesis of brain tumors have been summarized.

5.2.1 Genetic Etiology

See Table 5.3.

5.2.2 miRNA and Brain & CNS Cancers

See Table 5.4.

Table 5.4 A comprehensive list of miRNAs reported to be involved in brain tumor pathogenesis

miRNAs	Direct targets	miRNA_function	Effect	References
miR-7	EGFR	– Induces apoptosis – Inhibits cell proliferation – Inhibits cell migration – Inhibits cell invasion	Tumor-suppressive	Wang et al. (2012a, b, c, d, e, f, g)
miR-218	VOPP1 REST CDK6 RICTOR CTSB	– Induces apoptosis – Inhibits cell viability – Inhibits cell proliferation – Inhibits tumorigenicity	Tumor-suppressive	Xia et al. (2012a, b), Venkataraman et al. (2013)
miR-376*	RAP2A	– Promotes cell migration – Promotes cell invasion	Oncogenic	Choudhury et al. (2012)
miR-330	SH3GL2	– Promotes cell proliferation – Promotes cell migration – Promotes cell invasion – Activates cell cycle – Inhibits apoptosis	Oncogenic	Qu et al. (2012)
miR-23a	PTEN	– Promotes cell growth – Promotes cell survival	Oncogenic	Tan et al. (2012)
miR-125b	E2F2	– Inhibits cell proliferation	Tumor-suppressive	Wu et al. (2012a, b, c)

(continued)

Table 5.4 (continued)

miRNAs	Direct targets	miRNA_function	Effect	References
miR-136	MTDH BCL2	– Promotes apoptosis induced by chemotherapy	Tumor-suppressive	Wu et al. (2014)
miR-524-5p	JAG1 HES1	– Inhibits cell proliferation – Inhibits cell invasion	Tumor-suppressive	Chen et al. (2012a, b, c)
miR-218	LEF1	– Inhibits cell invasion	Tumor-suppressive	Tie et al. (2010)
miR-23b	PYK2	– Inhibits cell migration – Inhibits cell invasion	Tumor-suppressive	Chen et al. (2014a, b)
miR-1275	CLDN11	– Increases cell growth	Oncogenic	Katsushima et al. (2012)
miR-221	TIMP3	– Increases cell invasion	Oncogenic	Zhang et al. (2010a, b, c, d)
miR-222	TIMP3	– Increases cell invasion	Oncogenic	Zhang et al. (2010a, b, c, d)
miR-23b	VHL	– Increases cell growth – Inhibits apoptosis – Increases cell invasion – Promotes beta-catenin/Tcf-4 and HIF-1alpha/VEGF signaling	Oncogenic	Chen et al. (2012a, b, c)
miR-27a	NPA	– Promotes cell proliferation – Reduces the cell cycle G1 arrest – Promotes cell invasion	Oncogenic	Feng et al. (2012a, b, c)
miR-34a	PDGFRA	– Inhibits cell growth	Tumor-suppressive	Guessous et al. (2010), Silber et al. (2012)
miR-483-5p	MAPK3	– Inhibits cell proliferation – Induces cell cycle G0/G1 arrest	Tumor-suppressive	Soon et al. (2009), Wang et al. (2012a, b, c, d, e, f, g)
miR-128	RPS6KB1	– Inhibits cell proliferation – Inhibits tumor growth – Inhibits angiogenesis	Tumor-suppressive	Papagiannakopoulos et al. (2012)
miR-25	MDM2 TSC1	– Inhibits cell proliferation – Induces cell cycle arrest	Tumor-suppressive	Suh et al. (2012)
miR-32	MDM2 TSC1	– Inhibits cell proliferation	Tumor-suppressive	Suh et al. (2012)

(continued)

Table 5.4 (continued)

miRNAs	Direct targets	miRNA_function	Effect	References
		– Induces cell cycle arrest		
miR-137	COX2	– Inhibits cell proliferation – Inhibits cell invasion	Tumor-suppressive	Silber et al. (2008)
miR-221	GJA1	– Promotes cell proliferation – PromoteS cell invasion – Inhibits apoptosis	Oncogenic	Hao et al. (2012)
miR-222	GJA1	– Promotes cell proliferation – Promotes cell invasion – Inhibits apoptosis	Oncogenic	Hao et al. (2012)
miR-124	SNAI2	– Reduces neurosphere formation – Reduces CD133(+) cell subpopulation – Inhibits cell invasion	Tumor-suppressive	Silber et al. (2008)
miR-181d	KRAS BCL2	– Inhibits cell proliferation – Induces cell cycle arrest – Induces apoptosis	Tumor-suppressive	Wang et al. (2012a, b, c, d, e, f, g)
miR-451	CAB39	– Inhibits PI3 K/AKT signaling – Inhibits tumor growth	Tumor-suppressive	Tian et al. (2012), Zhao et al. (2017)
miR-205	VEGFA	– Induces apoptosis – Induces cell cycle arrest – Reduces cell viability – Reduces clonability – Inhibits cell invasion	Tumor-suppressive	Yue et al. (2012)
miR-30e*	NFKBIA	– Activates NF-kB signaling – Increases cell invasion	Oncogenic	Jiang et al. (2012)
miR-135a	STAT6 SMAD5 BMPR2	– Induces mitochondria-dependent apoptosis	Tumor-suppressive	Wu et al. (2012a, b, c)
miR-31	RDX	– Inhibits cell migration – Inhibits cell invasion	Tumor-suppressive	Hua et al. (2012)
miR-7	PTK2	– Inhibits cell migration – Inhibits cell invasion	Tumor-suppressive	Okuda et al. (2013)
miR-34a	DLL1	– Inhibits cell proliferation – Induces apoptosis	Tumor-suppressive	Guessous et al. (2010)

(continued)

Table 5.4 (continued)

miRNAs	Direct targets	miRNA_function	Effect	References
		– Induces neural differentiation		
miR-27b	TFAP2A	– Promotes cell growth – Inhibits apoptosis – Promotes cell invasion	Oncogenic	Lee et al. (2012)
miR-128	EGFR PDGFRA	– Represses cell growth – Promotes neuronal differentiation	Tumor-suppressive	Papagiannakopoulos et al. (2012)
miR-17	CAMTA1	– Promotes neurosphere formation – Inhibits cell differentiation	Oncogenic	Schraivogel et al. (2011)
miR-9*	CAMTA1	– Promotes neurosphere formation – Inhibits cell differentiation	Oncogenic	Schraivogel et al. (2011)
miR-9	CAMTA1	– Promotes neurosphere formation – Inhibits cell differentiation	Oncogenic	Schraivogel et al. (2011)
miR-491-5p	MMP9	– Inhibits cell invasion	Tumor-suppressive	Li et al. (2015a, b, c, d)
miR-221	PTPRM	– Increases cell migration – Increases cell growth	Ooncogenic	Quintavalle et al. (2012)
miR-222	PTPRM	– Increases cell migration – Increases cell growth	Oncogenic	Quintavalle et al. (2012)
miR-34a	NOTCH1	– Inhibits cell proliferation – Induces apoptosis	Tumor-suppressive	Li et al. (2011a, b, c, d, e)
miR-146a	NOTCH1	– Reduces cell proliferation – Reduces cell migration – Reduces formation and migration of glioma stem-like cells	Tumor-suppressive	Li et al. (2011a, b, c, d, e)
miR-302a	CXCR4	– Inhibits self-renewal – Inhibits cell infiltration	Tumor-suppressive	(Fareh et al. 2012)
miR-106a	E2F1	– Inhibits cell proliferation – Induces apoptosis	Tumor-suppressive	Zhi et al. (2010)
miR-335	DAAM1	– Increases cell viability	Oncogenic	Shu et al. (2011)

(continued)

Table 5.4 (continued)

miRNAs	Direct targets	miRNA_function	Effect	References
		– Increases colony-forming ability – Increases cell invasion		
miR-10b	BCL2L11 TFAP2C CDKN1A CDKN2A	– Promotes cell growth – Reduces cell cycle arrest – Inhibits apoptosis – Reduces tumor growth	Oncogenic	Gabriely et al. (2011)
miR-381	LRRC4	– Increases cell proliferation	Oncogenic	Tang et al. (2011a, b)
miR-10b	HOXD10	– Promotes cell invasion	Oncogenic	Guessous et al. (2013)
miR-21	SPRY2	– Promotes cell invasion	Oncogenic	Chan et al. (2005)
miR-26b	EPHA2	– Inhibits cell proliferation – Inhibits cell migration – Inhibits cell invasion – Inhibits vasculogenic mimicry process	Tumor-suppressive	Wu et al. (2011a, b)
miR-34a	MAGEA2 MAGEA3 MAGEA6 MAGEA12	– Induces apoptosis – Induces cell cycle G2 arrest – Induces senescence – Increases chemosensitivity	Tumor-suppressive	Weeraratne et al. (2011)
miR-218	IKBKB	– Reduces cell migration – Reduces cell invasion	Tumor-suppressive	Xia et al. (2012a, b, c), Feng et al. (2017)
miR-146b-5p	EGFR	– Decreases cell invasion – Decreases cell migration	Tumor-suppressive	Katakowski et al. (2010)
miR-221	BBC3	– Inhibits apoptosis – Induces cell survival	Oncogenic	Zhang et al. (2010a, b, c, d)
miR-222	BBC3	– Inhibits apoptosis – Induces cell survival	Oncogenic	Zhang et al. (2010a, b, c, d)
miR-326	PKM	– Induces apoptosis – Reduces metabolic activity	Tumor-suppressive	Kefas et al. (2009)
miR-125a	PDPN	– Inhibits cell invasion – Induces apoptosis – Inhibits cell proliferation	Tumor-suppressive	Cortez et al. (2010)
miR-26a	PTEN RB1 MAP3K2	– Increases AKT signaling	Oncogenic	Huse et al. (2009)

(continued)

Table 5.4 (continued)

miRNAs	Direct targets	miRNA_function	Effect	References
		– Promotes cell proliferation – Decreases apoptosis – Promotes tumor growth		
miR-326	NOTCH1 NOTCH2	– Induces cytotoxicity – Reduces tumorigenicity	Tumor-suppressive	Kefas et al. (2009)
miR-34a	MET NOTCH1 NOTCH2	– Inhibits cell proliferation – Inhibits cell cycle progression – Inhibits cell survival – Inhibits cell invasion	Tumor-suppressive	Guessous et al. (2010)
miR-200a	CTNNB1	– Inhibits cell growth	Tumor-suppressive	Saydam et al. (2009)
miR-153	BCL2 MCL1	– Inhibits cell proliferation – Increases apoptosis	Tumor-suppressive	Zhao et al. (2013a, b)
miR-125b	BMF	– Increases cell viability – Increases cell proliferation – Inhibits apoptosis – Reduces ATRA sensitivity	Oncogenic	Xia et al. (2009a, b)
miR-124	SLC16A1	– Inhibits cell proliferation	Tumor-suppressive	Xia et al. (2012a, b, c)
miR-199b-5p	HES1	– Inhibits cell proliferation	Tumor-suppressive	Garzia et al. (2009)
miR-146b	MMP16	– Inhibits cell migration – Inhibits cell invasion	Tumor-suppressive	Li et al. (2013)
miR-15b	CCNE1	– Induces cell cycle G0/G1 arrest	Tumor-suppressive	Xia et al. (2009a, b)
miR-296	HGS	– Promotes angiogenesis	Oncogenic	Würdinger et al. (2008)
miR-125a	NTRK3	– Inhibits cell growth – Induces apoptosis	Tumor-suppressive	(Cortez et al. 2010)
miR-9	NTRK3	– Inhibits cell growth – Induces apoptosis	Tumor-suppressive	Nass et al. (2009)
miR-128	E2F3	– Inhibits cell proliferation	Tumor-suppressive	Papagiannakopoulos et al. (2012)
miR-124a	CDK6	– Decreases cell growth	Tumor-suppressive	Pierson et al. (2008)
miR-7	EGFR	– Decreases cell viability – Decreases cell invasion	Tumor-suppressive	Kefas et al. (2008)

5.3 Genetic Aspects of Breast Cancer

Breast cancer (BC) is developed in the breast tissue containing ducts (tubes that carry milk to the nipple) and lobules (milk producing glands). This cancer occurs in both women and men; however, it is rare in men. BC is the leading cause of cancer death in women, the second most common cancer worldwide, and the fifth most common cause of cancer-related deaths (Turnbull and Rahman 2008). Accumulation of mutations in critical genes including genes involved in control of cell growth and division or repair of damaged DNA allows cells to grow and divide uncontrollably to form a tumor. According to the micro-evolutionary process of cancer, these genetic changes are acquired during a person's lifetime and are present only in certain cells in the breast that ultimately turn a normal breast cell to a cancerous one (Vogelstein and Kinzler 2002). Most of these alterations, as the somatic mutations, are not inherited. Somatic mutations in many different genes have been detected in breast tumors. In other hand, some gene mutations, less commonly, present in all of the body's cells that increase the risk of developing breast cancer. These genetic changes are typically inherited from one of the parents. Cancer is a multifactorial disease in which even in individuals with germline mutations, through alteration of other genes, together with environmental and lifestyle factors, has influential role on development of BC (Golshan 2015). In this section we aimed to discuss about the genes that lead a normal breast epithelial cell to initiate the process of tumor initiation and probably delineate them to advanced metastatic disease. According to Online Mendelian Inheritance in Man (OMIM), most important genes that are related to breast cancer are demonstrated briefly in Table 5.5.

5.3.1 Genetic Etiology of Breast Cancer

See Table 5.5.

5.3.2 Genetic Aspects of Breast Cancer Based on Penetrance

In recent years, our understanding of genetic predisposition to breast cancer has been significantly advanced. Observational epidemiology has also provided essential foundations to our understanding, both in quantifying the contribution of genetic factors to BC and in predicting how these factors will act independently and dependently. Genetic modeling has been performed to predict the profiles of the genes involved, which in turn facilitates the selection of molecular methods appropriate for identifying them. Linkage analysis, mutational screening of

Table 5.5 Summary of known breast cancer predisposition genetic factors in BC

Gene	Location	Function	Other related disease	No. of mutations reported based on HGMD	References
BRCA1	17q21	– E3 ubiquitin-protein ligase that specifically mediates the formation of 'Lys-6'-linked polyubiquitin chains—plays a central role in DNA repair by facilitating cellular responses to DNA damage	– Breast-ovarian cancer, familial, 1 (BROVCA1), Ovarian cancer (OC) – Pancreatic cancer 4 (PNCA4)	1901	Ford et al. (1994), Karami and Mehdipour (2013)
BRCA2	13q12.3	– Involved in double-strand break repair and/or homologous recombination	– Pancreatic cancer – Breast-ovarian cancer – Fanconi anemia – Glioma 3	1643	Wooster et al. (1995)
TP53	17p13.1	– Acts as a tumor suppressor in many tumor types – Induces growth arrest or apoptosis depending on the physiological circumstances and cell type	– Esophageal cancer – Li-Fraumeni syndrome – Squamous cell carcinoma of the head and neck – Lung cancer – Papilloma of choroid plexus, Adrenocortical carcinoma – Basal cell carcinoma 7	360	Børresen-Dale (2003)
ATM	11q22-q23	– Serine/threonine protein kinase which activates checkpoint signaling upon double strand breaks (DSBs), apoptosis and genotoxic stresses such as ionizing ultraviolet A light (UVA), thereby acting as a DNA damage sensor	– Ataxia telangiectasia	813	Ahmed and Rahman (2006), Mehdipour et al. (2011a, b)
ESR	6q25.1	– A nuclear hormone receptor involved in the regulation of eukaryotic gene expression – Affect cellular proliferation and differentiation in target tissues	– Estrogen resistance	25	Dontu et al. (2004)
K-RAS	12p12.1	– Ras proteins bind GDP/GTP and possess intrinsic GTPase activity – Plays an important role in the regulation of cell proliferation	– Leukemia, acute myelogenous – Leukemia, juvenile – Myelomonocytic – Noonan syndrome – Gastric cancer – Cardiofaciocutaneous syndrome	33	Pereira et al. (2013)

(continued)

Table 5.5 (continued)

Gene	Location	Function	Other related disease	No. of mutations reported based on HGMD	References
RAD54L	1p32	– Involved in transcriptional regulation and chromatin remodeling	– Alpha-thalassemia mental retardation syndrome – X-linked (ATRX), Mental retardation, Alpha-thalassemia – Myelodysplasia syndrome	2	Gonzalez et al. (1999)
PALB2	16p12.1	– Plays a critical role in homologous recombination repair (HRR) through its ability to recruit BRCA2 and RAD51 to DNA breaks	– Fanconi anemia complementation group N – Pancreatic cancer	183	Rahman et al. (2007)
CDH1	16q22.1	– Cadherins are calcium-dependent cell adhesion proteins – DH1 is involved in mechanisms regulating cell-cell adhesions, mobility and proliferation of epithelial cells	– Hereditary diffuse gastric cancer – Endometrial cancer – Ovarian cancer	159	Petridis et al. (2014)
CHEK2	22q12.1	– Serine/threonine-protein kinase which is required for checkpoint-mediated cell cycle arrest, activation of DNA repair and apoptosis in response to the presence of DNA double-strand breaks	– Li-Fraumeni syndrome 2 – Prostate cancer – Osteogenic sarcoma	97	Cybulski et al. (2006)
BRIP1	17q22-q24	– DNA-dependent ATPase and 5′ to 3′ DNA helicase required for the maintenance of chromosomal stability	– Fanconi anemia complementation group J	72	Walsh and King (2007)
PIK3CA	3q26.3	– The protein encoded by this gene represents the catalytic subunit, which uses ATP to	– Colorectal cancer – Ovarian cancer – Hepatocellular carcinoma – Keratosis	Not registered	Bachman et al. (2004)

(continued)

Table 5.5 (continued)

Gene	Location	Function	Other related disease	No. of mutations reported based on HGMD	References
		phosphorylate certain signaling molecules, which triggers a series of additional reactions that transmit chemical signals within cells. This gene has been found to be oncogenic	– Megalencephaly-capillary malformation-polymicrogyria syndrome – Congenital lipomatous overgrowth, vascular malformations, and epidermal nevi (CLOVE) – Cowden syndrome 5		
CASP8	2q33-q34	– Most upstream protease of the activation cascade of caspases responsible for the TNFRSF6/FAS mediated and TNFRSF1A induced cell death	– Caspase-8 deficiency	5	Cox et al. (2007)
BARD1	2q34-q35	– The BRCA1-BARD1 heterodimer specifically mediates the formation of 'Lys-6'-linked polyubiquitin chains and coordinates a diverse range of cellular pathways such as DNA damage repair, ubiquitination and transcriptional regulation to maintain genomic stability	– Fallopian tube carcinoma – Lung cancer – Ovarian cancer	40	Jakubowska et al. (2008)
HMMR	5q34	– Involved in cell motility. It may also be involved in cellular transformation and metastasis formation, and in regulating extracellular-regulated kinase (ERK) activity	Non-reported	Not registered	Maxwell et al. (2011)
NQO2	6pter-q12	– The enzyme apparently serves as a quinone reductase in connection with conjugation reactions of hydroquinones involved in detoxification pathways – As well as in biosynthetic processes such as the vitamin	– Delayed memory recall – Parkinson disease	3	Choi et al. (2009)

(continued)

Table 5.5 (continued)

Gene	Location	Function	Other related disease	No. of mutations reported based on HGMD	References
		K-dependent gamma-carboxylation of glutamate residues in prothrombin synthesis			
RB1CC1	8q11	– Plays a role as a modulator of TGF-beta-signaling by restricting substrate specificity of RNF111 – Involved in autophagy. Inhibits PTK2/FAK1 and PTK2B/PYK2 activity and activation of downstream signaling pathways	– Leukaemia – Schizophrenia	4	Chano et al. (2002)
SLC22A18	11p15.5	– May act as a transporter of organic cations based on a proton efflux antiport mechanism – May play a role in the transport of chloroquine and quinidine-related compounds in kidney	– Lung cancer – Rhabdomyosarcoma	Not registered	Gallagher et al. (2006)
TSG101	11p15.1	– Component of the ESCRT-I complex – A regulator of vesicular trafficking process – May be involved in cell growth and differentiation – Acts as a negative growth regulator	– Non-reported	Not registered	Li et al. (1997)
XRCC3	14q32.3	– Involved in the homologous recombination repair (HRR) pathway of double-stranded DNA, thought to repair chromosomal fragmentation, translocations and deletions	– Melanoma – Epithelial – Ovarian cancer, Mitomycin-C resistance	7	Krupa et al. (2009)

(continued)

Table 5.5 (continued)

Gene	Location	Function	Other related disease	No. of mutations reported based on HGMD	References
AKT1	14q32.32	– AKT1 is one of 3 closely related serine/threonine-protein kinases (AKT1, AKT2 and AKT3) called the AKT kinase, and which regulate many processes including metabolism, proliferation, cell survival, growth and angiogenesis	Colorectal cancer, Proteus syndrome, Cowden syndrome 6	7	Ju et al. (2007)
PHB	17q21	– Prohibiting inhibits DNA synthesis – It has a role in regulating proliferation – May play a role in regulating mitochondrial respiration activity and in aging	– Non-reported	2	Webster et al. (2013)
PPM1D	17q23.2	– Required for the relief of p53-dependent checkpoint mediated cell cycle arrest	– Autism?	47	Li et al. (2002)
Androgen receptor (AR)	Xq11-q12	The protein functions as a steroid-hormone activated transcription factor. Upon binding the hormone ligand, the receptor dissociates from accessory proteins, translocates into the nucleus, dimerizes, and then stimulates transcription of androgen responsive genes	– SBMA – Complete androgen insensitivity (CAIS) Prostate cancer	577	Mehdipour et al. (2011a, 2011b)

candidate genes, and association studies have been used to identify predisposition factors of three distinct risk-prevalence profiles: rare high-penetrance alleles, rare intermediate-penetrance alleles, and common low-penetrance alleles (Turnbull and Rahman 2008). Bellow, we have summarized the involved genes in breast cancer according to their power and penetrance (Table 5.6).

Table 5.6 Summary of genetic factors involved in BC based on their penetrance

High-penetrance	Gene	Chr. location	Function	Site of expression	Breast cancer risk	Other related cancers and disease	OMIM	References
	BRCA1	17q21	– Plays a role in maintaining genomic stability – It also acts as a tumor suppressor	Isoform 1 and isoform 3 are widely expressed. Isoform 3 is reduced or absent in several breast and ovarian cancer cell lines	>10%	– Ovarian	113705	Turnbull and Rahman (2008)
	BRCA2	13q12.3	– Involved in maintenance of genome stability, specifically the homologous recombination pathway for double-strand DNA repair – BRCA2 is considered as a tumor suppressor gene	Highest levels of expression in breast and thymus, with slightly lower levels in lung, ovary and spleen	>10%	– Ovarian – Prostate	600185	Turnbull and Rahman (2008)
	TP53	17p13.1	– Acts as a tumor suppressor in many tumor types – Induces growth arrest or apoptosis depending on the physiological circumstances and cell type	Isoforms are expressed in a wide range of normal tissues but in a tissue-dependent manner	>10%	– Sarcomas – Adrenal – Brain	191170	Chompret et al. (2000), Turnbull and Rahman (2008)
Intermediate penetrance	ATM	11q22-q23	– ATM is a central player in the response to double-strand DNA breaks through initiation of a signaling cascade that involves phosphorylation of multiple proteins including p53, BRCA1, and CHK2	Found in pancreas, kidney, skeletal muscle, liver, lung, placenta, brain, heart, spleen, thymus, testis, ovary, small intestine, colon and leukocytes.	2–3%	– Ataxia telangiectasia (AT) – T-cell acute–lymphoblastic –leukemia (TALL) – T-prolymphocytic leukemia (TPLL)	607585	Renwick et al. (2006)
	BRIP1	17q22.2	– DNA-dependent ATPase and 5′ to 3′ DNA helicase required for the maintenance of chromosomal stability. - Involved in the repair of DNA double-strand breaks by homologous	Ubiquitously expressed, with highest levels in testis	2–3%	– Fanconi anemia complementation group J (FANCJ)	605882	Seal et al. (Seal et al. 2006)

(continued)

Table 5.6 (continued)

High-penetrance	Gene	Chr. location	Function	Site of expression	Breast cancer risk	Other related cancers and disease	OMIM	References
			recombination in a manner that depends on its association with BRCA1					
	PALB2	16p12.2	– Plays a critical role in homologous recombination repair (HRR) through its ability to recruit BRCA2 and RAD51 to DNA breaks	This gene has expression in most organs and most developmental stages	2–4%	– Fanconi anemia complementation group N (FANCN) – Pancreatic cancer (PNCA)	610355	Rahman et al. (2007)
	CHEK2	22q12.1	– This Serine/ threonine- protein kinase is required for checkpoint-mediated cell cycle arrest, activation of DNA repair and apoptosis in response to the presence of DNA double-strand breaks	High expression is found in testis, spleen, colon and peripheral blood leukocytes. Low expression is found in other tissues	2–3%	– Li-Fraumeni syndrome 2 – Osteogenic sarcoma – Prostate cancer (PC)	604373	Vahteristo et al. (2002)
	RAD50	5q31	– Component of the MRN complex, which plays a central role in double-strand break (DSB) repair, DNA recombination, maintenance of telomere integrity and meiosis	Expressed at very low level in most tissues, except in testis where it is expressed at higher level. Expressed in fibroblasts	2–3%	– Nijmegen breakage syndrome-like disorder (NBSLD)	604040	Heikkinen et al. (2006)
Uncertain Penetrance	PTEN	10q23.3	– A lipid phosphatase that functions as a tumor suppressor through negative regulation of a cell-survival signaling pathway	Expressed at a relatively high level in all adult tissues, including heart, brain, placenta, lung, liver, muscle, kidney and pancreas	2–10%	– Cowden syndrome 1 (CWS1) – Thyroid – Endometrial cancer – Glioma 2 (GLM2)	601728	Lynch et al. (1997)
	STK11	19p13.3	– This gene, which encodes a member of the serine/threonine kinase family, regulates cell polarity and – Functions as a tumor suppressor	Ubiquitously expressed. Strongest expression in testis and fetal liver	2–10%	Peutz-Jeghers syndrome (PJS)	602216	Giardiello et al. (2000)

(continued)

Table 5.6 (continued)

High-penetrance	Gene	Chr. location	Function	Site of expression	Breast cancer risk	Other related cancers and disease	OMIM	References
						– Testicular germ cell tumor (TGCT)		
	CDH1	16q22.1	– A calcium-dependent cell adhesion protein which is involved in mechanisms regulating cell-cell adhesions, mobility and proliferation of epithelial cells	Non-neural epithelial tissues.	2–10%	– Hereditary diffuse gastric cancer (HDGC) – Endometrial cancer (ENDMC) – Ovarian cancer (OC)	192090	Masciari et al. (2007)
Low penetrance	FGFR2	10q26	– Tyrosine-protein kinase that acts as cell-surface receptor for fibroblast growth factors – Plays an essential role in the regulation of cell proliferation, differentiation, migration and apoptosis, and in the regulation of embryonic development	This gene is expressed in many tissues and embryogenesis stages	OR = 1.2	– Crouzon syndrome – Pfeiffer syndrome – Craniosynostosis – Apert syndrome – Jackson-Weiss syndrome	176943	Hunter et al. (2007)
	TOX3	16q12.1	– Involved in bending and unwinding of DNA and alteration of chromatin structure	Expressed mainly in epithelial cells. Expressed in the central nervous system (CNS), in the ileum and within the brain in the frontal and occipital lobe	OR = 1.24	– Uterine cancer – Lung cancer – Skin cancer	611416	Ruiz-Narváez et al. (2010)
	MAP3K1	5q11.2	– A serine/threonine kinase and is part of some signal transduction cascades	This gene is expressed in many tissues and embryogenesis stages	OR = 1.13	– 46, XY sex reversal 6 (SRXY6)	600982	Rebbeck et al. (2009)
	CASP8	2q33-q34	– Most upstream protease of the activation cascade of caspases responsible for the TNFRSF6/FAS mediated and TNFRSF1A induced cell death	Isoform 1, isoform 5 and isoform 7 are expressed in a wide variety of tissues. Highest expression in peripheral blood leukocytes, spleen, thymus and liver. Barely detectable in brain, testis and skeletal muscle	OR = 1.1	– Caspase-8 deficiency	601763	Cox et al. (2007), Motevalizadeh et al. (2017)

5.3.3 Breast Cancer Sub-classification Based on Expression Signature

Breast cancer is a heterogeneous and complex diseases, with a spectrum of many subtypes and distinct biological features that lead to the diverse response patterns to various treatment modalities and clinical outcomes. Traditional classification systems regarding biological characteristics may have limitations for patient-tailored treatment strategies. Tumors with similar clinical and pathological characteristics may behave differently. Analyses of BC with new molecular techniques now hold promises for the development of more accurate tests for the prediction of recurrence. Gene signatures have been developed as the predictors of response to therapy and protein gene products that play direct roles in driving the biology and clinical behavior of cancer cells (Schnitt 2010; Yersal and Barutca 2014). These are potential targets for the development of novel therapeutics. HER2, ESR, PR and Ki67 are the most popular and studied genes through which, breast cancers are classified on the basis of their expression signatures (Table 5.7).

5.3.4 Micro RNAs (miRNAs) as a Non-coding Players of BC Tumorogenesis

MicroRNAs (miRNAs) are a recently discovered class of endogenously expressed, single stranded, non-coding RNAs of about 19–25 nucleotides in length, that regulate gene expression through binding to the 3′ un-translated regions (UTR) of target messenger RNAs (mRNAs). Therefore, mis-regulation of them can be involved in diverse biological and pathological processes. To date, a large number of miRNAs and predicted mRNA target sites have been identified. Numerous miRNAs are involved in controlling and regulating multiple processes linked to cancer pathogenesis such as proliferation, apoptosis, angiogenesis and immune function. So, they have potential values in cancer classification, diagnostic biomarkers, predictive markers, prognosis, response to therapy, and innovation of therapeutic targets (Calin and Croce 2006; Fatica and Bozzoni 2014).

Epithelial to mesenchymal transition (EMT) process causes metastasis, tumor progression and distribution in the body. Some of miRNAs are involved in these process. Some miRNAs including miR-7, 50, -52, 17/20, 22 and miR-30 are involved in metastasis commonly called **metastatic miRNAs**. Other miRNAs which act as oncogene are known as oncomir and those involved in inhibition of cell proliferation, invasion and metastasis are known as **tumor suppressor miRNAs** (Allison 2012). In Table 5.8, some more important and involved miRNAs in breast cancer are listed.

Table 5.7 Breast cancer sub-classification based on expression signature

Class	Expression signature	Prevalence (%)	Clinical property	Treatment	References
Luminal A	ER-positive and/or PR-positive HER2-negative Low Ki67	50–60	– These tumors frequently have low histological grade, - low degree of nuclear pleomorphism – Low mitotic activity – Include special histological types (i.e., tubular, invasive cribriform, mucinous and lobular) with good prognosis	– Hormonal therapy – Chemotherapy	Carey et al. (2006)
Luminal B	ER-positive and/or PR-positive HER2-positive (or HER2-negative with high Ki67)	15–20	– More aggressive phenotype – Higher histological grade – Proliferative index – Worse prognosis	– Endocrine therapy – Chemotherapy	Creighton (2012)
Triple negative/basal-like	ER-negative PR-negative HER2-negative Ki67-positive	8–37	– High histological and nuclear grade – Poor tubule formation – Presence of central necrotic or fibrotic zones – Pushing borders, conspicuous – Lymphocytic infiltrate – Medullary features with exceptionally high mitotic and proliferative indices	– No response to endocrine therapy or trastuzumab – Appear to be sensitive to platinum-based chemotherapy and PARP inhibitors	Venkitaraman (2010)

(continued)

Table 5.7 (continued)

Class	Expression signature	Prevalence (%)	Clinical property	Treatment	References
HER2 type	ER-negative PR-negative HER2-positive	15–20	– Increased sensitivity to certain cytotoxic agents such as doxorubicin – Relative resistance to hormonal agents – Lymph node-positive and – Poorer tumor grade	– Respond to trastuzumab (Herceptin) – Respond to anthracycline-based chemotherapy	Engel and Kaklamani (2007)
Normal breast-like	ER-negative PR-negative HER2-negative	5–10	– Presenting an intermediate prognosis between luminal and basal-like cancers	– Do not respond to neoadjuvant chemotherapy	Barutca (2014)

Table 5.8 The most important miRNAs reported in breast cancer development and progression

miRNA	Target	Role in breast cancer	Act as: metastatic/oncogene/tumor suppressor	References
miRNA-21	PDCD4	– Enhanced levels of miRNA-21 resulted in increased invasive capacity of HER2/neu expressing breast cancer cells – Also, it enhances proliferation, survival and migration in cancer cells through targeting of PTEN	Oncogene/metastatic	Kumar et al. (2010), Fang et al. (2017)
miRNA-200 family	ZEB-1/2 PI3 K/AKT	– **Inhibition** of the epithelial to mesenchymal transition process (**EMT**)	Tumor suppressor	Gregory et al. (2008)
miRNA-9		– Inducers of epithelial to mesenchymal phenotype (EMT)	Oncogene	Gravgaard et al. (2012)
miRNA-24	Net1A	– **Inducers of (EMT)**. Also, miRNA-24 levels are up-regulated in metastatic compared with primary breast tumor samples with mesenchymal phenotype	Oncogene	Papadimitriou et al. (2012)
miRNA-29	N-myc interactor (NMI)	– Inducers of EMT. Increased levels of miRNA-29 increases invasion	Oncogene	Rostas et al. (2014)
miRNA-29a	Tristetraprolin (TTP)	– Enhanced miRNA-29a has observed in breast cancer patient samples with invasive phenotype. Also, it induces EMT	Oncogene	Gebeshuber et al. (2009)
miRNA-103/107	RNase III endonuclease dicer	– Over-expression of miRNA103/107 induced Dicer down-regulation and **induction of EMT**, with subsequent enhancement in invasive capacity – Furthermore, miRNA103/107 could induce EMT by decreasing miRNA-200 (which negatively regulates EMT)	Oncogene	Grelier et al. (2009)

(continued)

Table 5.8 (continued)

miRNA	Target	Role in breast cancer	Act as: metastatic/oncogene/tumor suppressor	References
miRNA-106b-25 cluster	E-cadherin, β-catenin, Jag1, MMP-9, vimentin, TGF-β and six1	– Correlates with metastatic phenotype and shorter relapse free survival and is involved in **EMT induction**	Oncogene	Smith et al. (2012)
miRNA-155	TGFβ, C/EBPβ, E-cadherin and vimentin	– **Induction of EMT**, enhancing metastasis and invasion. miRNA-155 was the first to be found to actually induce tumorigenesis	Oncogene/Metastatic	Johansson et al. (2013)
miRNA 221/222	Fra-1, E- Cadherin, ZEB2 and SLUG	– **Induces EMT** and subsequent enhancement in invasion – Induction of tamoxifen resistance in the ER + breast cancer cell line MCF-7	Oncogene/Metastatic	Hwang et al. (2013)
miRNA-7	SETDB1, STAT3	– Over-expression of miRNA-**7 suppressed the EMT-like characteristics** of MDA-MB-231 cells, as reflected in the observation that these cells became less scattered and lost their spindle-like morphology, increased E-cadherin and reduced vimentin expression. It also has anti-metastatic action	tumor suppressor	Zhang et al. (2014a, b, c, d, e, f)
miRNA-124	E-cadherin, vimentin, SLUG, Fibronectin	– **Inhibition of EMT** – It plays a crucial role in neural development. Recent evidence suggests that it is also involved in cancer pathogenesis, with reduced expression seen in various cancers, including the breast – It also shows anti-metastatic action	Tumor suppressor	Liang et al. (2012)

(continued)

Table 5.8 (continued)

miRNA	Target	Role in breast cancer	Act as: metastatic/oncogene/tumor suppressor	References
miRNA-145	Snail, ZEB1/2, OCT4, fibronectin, E-cadherin	– Decreasing of miRNA-145 expression has observed in breast tumor tissues with invasive phenotype – Inhibition of EMT by targeting Oct4. It also shows anti-metastatic action	Tumor suppressor	Hu et al. (2012)
miRNA-375	MTDH	– Significantly down-regulated in tamoxifen-resistant (TamR) MCF-7 cells – EMT inhibitor – Reduction of invasiveness	Tumor suppressor	Ward et al. (2013)
miRNA-448	SATB1, amphiregulin, Twist, NFkB	– Inhibition of EMT – Enhanced invasive capacity was also observed upon miRNA-448 inhibition in vitro	Tumor suppressor	Li et al. (2011a, b, c, d, e)
miRNA-18a	HIF1A	– Reduces cell invasiveness and sensitivity to anoikis and hypoxia in vitro, and primary tumor growth and lung metastasis in vivo	Tumor suppressor	Krutilina et al. (2014)
miRNA-31	Fzd3, ITGA5, RDX, RhoA	– Expression of miRNA-31 is reduced in several metastatic breast cancer cell lines – Correlates inversely with metastasis in human breast cancer patients	Tumor suppressor	Valastyan et al. (2009)
miRNA-107	CDK8	– Significantly inhibited cell migration and invasion in MDA-MB-231 cells	Tumor suppressor	Li et al. (2014a, b, c, d)
miRNA-146a/b	NFkB, IRAK, TRAF6	– Over-expression of miRNA146a/b in MDA-MB-231 resulted in marked inhibition of migration and invasion due to reduced NF-κB activity	Tumor suppressor	Bhaumik et al. (2008)

(continued)

Table 5.8 (continued)

miRNA	Target	Role in breast cancer	Act as: metastatic/oncogene/tumor suppressor	References
miRNA-302	CXCR4	– Enforced expression of miRNA302a significantly inhibited both in vitro and in vivo cell invasion and metastasis, by inhibiting the CXCR4 gene	Tumor suppressor	Liang et al. (2014)
miRNA-10b	RHOC, TIAM1	– This miRNA is highly expressed in metastatic breast cancer cells – Correlates with poor clinical progression in patients with breast cancer	Oncogene	Moriarty et al. (2010)
miRNA-18b	NLRP7, KLK3, OLFM3, POSTN, MAGED4B, KIR3DL3, CRX, SEMG1, CEACAM5	– Inhibition of miRNA18b in breast cancer cell lines significantly suppressed their invasive capacity by modulating several target genes	Oncogene/metastatic	Fonseca-Sanchéz et al. (2013)
miRNA-495	JAM-A	– Over-expression of miRNA-495 significantly enhanced invasive capacity of breast cancer cells	Oncogene/metastatic	Cao et al. (2014)
miRNA-Has-30c	NOV, CCN3	– It has pro-metastatic function and enhances invasive capacity toward	Oncogene/metastatic	Dobson et al. (2014)
MiRNA-373/520	E-cadherin, CD44	– Stable over-expression of miRNA-373 and-520c stimulated breast cancer cell migration in vitro and in vivo by the suppression of the cell surface glycoprotein CD44 through binding to the 3′ UTR region of its mRNA – In addition, miRNA-373 promotes cell invasion through targeting of sites in the promoter of E-cadherin mRNA	Oncogene/metastatic	Huang et al. (2008)

(continued)

Table 5.8 (continued)

miRNA	Target	Role in breast cancer	Act as: metastatic/oncogene/tumor suppressor	References
miRNA-26a/b	CHD1, GREB1, KPNA2	– Inhibition of breast cancer proliferation	Tumor suppressor	Tan et al. (2014)
miRNA-93	TGFb, JAK1,STAT3, AKT3, SOX3, EZH1, HMGA2	– Inhibition of breast cancer proliferation	Tumor suppressor	Liu et al. (2012a, b, c)
miRNA-124	Ets-1	– Inhibition of breast cancer proliferation	Tumor suppressor	Li et al. (2014a, b, c, d)
miRNA-145	PUMA	– Inhibition of breast cancer proliferation	Tumor suppressor	Spizzo et al. (2010)
miRNA-Let-7	RB1, E2F, HRAS, HMGA2	– Inhibition of breast cancer proliferation	Tumor suppressor	Johnson et al. (2007)
miRNA-769-3p	NDRG1	– Inhibition of breast cancer proliferation	Tumor suppressor	Iorio et al. (2005)
miRNA-107	CDK8	– Inhibition of breast cancer proliferation	Tumor suppressor	Li et al. (2014a, b, c, d)
miRNA-27a	SP, myt-1, ZBTB10, survivin, VGED, VGEFR1	– Enhances of breast cancer proliferation	Oncogene	Alvarez-Saavedra and Horvitz (2010)
miRNA-221/222	Kip1, PTEN, EGFR, RAS, RAF, MEK	– Enhances of breast cancer proliferation	Oncogene	Garofalo et al. (2009)
miRNA-205	E-cadherin, ZEB1, ZEB2, HER3, VEGF	– Inhibition o EMT and tumor invasion	Tumor suppressor	Gregory et al. (2008) Savad et al. (2012)
miRNA-192	MDM2, CDC7, IGF1, DHFR1	– Anti-proliferative effect	Tumor suppressor	Zhang et al. (2014a, b, c, d, e, f)

(continued)

Table 5.8 (continued)

miRNA	Target	Role in breast cancer	Act as: metastatic/oncogene/tumor suppressor	References
miRNA-34	SIRT1, BCL2, CCND1, NMYC	– Anti-proliferative effect	Tumor suppressor	Achari et al. (2014)
miRNA-17		– Promote tumor development and cell proliferation	Oncogene	Li et al. (2011a, b, c, d, e)

5.3.5 *Breast Cancer Methylome at a Glance*

All heritable changes in gene expression and chromatin structure but not in DNA sequences are known as epigenetic events). Epigenetic mechanisms are the main player in the triggering and maintenance of differentiation processes. DNA methylation, histone modifications and RNA-mediated silencing are the main principles of epigenetic inheritance (Brooks et al. 2009). Disruption and mutually reinforcing of any of these epigenetic mechanisms leads to inappropriate gene expression, resulting in diseases. Despite the uncertainty about the precise underlying mechanisms of epigenetic alteration, a tremendous researches have recently revealed the role of epigenetic disruption in cancers (Izadi and Noruzinia, 2015; Mehdiour 2015). Great advances have been achieved to unmask the epigenetic region involved in tumorigenesis of BC and help the researchers characterize the developmental strategies for cancer prevention and treatment (Esteller 2007). Here, we have classified the status of methylation in some involved genes in breast cancer (Table 5.9).

5.4 Stomach (Gastric) Cancer

The Gastric (stomach) (GC) is located in the upper abdomen and avails digest victuals. Virtually all gastric cancers are adenocarcinomas (cancers that commence in cells that make and surrender mucus and other fluids). Other types of GC include gastrointestinal carcinoid tumors, gastrointestinal stromal tumors, and lymphomas. Infection with H. pylori is a mundane cause of gastric cancer. Diagnosis of gastric cancer is often performed at an advanced stage of the disease because there are no early signs or symptoms (Alberts et al. 2003). This disease remains a major cause of cancer death in many developing countries, and, globally, ranks just below lung cancer as the second most frequent cause of total cancer deaths. Besides, environmental factors play a consequential role in GC risk and development (Rugge et al. 2015).

Most gastric adenocarcinomas are known as the advanced lesions, and consequently have a high mortality rate. The intestinal form is more proximately associated with environmental agents, and cancers of this type appear to acquire a distinct pattern of genetic variations. The diffuse form has been more proximately linked to heritable factors (Lynch et al. 2005). Here, we endeavored to summarize both somatic and heritable genetic and molecular alterations involved in stomach cancer.

Table 5.9 Epigenetically deregulated genes involved in breast cancer

Gene	Location	Function	Site of expression	Other related disease	Hypo/hyper-methylation	References
CDKN2A	9p21	– Acts as a negative regulator of the proliferation of normal cells by interacting strongly with CDK4 and CDK6. This inhibits their ability to interact with cyclins D and to phosphorylate the retinoblastoma protein	Widely expressed but not detected in brain or skeletal muscle. Isoform 3 is pancreas-specific	– Li-Fraumeni syndrome – Familial atypical multiple mole melanoma-pancreatic carcinoma syndrome – Melanoma	Hyper	Fackler et al. (2004), Spitzwieser et al. (2017)
DAPK1	9q21.33	– Calcium/calmodulin-dependent serine/threonine kinase involved in multiple cellular signaling pathways that trigger cell survival, apoptosis, and autophagy	Isoform 2 is expressed in normal intestinal tissue as well as in colorectal carcinomas	– Alzheimer disease	Hyper	Parrella et al. (2004)
MGMT	10q26	– DNA repair protein that is involved in cellular defense against mutagenesis and toxicity from alkylating agents	This gene has expression in most tissues	– Cervical carcinoma – Endometrial cancer – Lung cancer	Hyper	Fumagalli et al. (2012)
MLH1	3p21.3	– Heterodimerizes with PMS2 to form MutL alpha, a component of the post-replicative DNA mismatch repair system (MMR)	Colon, lymphocytes, breast, lung, spleen, testis, prostate, thyroid, gall bladder and heart	– Hereditary non-polyposis colorectal cancer – Muir-Torre syndrome – Endometrial cancer	Hyper	Park et al. (2011a, b)
GSTP1	11q13	– Conjugation of reduced glutathione to a wide number of exogenous and endogenous hydrophobic electrophiles – Regulates negatively CDK5 activity via p25/p35 translocation to prevent neurodegeneration	Wide range of tissues	– Oral cancer	Hyper	Lin and Nelson (2003)

(continued)

Table 5.9 (continued)

Gene	Location	Function	Site of expression	Other related disease	Hypo/hyper-methylation	References
RARB	3p24.2	– This gene encodes retinoic acid receptor beta, a member of the thyroid-steroid hormone receptor superfamily of nuclear transcriptional regulators, which mediates cellular signaling in embryonic morphogenesis, cell growth and differentiation	Wide range of tissues	– Microphthalmia	Hypo	Stefanska et al. (2010)
APC	5q21-q22	– Tumor suppressor. Promotes rapid degradation of CTNNB1 – Participates in Wnt signaling as a negative regulator	Expressed in a variety of tissues	– Familial adenomatous polyposis – Medulloblastoma, gastric cancer – Hepatocellular carcinoma	Hyper	Brooks et al. (2010)
ESR2	14q23.2	– This gene encodes a member of the family of estrogen receptors and superfamily of nuclear receptor transcription factors	– Isoform beta-1 is expressed in testis and ovary – Isoform beta-2 is expressed in spleen, thymus, testis and ovary. Isoform beta-3 is found in testis – Isoform beta-4 is expressed in testis and – Isoform beta-5 is expressed in testis, placenta, skeletal muscle, spleen and leukocytes	– Rheumatoid arthritis, ovulatory defects – Hypospadias, Alzheimer disease – High systolic blood pressure	Hypo/hyper	Hervouet et al. (2013)
CDH1	16q22.1	– CDH1 is involved in mechanisms regulating cell-cell adhesions, mobility and proliferation of epithelial cells – Has a potent invasive suppressor role	Non-neural epithelial tissues	– Hereditary diffuse gastric cancer – Endometrial cancer, ovarian cancer	Hyper	Li et al. (2006)

(continued)

Table 5.9 (continued)

Gene	Location	Function	Site of expression	Other related disease	Hypo/hyper-methylation	References
RASSF1	3p21.3	– Potential tumor suppressor – Required for death receptor-dependent apoptosis	– Isoform A and isoform C are ubiquitously expressed in all tissues tested – Isoform A is absent in many corresponding cancer cell lines – Isoform B is mainly expressed in hematopoietic cells	– Lung cancer	Hyper	Fackler et al. (2003), Pirouzpanah et al. (2015)
CDH13	16q23.3	– A calcium-dependent cell adhesion protein that may act as a negative regulator of neural cell growth.	– Highly expressed in heart. In the CNS, expressed in cerebral cortex, medulla, hippocampus, amygdala, thalamus and substantia nigra. No expression detected in cerebellum or spinal cord	– Amyotrophic lateral sclerosis – Higher adiponectin levels	Hyper	Shinozaki et al. (2005)
TWIST1	7p21.2	– The protein encoded by this gene is a bHLH transcription factor which implicated in cell lineage determination and differentiation	Subset of mesodermal cells	– Saethre-Chotzen syndrome – Robinow-Sorauf syndrome – Craniosynostosis 1	Hyper	Fackler et al. (2003)
ATM	11q22-q23	– Serine/threonine protein kinase which activates checkpoint signaling upon double strand breaks (DSBs), apoptosis and genotoxic stresses such as ionizing ultraviolet A light (UVA), thereby acting as a DNA damage sensor	Found in pancreas, kidney, skeletal muscle, liver, lung, placenta, brain, heart, spleen, thymus, testis, ovary, small intestine, colon and leukocytes	– Ataxia telangiectasia	Hyper	Flanagan et al. (2009)

(continued)

Table 5.9 (continued)

Gene	Location	Function	Site of expression	Other related disease	Hypo/hyper-methylation	References
BRCA1	17q21.31	– Plays critical roles in DNA repair, cell cycle checkpoint control, and maintenance of genomic stability	– Isoform 1 and isoform 3 are widely expressed – Isoform 3 is reduced or absent in several breast and ovarian cancer cell lines	– Ovarian cancer – Pancreatic cancer	Hyper	Zhang and Long (2015)

5.4.1 Genetic Etiology

See Table 5.10.

5.4.2 Cytogenetic Abberations

Various structural and numerical chromosome aberrations have been reported in gastric cancers albeit no categorical aberration has yet been reported. Chromosomes X, Y, 1, 7, 8, 9, 17 and 20 are the more commonly targets in those numerical aberrations occur. Chromosome 17 polysomy has been reported to be associated with lymphovascular invasion and nodal metastases. Conventional cytogenetic analysis of gastric cancers utilizing G-banding disclosed structural abnormalities more commonly involving chromosomes 1, 11, 14, 7, 17, 6, 8 and 13. Multicolor spectral karyotyping has been applied to identify recurrent chromosomal rearrangements in gastric cancer cell lines. Chromosome 8 was most commonly involved in rearrangements. Recurrent translocations, mostly, occurred in the key oncogene and tumor-suppressor gene loci, including chromosomes' bands 8q24 and 11q13 as the more common breakpoints (Ochi et al. 1986; Han et al. 1996; Panani 2008).

Comparative genomic hybridization has been employed to screen for abnormalities of copy number alterations in gastric cancers. According to the literature, 84% of tumors and all of the cell lines showed aberrations in DNA copy number. Concrete patterns of allelic gains and losses appeared to associate with different histopathological and disease-cognate features, such as metastases. Studies onLoss of heterozygosity (LOH) in gastric adenocarcinomas have revealed high LOH at 1p, 3p, 4p, 5q, 7p, 8p, 9p, 12q, 13q, 17p, 18q and 22q. The comparison of the intra-tumoural distribution of LOH between early and advanced GC suggests that numerous LOH is an early event in tumorigenesis while a few sporadic losses occurring later through disease progression (Wu et al. 2001; Vauhkonen et al. 2006).

5.4.3 Micro RNA Profile of Gastric Cancer

In spite of the promising developed therapeutic strategies, the overall outcome of gastric cancer remains poor. Current tumor markers are not ideal due to relatively low sensitivity and specificity. There is an exigent desideratum for identifying more concrete and more sensitive novel markers in the clinical management of GC. Expression profiling of different cancers could be considered as a supportive insight for the therapeutic monitoring and stratification of patients. The accumulative data suggests that expression analysis of minuscule noncoding-RNAs such as

Table 5.10 Most reported cancer genes that are involved in the process of stomach cancer development

Gene	Location	Function	Other-related diseases	OMIM	References
CASP10	2q33-q34	– Involved in the activation cascade of caspases responsible for apoptosis execution	– Autoimmune lymphoproliferative syndrome, type II – Non-Hodgkin lymphoma	601762	Oliveira et al. (2004) Park et al. (2002)
APC	5q21-q22	– This gene encodes a tumor suppressor protein – Acts as an antagonist of the Wnt signaling pathway – It is also involved in other processes including cell migration and adhesion, transcriptional activation, and apoptosis	– Familial adenomatous polyposis (FAP) – Hereditary desmoid disease (HDD) – Medulloblastoma (MDB) – Hepatocellular carcinoma (HCC)	611731	Horii et al. (1992)
IRF1	5q31.1	– RF1 serves as an activator of interferons alpha and beta transcription – It has been shown to be required for double-stranded RNA induction of these genes – IRF1 also functions as a transcription activator of genes induced by interferons alpha, beta, and gamma – Further, IRF1 has been shown to play roles in regulating apoptosis and tumor-suppression	– Preleukemic myelodysplastic syndrome (PMS) – Acute myelogenous leukemia (AML), – Somatic nonsmall cell lung cancer (SNCLC)	147575	Gao et al. (2012) Yamashita et al. (2010)

(continued)

Table 5.10 (continued)

Gene	Location	Function	Other-related diseases	OMIM	References
KLF6	10p15	– This gene encodes a member of the Kruppel-like family of transcription factors – The zinc finger protein is a transcriptional activator, and functions as a tumor suppressor	Prostate cancer	602053	Zhang et al. (2012a, b, c, d) Chen et al. (2011a, b, c)
FGFR2	10q26	– Tyrosine-protein kinase that acts as cell-surface receptor for fibroblast growth factors – Plays an essential role in the regulation of cell proliferation, differentiation, migration and apoptosis, and in the regulation of embryonic development	– Crouzon syndrome (CS) – Jackson-Weiss syndrome (JWS) – Apert syndrome (APRS) – Pfeiffer syndrome (PS) – Beare-Stevenson cutis gyrata syndrome (BSTVS) – Familial scaphocephaly syndrome (FSPC) – Lacrimo-auriculo-dento-digital syndrome (LADDS) – Antley-Bixler syndrome – Bent bone dysplasia syndrome (BBDS)	176943	Das et al. (2014) Tajiri et al. (2014) Su et al. (2014a, b)
MUTYH	1p34.1	– Involved in oxidative DNA damage repair – Initiates repair of A*oxoG to C*G by removing the inappropriately paired adenine base from the DNA backbone – Possesses both adenine and 2-OH-A DNA glycosylase activities	– Adenomas, multiple colorectal – Colorectal adenomatous polyposis	604933	Shinmura et al. (2011)

(continued)

Table 5.10 (continued)

Gene	Location	Function	Other-related diseases	OMIM	References
ERBB2	17q12	– Protein tyrosine kinase that is part of several cell surface receptor complexes, but that apparently needs a coreceptor for ligand binding – Essential component of a neuregulin-receptor complex, although neuregulins do not interact with it alone. GP30 is a potential ligand for this receptor – Regulates outgrowth and stabilization of peripheral microtubules (MTs)	– Adenocarcinoma of lung – Glioblastoma – Ovarian cancer – Breast cancer	164870	Li et al. (2015a, b, c, d) Bayrak et al. (2013) Lee et al. (2005a, b)
CDH1	16q22.1	– Cadherins are calcium-dependent cell adhesion proteins. They preferentially interact with themselves in a homophilic manner in connecting cells; cadherins may thus contribute to the sorting of heterogeneous cell types – CDH1 is involved in mechanisms regulating cell-cell adhesions, mobility and proliferation of epithelial cells – Has a potent invasive suppressor role	– Endometrial cancer (ENDMC) – Ovarian cancer (OC) – Breast cancer – Prostate cancer	192090	Yanjun et al. (2013) Corso et al. (2014) Jing et al. (2014)

(continued)

Table 5.10 (continued)

Gene	Location	Function	Other-related diseases	OMIM	References
BAX	19q13.33	– Accelerates programmed cell death by binding to, and antagonizing the apoptosis repressor BCL2 or its adenovirus homolog E1B 19k protein – Under stress conditions, undergoes a conformation change that causes translocation to the mitochondrion membrane, leading to the release of cytochrome c that then triggers apoptosis – Promotes activation of CASP3, and thereby apoptosis	– Colorectal cancer – T-cell acute lymphoblastic leukemia	600040	Wang et al. (2014a, b) Lv et al. (2012)
KRAS	12p12.1	– Ras proteins bind GDP/GTP and possess intrinsic GTPase activity – Plays an important role in the regulation of cell proliferation	– Bladder cancer – Breast cancer – Leukemia – Lung cancer – Pancreatic carcinoma	190070	Lu et al. (2015) Das et al. (2014)
RUNX3	1p36.11	– This gene encodes a member of the runt domain-containing family of transcription factors – A heterodimer of this protein and a beta subunit forms a complex that binds to the core DNA sequence 5′-PYGPYGGT-3′ found in a	– Testicular yolk sac tumor – Esophagus squamous cell carcinoma	600210	Wei et al. (2005) Liu et al. (2014a, b, c) Liu et al. (2014a, b, c)

(continued)

Table 5.10 (continued)

Gene	Location	Function	Other-related diseases	OMIM	References
		number of enhancers and promoters, and can either activate or suppress transcription – It also interacts with other transcription factors. It functions as a tumor suppressor, and the gene is frequently deleted or transcriptionally silenced in cancer			
PTEN	10q23.31	– Tumor suppressor – Acts as a dual-specificity protein phosphatase, dephosphorylating tyrosine-, serine- and threonine-phosphorylated proteins – Also acts as a lipid phosphatase. It functions as a tumor suppressor by negatively regulating AKT/PKB signaling pathway	– Cowden syndrome – Lhermitte-Duclos disease – Bannayan-Riley-Ruvalcaba syndrome – Squamous cell carcinoma of the head and neck – Endometrial cancer – Prostate cancer	601728	Wang et al. (2015a, b) Xu et al. (2014) Mina et al. (2012) Miller et al. (2005)
MSH2	2p21-p16	– Component of the post-replicative DNA mismatch repair system (MMR) – MutS alpha may also play a role in DNA homologous recombination repair	– Muir-torre syndrome – Mismatch repair cancer syndrome – Colorectal cancer	609309	Laitman et al. (2012) Fan et al. (2006)

(continued)

Table 5.10 (continued)

Gene	Location	Function	Other-related diseases	OMIM	References
		– In melanocytes may modulate both UV-B-induced cell cycle regulation and apoptosis			
DCC	18q21.2	– Receptor for netrin required for axon guidance – Mediates axon attraction of neuronal growth cones in the developing nervous system upon ligand binding – Its association with UNC5 proteins may trigger signaling for axon repulsion – It also acts as a dependence receptor required for apoptosis induction when not associated with netrin ligand – Implicated as a tumor suppressor gene	– Mirror movements 1 – familial congenital mirror movements	120470	Hibi et al. (2010) Wang et al. (1999) Kataoka et al. (2000) Kataoka et al. (1995)
PIK3CA	3q26.32	– PI 3-Kinases (phosphoinositide 3-kinases, PI 3-Ks) are a family of lipid kinases capable of phosphorylating the 3'OH of the inositol ring of phosphoinositides – They are responsible for coordinating a diverse range of cell functions including proliferation and survival	– Breast cancer – CLOVE syndrome – Colorectal cancer – Cowden syndrome 5 – Hepatocellular carcinoma – Nonsmall cell lung cancer – Ovarian cancer	171834	Zhang et al. (2014a, b, c, d, e, f) Li et al. (2005a, b) Yoshino et al. (2012)

(continued)

Table 5.10 (continued)

Gene	Location	Function	Other-related diseases	OMIM	References
CDKN1B	12p13.1	– Important regulator of cell cycle progression. Involved in G1 arrest – Potent inhibitor of cyclin E- and cyclin A-CDK2 complexes – Forms a complex with cyclin type D-CDK4 complexes and is involved in the assembly, stability, and modulation of CCND1-CDK4 complex activation – Acts either as an inhibitor or an activator of cyclin type D-CDK4 complexes depending on its phosphorylation state and/or stoichometry	– Multiple endocrine neoplasia	600778	Wei et al. (2011) Zheng et al. (2005) Al-Moundhri (2005)
VEGFA	6p21.1	– Growth factor active in angiogenesis, vasculogenesis and endothelial cell growth – Induces endothelial cell proliferation, promotes cell migration, inhibits apoptosis and induces permeabilization of blood vessels	– Microvascular complications of diabetes 1	192240	Cao et al. (2010) Angelescu et al. (2013)
MMP2	16q12.2	– Matrix metalloproteases (MMPs), also called matrixins, are zinc-dependent endopeptidases and the major proteases in ECM degradation	– Multicentric osteolysis, nodulosis, and arthropathy (MONA)	120360	Ji et al. (2005) Zhang et al. (2004) Miao et al. (2003)

(continued)

Table 5.10 (continued)

Gene	Location	Function	Other-related diseases	OMIM	References
		– MMPs are capable of degrading several extracellular molecules and a number of bioactive molecules			
CDH17	8q22.1	– Cadherins are calcium-dependent cell adhesion proteins – They preferentially interact with themselves in a homophilic manner in connecting cells; cadherins may thus contribute to the sorting of heterogeneous cell types – LI-cadherin may have a role in the morphological organization of liver and intestine – Involved in intestinal peptide transport	– Epithelial predominant wilms' tumor – Hepatocellular carcinoma	603017	Qiu et al. (2013) Lee et al. (2010) Zhang et al. (2011a, b)
TOP2A	17q21-q22	– Topoisomerases are ubiquitously expressed enzymes that overcome topological problems in genomic DNA, which can result from DNA replication, transcription and repair – Common problems such as knots and tangles are often resolved by topoisomerases	– Cervix endometriosis – Breast adenoid cystic carcinoma	126430	Liang et al. (2008) Miura et al. (2014)

(continued)

Table 5.10 (continued)

Gene	Location	Function	Other-related diseases	OMIM	References
KLF4	9q31	– Transcription factor; can act both as activator and as repressor – The encoded zinc finger protein is required for normal development of the barrier function of skin. – The encoded protein is thought to control the G1-to-S transition of the cell cycle following DNA damage by mediating the tumor suppressor gene p53	– Venous hemangioma – Skin squamous cell carcinoma	602253	Ji et al. (2013) Hsu et al. (2013) Cho et al. (2007)
SIRT1	10q21.3	– The functions of human sirtuins have not yet been determined; however, yeast sirtuin proteins are known to regulate epigenetic gene silencing and suppress recombination of rDNA – Studies suggest that the human sirtuins may function as intracellular regulatory proteins with mono-ADP-ribosyltransferase activity	– Xeroderma pigmentosum, group d – Tauopathy	604479	Li et al. (2016a, b) Yang et al. (2013a, b, c, d, e, f, g) Kim et al. (2010a, b)

(continued)

Table 5.10 (continued)

Gene	Location	Function	Other-related diseases	OMIM	References
MYC	8q24.21	– Transcription factor that binds DNA in a non-specific manner, yet also specifically recognizes the core sequence 5-CAC [GA] TG-3 – Activates the transcription of growth-related genes	– Burkitt lymphoma – Myoclonus, familial cortical	190080	Zhang et al. (2010a, b, c, d) de Souza et al. (2013)
FGFR4	5q35.2	– These receptor proteins play a role in important processes such as cell division, regulating cell growth and maturation, formation of blood vessels, wound healing, and embryo development	– Prostate cancer (PC)	134935	Shoji et al. (2015) Liang et al. (2014)
MUC5B	11p15.5	– Gel-forming mucin that is thought to contribute to the lubricating and viscoelastic properties of whole saliva and cervical mucus	– Pulmonary fibrosis	600770	Leteurtre et al. (2006) Perrais et al. (2001)
FAT4	4q28.1	– The precise function of the FAT4 protein is largely unknown; however, research shows that the FAT4 protein is likely involved in determining the position of various components within cells (cell polarity) – The FAT4 protein is also thought to function as a tumor suppressor	– Head and neck squamous cell carcinoma – Melanoma – Hepatocellular carcinoma – Hennekam syndrome	612411	Cai et al. (2015) Lin et al. (2015)

(continued)

Table 5.10 (continued)

Gene	Location	Function	Other-related diseases	OMIM	References
MSI2	17q22	– RNA binding protein that regulates the expression of target mRNAs at the translation level – May play a role in the proliferation and maintenance of stem cells in the central nervous system	– Chronic myeloid leukemia	607897	Emadi-Baygi et al. (2013)
IL1RN	2q14.2	– Inhibits the activity of interleukin-1 by binding to receptor IL1R1 and preventing its association with the coreceptor IL1RAP for signaling – Has no interleukin-1 like activity. Binds functional interleukin-1 receptor IL1R1 with greater affinity than decoy receptor IL1R2; however, the physiological relevance of the latter association is unsure	– Interleukin 1 receptor antagonist deficiency – Microvascular complications of diabetes 4	147679	Camargo et al. (2006) El-Omar et al. (2000)

microRNAs (miRNAs) may be utilized as the potential biomarkers for the diagnosis and prognosis of a range of diseases such as cancer (Qiaoqiong et al. 2011, Tian et al. 2014). Here, we have highlighted the miRNAs which their expression profiles are cognate with GC phenotypes. Also, biological activity and target molecules of these diminutive biomolecules have been reported (Table 5.11).

5.5 Esophageal Cancer

Esophageal cancer is the eighth more common cancer worldwide that occurs mostly in men. Unfortunately, due to late diagnosis of this cancer, it is often fatal (Holmes and Vaughan 2007). Environmental factors such as tobacco smoke, gastrointestinal esophageal reflux disease and genetic changes are involved in the development of this cancer. Regardless of whether cancer occurs sporadically in an individual or frequently in members of the family as a familial trait, it is a genetic disease (Deere 2007). Different types of genes play role in the cancer initiation including genes encoding proteins of signaling pathways, cytoskeleton components involved in maintaining contact inhibition, regulating of mitotic cycle, programmed cell death machinery components and repair proteins. After the onset of cancer, additional genetic damages due to epigenetic alterations, genes mutations and chromosomal instability will be accumulated and cancer will be evolved. Different types of mutations are responsible for causing this cancer which include gain of functional mutations in proto-oncogenes, loss of functional mutations in tumor suppressor genes, and chromosomal translocation (LeVea 2015). Hence, we summarized the most important and reported genes involved in esophageal cancer in Table 5.12.

5.5.1 Genetic Etiology

See Table 5.12.

5.5.2 Micro RNAs and Esophageal Cancer

Micro-ribonucleic acids (micro RNA) are the non-coding ribonucleic acids that are evolutionary conserved and has a length of 18–25 nucleotides. Micro-RNAs control the gene expression through degradation or translation inhibition of mRNA after transcription. These molecular structures participate in the control of physiological and pathological procedures and can act as oncogenes or tumor inhibitors. Therefore, mutations in the open reading frames could lead to cancer. Identifying of micro RNA and target molecules has provided a clear horizon to recognize the routes that lead to cancer. Till now, many miRNAs are shown to be functionally

Table 5.11 miRNAs which reported to be effective on Gastric Cancer. NPA (non-predicted target)

miRNAs	Targets	miRNA functions	Main effect	References
miR-21	PTEN	– Increase cell proliferation – Decrease apoptosis	Oncogenic	Yang et al. (2013a, b, c, d, e, f, g)
miR-146a	WASF2	– Inhibit cell migration – Inhibit cell invasion	Tumor-suppressive	Yao et al. (2013)
miR-9	CCND1 ETS1	– Inhibit cell proliferation – Inhibit cell invasion – Inhibit metastasis	Tumor-suppressive	Zheng et al. (2013), Gao et al. (2017)
miR-296-5p	CDX1	– Promote cell growth – Inhibit apoptosis – Change cell cycle distribution	Oncogenic	Li et al. (2014a, b, c, d)
miR-301a	RUNX3	– Promote cell growth – Promote soft agar clonogenicity – Promote cell migration – Promote cell invasion – Decrease apoptosis induced by cisplatin – Promote the percentage of G2/M phase cells	Oncogenic	Wang et al. (2013a, b, c, d)
miR-17-5p	CDKN1A TP53INP1	– Promotes cell cycle progression – Inhibits apoptosis	Oncogenic	Wang et al. (2013a, b, c, d)
miR-20a	CDKN1A TP53INP1	– Promote cell cycle progression – Inhibit apoptosis	Oncogenic	Wang et al. (2013a, b, c, d)
miR-133b	FGFR1 (2260)	– Inhibit cell proliferation – Inhibti colony formation	Tumor-suppressive	Wen et al. (2013)
miR-34a	NPA	– Reduce cell viability – Inhibit cell proliferation – Induce apoptosis – Inhibit cell migration	Tumor-suppressive	Cao et al. (2013a, b)
miR-372	TNFAIP1	– Apoptosis – Cell growth	Oncogenic	Zhou et al. (2013a, b)
miR-27a	NPA	– Promote cell growth	Oncogenic	Liu et al. (2013)
miR-149	ZBTB2	– Inhibit cell proliferation – Inhibit cell cycle progression	Tumor-suppressive	Wang et al. (2012a, b, c, d, e, f, g)

(continued)

Table 5.11 (continued)

miRNAs	Targets	miRNA functions	Main effect	References
miR-125b	NPA	– Increase cell proliferation – Inhibit apoptosis	Oncogenic	Yang et al. (2013a, b, c, d, e, f, g)
miR-101	COX2	– Inhibit cell proliferation – Induce apoptosis – Inhibit tumor growth	Tumor-suppressive	He et al. (2012)
miR-29c	MCL1	– Induce apoptosis	Tumor-suppressive	Saito et al. (2013)
miR-146a	CARD10 COPS8	– Inhibit NF-kB signaling	NPA	Crone et al. (2012)
miR-144	ZFX	– Increae 5-fu sensitivity	NPA	Akiyoshi et al. (2012)
miR-544	IRX1	– Promote cell proliferation – Promote cell cycle progression	Oncogenic	Zhi et al. (2013)
miR-181b	NPA	– Increase cell proliferation – Increase cell migration – Increase cell invasion – Induce apoptosis	Oncogenic	Guo et al. (2012)
miR-199a	SMAD4	– Block TGF-beta signaling – Inhibit cell growth arrest – Inhibit apoptosis	Oncogenic	Zhang et al. (2012a, b, c, d)
miR-146a	L1CAM	– Inhibit cell invasion – Inhibit metastasis	Tumor-suppressive	Hou et al. (2012a, b)
miR-574-3p	CUL2	– Inhibit cell proliferation – Inhibit cell migration – Inhibit cell invasion	Tumor-suppressive	Su et al. (2012)
miR-625	ILK	– Inhibit cell invasion – Inhibit metastasis	Tumor-suppressive	Wang et al. (2012a, b, c, d, e, f, g)
miR-7	IGF1R	– Reduce cell migration – Reduce cell invasion	Tumor-suppressive	Zhao et al. (2013a, b)
miR-181a	KLF6	– Promote cell proliferation – Promote colony formation – Promote cell migration – Promote cell invasion – Inhibit apoptosis	Oncogenic	Zhang et al. (2012a, b, c, d)
miR-181b	CREB1	– Inhibit cell proliferation – Inhibit colony formation	Tumor-suppressive	Chen et al. (2012a, b, c)
miR-223	STMN1	NPA	NPA	Kang et al. (2012)

(continued)

Table 5.11 (continued)

miRNAs	Targets	miRNA functions	Main effect	References
miR-495	PTP4A3	– Inhibit cell migration – Inhibit cell invasion	Tumor-suppressive	Li et al. (2012a, b, c, d, e)
miR-551a	PTP4A3	– Inhibit cell migration – Inhibit cell invasion	Tumor-suppressive	Li et al. (2012a, b, c, d, e)
miR-21	SERPINI1	NPA	Oncogenic	Yamanaka et al. (2012)
miR-101	NPA	– Cause E-cadherin dysfunction	NPA	Carvalho et al. (2012)
miR-124	SPHK1	– Inhibit cell proliferation – Inhibit tumorigenicity – Inhibit AKT signaling	Tumor-suppressive	Xia et al. (2012a, b, c)
miR-155	SMAD2	– Inhibit cell migration – Inhibit cell invasion – Reduce cell adhesion	Tumor-suppressive	Li et al. (2012a, b, c, d, e)
miR-196a	NPA	– Promote epithelial-mesenchymal transition – Increase cell migration – Increase cell invasion	Oncogenic	Tsai et al. (2012)
miR-409-3p	PHF10	– Inhibit cell proliferation – Induce cell apoptosis	Tumor-suppressive	Li et al. (2012a, b, c, d, e)
miR-145	CDH2	– Inhibit cell migration – Inhibit cell invasion – Inhibit metastasis – Impair local invasion	Tumor-suppressive	Gao et al. (2013a, b)
miR-196a	CDKN1B	– Increase cell cycle progression – Increase cell proliferation – Increase colony formation – Increase tumor growth	Oncogenic	Sun et al. (2012a, b)
miR-182	CREB1	– Inhibit cell proliferation – Inhibit colony formation	Tumor-suppressive	Kong et al. (2012)
miR-125a-3p	NPA	– Inhibit cell proliferation	Tumor-suppressive	Hashiguchi et al. (2012)
miR-222	RECK	– Promote cell proliferation – Promote colony formation	Oncogenic	Li et al. (2012a, b, c, d, e)

(continued)

Table 5.11 (continued)

miRNAs	Targets	miRNA functions	Main effect	References
miR-200b	NPA	– Change the cell morphology from – Fibroblast– to epithelial-like – Inhibit cell proliferation – Inhibit cell migration – Inhibit cell invasion	Tumor-suppressive	Kurashige et al. (2012)
miR-135a	JAK2	– Reduce cell proliferation – Reduce colony formation	Tumor-suppressive	Wu et al. (2012a, b, c)
miR-196b	NPA	– Increase cell migration – Increase cell invasion	Oncogenic	Liao et al. (2012)
miR-10b	HOXD10	– Increase cell invasion	Oncogenic	Liu et al. (2012a, b, c)
miR-223	FBXW7	– Inhibit apoptosis – Increase cell proliferation – Increase cell invasion	Oncogenic	Li et al. (2012a, b, c, d, e)
miR-21	PTEN	– Promote cell growth – Promote cell invasion – Promote cell migration	Oncogenic	Zhang et al. (2012a, b, c, d)
miR-610	VASP	– Reduce cell migration – Reduce cell invasion	Tumor-suppressive	Wang et al. (2012a, b, c, d, e, f, g)
miR-409-3p	RDX	– Reduce cell migration – Reduce cell invasion – Reduce distal pulmonary metastases – Reduce peritoneal dissemination	Tumor-suppressive	Zheng et al. (2012)
miR-148a	NPA	– Inhibit cell proliferation	Tumor-suppressive	Zhu et al. (2012)
miR-494	KIT	– Induce apoptosis – Inhibit cell growth – Affect cell cycle progression	Tumor-suppressive	Kim et al. (2011)
miR-146a	SMAD4	– Increase cell proliferation – Inhibit apoptosis	Oncogenic	Xiao et al. (2012)
miR-27	APC	– Promote metastasis – Promote pithelial-mesenchymal transition	Oncogenic	Zhang et al. (2011a, b)
miR-29a	SAPCD2	– Inhibit cell proliferation – Induce cell cycle arrest	Tumor-suppressive	Cui et al. (2011)
miR-191	NDST1	– Promote cell growth	Oncogenic	Shi et al. (2011)

(continued)

Table 5.11 (continued)

miRNAs	Targets	miRNA functions	Main effect	References
		– Inhibit apoptosis		
miR-9	NPA	– Inhibit cell proliferation – Inhibit cell migration – Inhibit cell invasion	Tumor-suppressive	Tsai et al. (2011)
miR-194	NPA	– Inhibit cell invasion	Tumor-suppressive	Song et al. (2012), Zhang et al. (2017)
miR-335	BCL2L2 SP1	– Inhibit cell invasion – Reduce metastasis	Tumor-suppressive	(Xu et al. 2012a, b)
miR-214	PTEN	– Reduce cell cycle G1 arrest	Oncogenic	Xiong et al. (2011)
miR-429	MYC	– Reduce cell viability – Inhibit cell proliferation – Inhibit cell attachment	Tumor-suppressive	Sun et al. (2011a, b)
miR-370	TGFBR2	– Promote cell migration – Promote cell oncogenic potential – Promote abdominal metastatic dissemination	Oncogenic	Lo et al. (2012)
miR-146a	EGFR IRAK1	– Inhibit cell migration – Inhibit cell invasion	Tumor-suppressive	Kogo et al. (2011)
miR-27a	CCND1	– Inhibit cell proliferation	Tumor-suppressive	Zhao et al. (2011a, b, c)
miR-148a	CDKN1B	– Promote cell proliferation – Promote cell cycle progression	Oncogenic	Guo et al. (2011a, b)
let-7f	MYH9	– Inhibit cell invasion – Inhibit cell migration – Inhibit metastasis	Tumor-suppressive	Liang et al. (2011)
miR-622	ING1	– Promote invasion – Promote tumorigenesis – Promote metastasis	Oncogenic	Guo et al. (2011a, b)
miR-449	GMNN MET CCNE2 SIRT1	– Inhibit cell proliferation – Promote apoptosis – Promote senescence	Tumor-suppressive	Kheir et al. (2011)
miR-146a	NPA	– Inhibit cell proliferation – Induce apoptosis	Tumor-suppressive	Hou et al. (2012a, b)
miR-126	SOX2	– Promote cell growth	Oncogenic	Otsubo et al. (2011)

(continued)

Table 5.11 (continued)

miRNAs	Targets	miRNA functions	Main effect	References
miR-107	CDK6	– Inhibit cell proliferation – Induce cell cycle G1 arrest – Inhibit cell invasion	Tumor-suppressive	Feng et al. (2012a, b, c)
miR-125a-5p	ERBB2	– Inhibit cell proliferation	Tumor-suppressive	Nishida et al. (2011)
miR-192	ALCAM	– Increase cell growth – Increase cell migration	Oncogenic	Xu et al. (2011)
miR-212	MYC	NPA	NPA	Xu et al. (2011)
miR-199a	MAP3K11	– Increase cell proliferation – Increase cell migration – Increase cell invasion	Oncogenic	Song et al. (2010)
miR-23a	IL6R	– Promote cell proliferation	Oncogenic	Zhu et al. (2010)
miR-126	CRK	– Inhibit cell growth – Induce cell cycle G0/G1 arrest – Inhibit cell migration – Inhibit cell invasion – Inhibit tumorigenicity – Inhibit metastasis	Tumor-suppressive	Feng et al. (2010)
miR-221	PTEN	– Promote cell growth – Promote cell invasion – Reduce radiosensitivity	Oncogenic	Chun-zhi et al. (2010), Shi et al. (2017)
miR-222	PTEN	– Promote cell growth – Promote cell invasion – Reduce radiosensitivity	Oncogenic	Chun-zhi et al. (2010)
miR-375	JAK2	– Inhibit cell proliferation	Tumor-suppressive	Ding et al. (2010)
miR-331-3p	E2F1	– Block cell cycle G1/S transition – Reduce colony formation – Reduce cell growth	Tumor-suppressive	Guo et al. (2010)
miR-650	ING4	– Promote tumorigenesis – Promote cell proliferation	Oncogenic	Zhang et al. (2010a, b, c, d)
miR-9	NFKB1	– Inhibit cell growth – Inhibit tumor growth	Tumor-suppressive	Wan and Liu (2010)
miR-181c	NOTCH4 KRAS	– Decrease cell growth	Tumor-suppressive	Hashimoto et al. (2010)
miR-150	EGR2	– Promote tumorigenesis – Promote cell proliferation	Oncogenic	Wu et al. (2010a, b)

(continued)

Table 5.11 (continued)

miRNAs	Targets	miRNA functions	Main effect	References
miR-212	MECP2	– Decrease cell growth	Tumor-suppressive	Wada et al. (2010)
miR-451	MIF	– Reduce cell proliferation – Increase radiotherapy	Tumor-suppressive	Bandres et al. (2009)
miR-106b	CDKN1A	– Increase cell cycle G1/S transition	Oncogenic	Kim et al. (2009)
miR-221	CDKN1A CDKN1C	– Increase cell cycle G1/S transition – Increase tumor growth	Oncogenic	Kim et al. (2009)
miR-222	CDKN1A CDKN1C	– Increase cell cycle G1/S transition – Increase tumor growth	Oncogenic	Kim et al. (2009)
miR-25	CDKN1C	– Increase cell cycle G1/S transition	Oncogenic	Kim et al. (2009)
miR-34	BCL2	– Inhibit cell growth – Induce cell cycle G1 arrest – Inhibit tumorshphere formation and growth	Tumor-suppressive	Ji et al. (2008), Jafari and Abediankenari (2017)
miR-15b	BCL2	– Increase drug sensitivity – Reduce multidrug resistance – Promote VCR-induced apoptosis	Tumor-suppressive	Xia et al. (2008)
miR-16	BCL2	– Increase drug sensitivity – Reduce multidrug resistance – Promote VCR-induced apoptosis	Tumor-suppressive	Xia et al. (2008)
miR-106b ~ 25	E2F1	– Impair the TGFbeta tumor suppressor pathway	NPA	Petrocca et al. (2008)

Table 5.12 Genetic pathology of Esophageal Cancer

Gene/locus	Location	Function	Other related disease	OMIM	References
TGFBR2	3p24.1	– Transduces the TGFB1, TGFB2 and TGFB3 signal from the cell surface to the cytoplasm – It thus regulates cell cycle arrest, control of mesenchymal cell proliferation and differentiation, wound healing, extracellular matrix production, immunosuppression and carcinogenesis	Hereditary non-polyposis colorectal cancer 6 Loeys-Dietz syndrome 2	190182	Jin et al. (2008)
DLEC1	3p22.2	– May act as a tumor suppressor by inhibiting cell proliferation	Lung cancer	604050	Zhang et al. (2009a, b, c)
LZTS1	8p21.3	– Involved in the regulation of cell growth – Contribute to the regulation of the cell cycle and the prevention of uncontrolled cell proliferation – May act as a tumor suppressor	Prostate cancer	606551	Vecchione et al. (2007)
DEC1	9q33.1	– Candidate tumor suppressor	Squamous cell carcinoma of the head and neck	604767	Xu et al. (2012a, b)
RNF6	13q12.13	– Mediating 'Lys-48'-linked polyubiquitination of LIMK1 and its subsequent targeting to the proteasome for degradation – Negatively regulates axonal outgrowth through regulation of the LIMK1 turnover. – Modulating its transcriptional activity – May also bind DNA and function as a transcriptional regulator		604242	Lo et al. (2002)

(continued)

Table 5.12 (continued)

Gene/locus	Location	Function	Other related disease	OMIM	References
WWOX	16q23.1-q23.2	– Putative oxidoreductase – Acts as a tumor suppressor and plays a role in apoptosis – Required for normal bone development (By similarity) – May function synergistically with p53/TP53 to control genotoxic stress-induced cell death – Plays a role in TGFB1 signaling and TGFB1-mediated cell death – May also play a role in tumor necrosis factor (TNF)-mediated cell death – Inhibits Wnt signaling, probably by sequestering DVL2 in the cytoplasm	Spinocerebellar ataxia Epileptic encephalopathy	605131	Kuroki et al. (2002)
DCC	18q21.2	– Tumor suppressor gene	Mirror movements 1 Colorectal cancer	120470	Lui Park et al. (2008)
TP53	17p13.1	– A tumor suppressor protein – Involved in cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism	Li-Fraumeni syndrome Squamous cell carcinoma of the head and neck Lung cancer Papilloma of choroid plexus Adrenocortical carcinoma Basal cell carcinoma 7	191170	Bellini et al. (2012)
EGFR	7p12	– The protein encoded by this gene is a transmembrane glycoprotein that is a member of the protein kinase superfamily – Binding of the protein to a ligand induces receptor dimerization and tyrosine autophosphorylation and leads to cell proliferation	Adenocarcinoma of lung Nonsmall cell lung cancer Inflammatory skin and bowel disease	131550	Ekman et al. (2007)

(continued)

Table 5.12 (continued)

Gene/locus	Location	Function	Other related disease	OMIM	References
ALDH2	12q24.2	– The second enzyme of the major oxidative pathway of alcohol metabolism	Acetaldehyde dehydrogenase deficiency	100650	Fang et al. (2011)
PTGS2	1q31.1	– Key enzyme in prostaglandin biosynthesis, and acts both as a dioxygenase and as a peroxidase	Bladder cancer Colorectal cancer Colorectal neoplasia Diabetes mellitus Lung cancer	600262	Shao et al. (2015)
MET	7q31	– Binds to hepatocyte growth factor/HGF ligand and regulates many physiological processes including proliferation, scattering, morphogenesis and survival	Hepatocellular carcinoma gastric cancer Renal cell carcinoma papillary Deafness	164860	Ozawa et al. (2015)
CDKN1A	6p21.2	– Binds to and inhibits the activity of cyclin-CDK2 or -CDK4 complexes, and thus functions as a regulator of cell cycle progression at G1	Breast cancer Multiple endocrine neoplasia 1 Systemic lupus erythematosus	116899	Lin et al. (2010a, b, c)
CD9	12p13.3	– The encoded protein functions in many cellular processes including differentiation, adhesion, and signal transduction – Expression of this gene plays a critical role in the suppression of cancer cell motility and metastasis		143030	Huan et al. (2015)
ADH1B	4q23	– Metabolizes a wide variety of substrates, including ethanol, retinol, other aliphatic alcohols, hydroxysteroids, and lipid peroxidation products	Reduced alcohol metabolism	103720	Gao et al. (2013a, b)

(continued)

Table 5.12 (continued)

Gene/locus	Location	Function	Other related disease	OMIM	References
PIK3CA	3q26.3	– The protein encoded by this gene represents the catalytic subunit, which uses ATP to phosphorylate PtdIns, PtdIns4P and PtdIns (4,5)P2 – This gene has been found to be oncogenic	Megalencephaly-capillary malformation Colorectal cancer Breast cancer Ovarian cancer Hepatocellular carcinoma, Keratosis, Congenital lipomatous overgrowth, vascular malformations, Epidermal nevi Cowden syndrome 5	171834	Baba et al. (2015)
PLCE1	10q23	– Involved in generating of two second messengers: inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG) and subsequently regulate various processes affecting cell growth, differentiation, and gene expression	Nephrotic syndrome, type 3 Glomerulosclerosis Gastric adenocarcinoma	608414	Guo et al. (2014)
FGF4	11q13.3	– Possesses broad mitogenic and cell survival activities and – Involved in a variety of biological processes including embryonic development, cell growth, morphogenesis, tissue repair, tumor growth and invasion		164980	Arai et al. (2003)
NOTCH1	9q34.3	– Play a role in a variety of developmental processes by controlling cell fate decisions – Regulates interactions between physically adjacent cells – This protein functions as a receptor for membrane bound ligands, and may play multiple roles during development	Adams-Oliver syndrome 5 Aortic valve disease 1	190198	Mandasari et al. (2016)

(continued)

Table 5.12 (continued)

Gene/locus	Location	Function	Other related disease	OMIM	References
CTTN	11q13	– Regulating the interactions between components of adherens-type junctions – Organizing the cytoskeleton and cell adhesion structures of epithelia and carcinoma cells – During apoptosis, the encoded protein is degraded in a caspase-dependent manner	Head and Neck Cancers Breast Cancer Oral Cavity Cancer	164765	Zhang et al. (2014a, b, c, d, e, f)
COL4A5	Xq22	– Type IV collagen is the major structural component of glomerular basement membranes (GBM), forming a 'chicken-wire' meshwork together with laminins, proteoglycans and entactin/nidogen	Alport syndrome	303630	Oohashi et al. (2011)
RB1	13q14.2	– A negative regulator of the cell cycle – First tumor suppressor gene found – The encoded protein also stabilizes constitutive heterochromatin to maintain the overall chromatin structure – The active, hypophosphorylated form of the protein binds transcription factor E2F1	Childhood cancer retinoblastoma Bladder cancer Osteosarcoma Lung cancer	614041	Müller et al. (2014)
CYP2A6	19q13.2	– Exhibits a high coumarin 7-hydroxylase activity – Can act in the hydroxylation of the anti-cancer drugs cyclophosphamide and ifosphamide – Competent in the metabolic activation of aflatoxin B1 – Constitutes the major nicotine C-oxidase – Acts as a 1,4-cineole 2-exo-monooxygenase – Possesses low phenacetin O-deethylation activity	Lung Cancer Colorectal Cancer Coumarin resistance Nicotine addiction	122720	Rossini et al. (2007)
SOX2	3q26.33	– Transcription factor that forms a trimeric complex with OCT4 on DNA and controls the expression of a number of genes involved in embryonic development such as YES1, FGF4, UTF1 and ZFP206 (By similarity)	Lung Cancer Brain Tumours Breast Cancer Microphthalmia	184429	Gao et al. (2015)

(continued)

Table 5.12 (continued)

Gene/locus	Location	Function	Other related disease	OMIM	References
SPARC	5q33.1	– This gene encodes a cysteine-rich acidic matrix-associated protein – The encoded protein is required for the collagen in bone to become calcified – Also involved in extracellular matrix synthesis and promotion of changes to cell shape	Osteogenesis imperfecta, type XVII	182120	Brabender et al. (2003)
CRP	1q23.2	– The protein encoded by this gene belongs to the pentaxin family – It is involved in several host defense related functions based on its ability to recognize foreign pathogens and damaged cells of the host and to initiate their elimination by interacting with humoral and cellular effector systems in the blood. Consequently, the level of this protein in plasma increases greatly during acute phase response to tissue injury, infection, or other inflammatory stimuli	Systemic lupus erythematosus Malaria Myocardial infarction	123260	Motoyama et al. (2013)

related to cancer initiation, development and therapy. To facilitate the study on cancer-related miRNAs, we provide a table aiming at annotating the experimentally verified oncogenic and tumor-suppressive miRNAs from literature (Patnaik et al. 2010; Gu and Wu 2011). This Table only presents items having direct functional evidences including: (1) the miRNA which regulates at least one cancer-related phenotype or cellular process (such as proliferation, apoptosis, migration and invasion, senescence and cell cycle regulation); or (2) the miRNA that directly regulates at least one oncogenic or tumor-suppressive gene. It may be a useful resource for both the computational analysis and the experimental study on miRNA regulations in cancer (Table 5.13).

Table 5.13 MicroRNA expression profiles reported in esophageal cancer

miRNAs	Target(s)	Function	Effect	References
miR-200c	NPA	– Reduce cisplatin sensitivity – Reduce cisplatin-induced. Apoptosis – Promote Akt signaling	NPA	Hamano et al. (2011)
miR-593	PLK1	– Reduce cell proliferation – Induce cell cycle G2/M arrest	Tumor-suppressive	Ito et al. (2011)
miR-25	BCL2L11	– Promote cell proliferation – Promote cell cycle progression – Inhibit apoptosis	Oncogenic	Kan et al. (2009)
miR-106b	CDKN1A	– Promote cell proliferation – Promote cell cycle progression – Inhibit apoptosis	Oncogenic	Kan et al. (2009)
miR-93	CDKN1A	– Promote cell proliferation – Promote cell cycle progression – Inhibit apoptosis	Oncogenic	Kan et al. (2009)
miR-196a	ANXA1	– Promote cell proliferation – Increase anchorage-independent growth – Inhibit apoptosis	Oncogenic	Luthra et al. (2008)
miR-451	NPA	– Induce apoptosis – Inhibit colony formation – Inhibit cell proliferation – Inhibit cell invasion – Inhibit metastasis	Tumor-suppressive	Wang et al. (2013a, b, c, d)

(continued)

Table 5.13 (continued)

miRNAs	Target(s)	Function	Effect	References
miR-223	ARTN	– Inhibit cell migration – Inhibit cell invasion	Tumor-suppressive	Zhang et al. (2011a, b)
miR-143	MAPK7	– Inhibit cell growth – Induce apoptosis – Inhibit cell migration – Inhibit cell invasion	Tumor-suppressive	Ni et al. (2013)
miR-210	FGFRL1	– Inhibit cell survival – Inhibit cell proliferation	Tumor-suppressive	Tsuchiya (2011)
miR-150	ZEB1	– Inhibit epithelial-mesenchymal-transition – Inhibit tumor growth	Tumor-suppressive	Yokobori et al. (2013)
miR-518b	RAP1B	– Inhibit cell proliferation – Induce apoptosis – Inhibit cell invasion	Tumor-suppressive	Zhang et al. (2011a, b)
miR-203	LASP1	– Inhibit cell migration – Inhibit cell invasion	Tumor-suppressive	Takeshita et al. (2012)
miR-133a	CD47	– Inhibit tumorigenesis	Tumor-suppressive	Suzuki et al. (2012a, b)
miR-25	CDH1	– Promote cell migration – Promote cell invasion	Oncogenic	Qu et al. (2012)
let-7	NPA	– Suppress cell proliferation	Tumor-suppressive	Liu et al. (2012a, b, c)
miR-29c	CCNE1	– Induce cell cycle G1/G0 arrest – Inhibit cell proliferation	Tumor-suppressive	Ding et al. (2011)
miR-205	ZEB2	– Inhibit cell invasion – Inhibit cell migration	Tumor-suppressive	Matsushima et al. (2011)
miR-203	TP63	– Inhibit cell proliferation	Tumor-suppressive	Yuan et al. (2011)
miR-141	YAP1	– Increase cell viability	Oncogenic	Imanaka et al. (2011), Xue et al. (2017)
miR-19a	TNFA	– Inhibit apoptosis – Promote tumor growth	Oncogenic	Liu et al. (2011)
miR-92a	CDH1	– Promote cell migration promote cell invasion	Oncogenic	Chen et al. (2011a, b, c)
miR-210	FGFRL1	– Inhibit cell survival – Inhibit cell proliferation – Induce cell death – Induce cell cycle G1/G0 and G2/M arrest	Tumor-suppressive	Tsuchiya et al. (2011)
miR-10b	KLF4	– Increase cell motility – Increase cell invasion	Oncogenic	Tian et al. (2010)
miR-373	LATS2	– Promote cell growth	Oncogenic	Lee et al. (2009)
miR-21	PDCD4	– Increase cell proliferation – Increase cell invasion	Oncogenic	Hiyoshi et al. (2009)

NPA (non-predicted target)

5.6 Lymphoma and Leukemia

5.6.1 Lymphoma

Lymphoma is a neoplasm that is developed in infection-fighting cells of the immune system, known as lymphocytes. The location of these cells are the lymph nodes, spleen, thymus, bone marrow, and other parts of the body (Müller-Hermelink et al. 2001). In lymphoma, lymphocytes change and grow out of control. There are two main types of lymphoma including Non-Hodgkin (most people are affected with this type) and Hodgkin (Shankland et al. 2012. There is a bimodal incidence of lymphoma with respect to age at diagnosis. Hodgkin lymphoma is a disease of young adulthood with a median age at diagnosis of 38. Approximately 12% of cases occur in individuals less than 20 years of age. A second peak of Hodgkin lymphoma occurs later in life. The most prevalent lymphomas are non-Hodgkin lymphomas, which are diagnosed at an average age of 67 Years Stewart and Wild (2014).

Chromosomal translocations are the most conspicuous genetic defects in lymphoma cells. These genetic alterations arise as a consequence of a high rate of gene recombination that is intrinsically cognate to the ontogeny of cells in the lymphoid lineage. Presumably, genes near the common breakpoints are deregulated as a result of their translocation. Many of the genes that drive these recurrent translocations remain unknown. In several cases, the genes that have been identified are unique to lymphoid cancers and are not, apparently, altered in other tumor types (KuÈppers and Dalla-Favera 2001; Vega and Medeiros 2003; Shankland et al. 2012, Zheng 2013). Here, we have summarized the key genes and their mechanisms in pathogenesis of lymphoma (Table 5.14).

5.6.2 Leukemia

The initiation of the cancer is related to an abnormal behavior in one cell of the body. There is millions of cells in our body which are distributed in tissues of organs (blood, muscles, bones, liver, lungs and etc.). Each cell contains genes involved in growth, regeneration and are responsible for death of cell. Typically, cells follow the specified manner and regulations and thereby maintain the health of the body. Cells sometimes miss-behave that leads to unusual and uncontrollable growth and proliferation. In most organs, these abnormal cells form solid masses known as the tumor. However, abnormal cells of the immune system or blood, are

Table 5.14 The most reported genes involved in pathogenesis of different types of Lymphoma

Gene	Location	Molecular pathology	Function	Phenotype	Other related disease	OMIM	References
BCL10	1p22	somatic mutations, t (1;14)(p22; q32)	– Involved in adaptive immune response – Promotes apoptosis, pro-caspase-9 maturation and activation of NF-kappa-B via NIK and IKK	Mucosa-associated lymphoid tissue (MALT) lymphomas	mmunodeficiency 37, Mesothelioma, Male germ cell tumor, Sezary syndrome	603517	Torres et al. (2014)
BCL2	18q21.33	t(8;14) and t (14;18)	– Suppresses apoptosis in a variety of cell systems including factor-dependent lymphohematopoietic and neural cells	Follicular non-Hodgkin lymphoma	Autoimmune disease, Cervical cancer, Prostate cancer	151430	Mahmoud and El-Sakhawy (2011)
RAD54L	1p34.1	Somatic mutations	– Involved in transcriptional regulation and chromatin remodeling	Non-Hodgkin lymphoma (NHL)	Breast cancer, Alpha-thalassemia mental retardation syndrome, X-linked (ATRX), Mental retardation	603615	Matsuda et al. (1999)
CASP10	2q33.1	Somatic mutations	– Involved in the activation cascade of caspases responsible for apoptosis execution	Non-Hodgkin lymphoma (NHL)	Autoimmune lymphoproliferatie syndrome 2A, Gastric cancer	601762	Shin et al. (2002)

(continued)

Table 5.14 (continued)

Gene	Location	Molecular pathology	Function	Phenotype	Other related disease	OMIM	References
RAD54B	8q22.1	Somatic mutations	– Involved in DNA repair and mitotic recombination – May play an active role in recombination processes in concert with other members of the RAD52 epistasis group	Non-Hodgkin lymphoma (NHL)	Colon cancer	604289	Hiramoto et al. (1999)
PRF1	10q22.1	Somatic mutations	– Plays a key role in secretory granule-dependent cell death, and in defense against virus-infected or neoplastic cells	Non-Hodgkin lymphoma (NHL)	Aplastic anemia, Hemophagocytic lymphohistiocytosis	170280	Clementi et al. (2005)
P53	17p13.1	Somatic mutations	– Acts as a tumor suppressor in many tumor types – Iduces growth arrest or apoptosis depending on the physiological circumstances and cell type	Non-Hodgkin lymphomas	Esophageal cancer, Li-Fraumeni syndrome, Squamous cell carcinoma of the head and neck, Lung cancer, Papilloma of choroid plexus, Adrenocortical carcinoma, Basal cell carcinoma	191170	Malkin et al. (1992)

(continued)

Table 5.14 (continued)

Gene	Location	Molecular pathology	Function	Phenotype	Other related disease	OMIM	References
ATM	11q22.3	Somatic mutations	– The ATM protein is a member of the phosphatidylinositol 3-kinase family of proteins that respond to DNA damage by phosphorylating key substrates involved in DNA repair and/or cell cycle control	Non-Hodgkin lymphoma (NHL)	Ataxia telangiectasia T-cell prolymphocytic leukemia, Breast cancer	607585	Gumy-Pause et al. (2006)
BCL7A	12q24.31	t(8;14;12) (q24.1;q32.3; q24.1)	– Negative regulation of transcription, DNA-templated	B-cell non-Hodgkin lymphoma, high-grade	Not reported	601406	Zani et al. (1996)
BCL6	3q27.3	t(3;14)(q27; q32), t(3;22) (q27;q11) t (3;16)(q27; p11) t(3;13) (q27;q14)	– Transcriptional repressor mainly required for germinal center (GC) formation and antibody affinity maturation which has different mechanisms of action specific to the lineage and biological functions	B-cell non-Hodgkin lymphomas (B-cell NHL)	B-cell leukemia	109565	Coco et al. (1994)

(continued)

Table 5.14 (continued)

Gene	Location	Molecular pathology	Function	Phenotype	Other related disease	OMIM	References
TCL1A	14q32.13	t(14;14)(q11;q32)t(7;14)(q35;q32); inv(14)(q11;q32)	– Enhances cell proliferation – Stabilizes mitochondrial membrane – Potential and promotes cell survival	T-cell lymphomas (T-CLL)	T-cell leukemias (T-CLL)	186960	Virgilio et al. (1994)
MYC	8q24.21	t(8;14), t(8;22) or t(2;8)	– Transcription factor that binds DNA in a non-specific manner – Activates the transcription of growth-related genes	Burkitt lymphoma	Jaw and kidney tumor, Abdominal tumors, B-cell chronic lymphocytic leukemia	190080	Adams et al. (1983)
CCND1	11q13.3	t(11;14)(q13;q32)	– Regulatory component of the cyclin D1-CDK4 (DC) complex that phosphorylates and inhibits members of the retinoblastoma (RB) protein family including RB1 and regulates the cell-cycle during G1/S transition	Mantle-cell lymphoma (MCL)	Multiple myeloma, parathyroid adenomas, Colorectal cancer, von Hippel-Lindau syndrome	168461	Gorczyca (2014)

(continued)

Table 5.14 (continued)

Gene	Location	Molecular pathology	Function	Phenotype	Other related disease	OMIM	References
IKZF1	7p12.2	t(3;7)(q27; p12), with BCL6	– Transcription regulator of hematopoietic cell differentiation	B-cell non-Hodgkin lymphomas			Hoffman et al. (2012)
REL	2p16.1	Gene amplification	– Proto-oncogene that may play a role in differentiation and lymphopoiesis	Hodgkin lymphomas	Lymphoproliferative syndrome	164910	Martín-Subero et al. (2002)
JAK2	9p24.1	Gene amplification, Translocation: t(8;9)(p22; p24) t(9;12) (p24;p13)	– JAK2 kinase is a member of a family of tyrosine kinases involved in cytokine receptor signaling	Hodgkin lymphomas	Leukemia, Erythrocytosis, Myelofibrosis, Polycythemia vera, Thrombocythemia 3, Budd-Chiari syndrome	147796	Engert and Younes (2015)
MDM2	12q15	Gene amplification	– Inhibits p53/TP53- and p73/TP73-mediated cell cycle arrest and apoptosis	Hodgkin lymphoma	soft tissue sarcomas, osteosarcomas, gliomas, ovarian and bladder carcinomas	164785	Engert and Younes (2015)
B-RAF	7q34	Mutation	– Protein kinase involved in the transduction of mitogenic signals from the cell membrane to the nucleus	Non-Hodgkin lymphoma	Colorectal cancer, Lung cancer, Familial non-Hodgkin lymphoma, Cardiofaciocutaneous syndrome 1, Noonan	164757	Lee et al. (2003)

(continued)

Table 5.14 (continued)

Gene	Location	Molecular pathology	Function	Phenotype	Other related disease	OMIM	References
			– Phosphorylates MAP2K1, and thereby contributes to the MAP kinase signal transduction pathway		syndrome 7, LEOPARD syndrome 3		
K-RAS	12p12.1	Mutation	– Ras proteins bind GDP/GTP and possess intrinsic GTPase activity – Plays an important role in the regulation of cell proliferation	Non-Hodgkin lymphoma	Leukemia, acute myelogenous (AML), Leukemia, juvenile myelomonocytic (JMML), Noonan syndrome 3, Gastric cancer, Cardiofaciocutaneous syndrome 2	190070	Rademaker (2007)

rarely make solid tumors. In leukemia, abnormal cells circulate into the blood, bone marrow and lymphatic system Stewart and Wild (2014).

There are two main families of leukemias including acute and chronic and each of those are further classified as lymphoblastic and myeloblastic (Vardiman et al. 2009, Horsboel et al. 2013). So the types and sub-types of leukemias are provided as followings:

1. Acute lymphoblastic leukemia (ALL): ALL is a rare type of non-Hodgkin lymphoma, as the result of abnormal adaptive immune cells, typically T-cells. It usually occurs in children. ALL is classified as: ALL-L1: small uniform cells; ALL-L2: large varied cells; ALL-L3: large varied cells with vacuoles (bubble-like features).
2. Chronic lymphocytic leukemia (CLL): In patients with CLL, the abnormal cells gather with other types of cells in the bone marrow. This crowding prevents the production of the healthy blood cells, including: (1) Red blood cells that carry oxygen; (2) Other types of white blood cells, such as neutrophils or granulocytes that fight infection; (3) Platelets, which are required for the clotting system. There are 2 general types of CLL based on whether the disease affects B cells or T cells. It is important for hematologists to find out whether the disease is caused by the overgrowth of T cells (T-cell prolymphocytic leukemia) or B (B-cell Prolymphocytic Leukemia and Hairy Cell Leukemia) cells.
3. Acute myelocytic leukemia (AML): Acute myelocytic cells are traceable in the bone marrow and, in some cases, has spread to other organs, such as the liver and spleen. Therefore, AML is not staged like most other cancers. The outlook for a patient with AML depends instead on other information, such as the subtype, the patient's age, and more complementary test results. Knowing the subtype of AML is very important, as it has impact on both a patient's outlook and the therapeutic choices. Two of the main systems that have been used to classify AML into subtypes are the French-American-British (FAB) classification and the newer World Health Organization (WHO) classification. AML subtypes according to FAB includes M0, M2, M3, M4, M4 eos, M5, M6, M7.
4. Chronic myeloblastic leukemia (CML): CML, also known as chronic myelogenous leukemia, is a type of cancer that starts in certain blood-forming cells of the bone marrow. In CML, a genetic change takes place in an early (immature) type of myeloid cells—the cells that make red blood cells, platelets, and most types of white blood cells (except lymphocytes). However, the malignant behavior of these cells are due to the presence of an abnormal fused gene called BCR-ABL. The leukemic cells grow and divide, building up in the bone marrow and migrating into the blood stream. These cells may also settle in other parts of the body, including the spleen. CML is a fairly slow growing leukemia, but it can also change into a fast-growing acute leukemia that is hard to treat.

Most cases of CML occur in adults, but very rarely it occurs in children too. In general, the classic treatment is as same as in adults.

At a glance, there are four main types of leukemia: acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), B-cell chronic lymphocytic leukemia (CLL) and chronic myelogenous leukemia (CML) (Table 5.15) (Chizuka et al. 2006; Smith et al. 2011). There are also other types that are less common. Blood cancer is part of a wider group called neoplastic that includes blood, bone marrow and lymphatic system known as lymphoid and hematopoietic tissue tumors. Blood cancer is diagnosed at many thousand individuals around the world and caused death of many patients. Over 200,000 individuals are affected with leukemias in the USA. While leukemia is the leading form of pediatric cancer, however, leukemia affects much more adults than children (Table 5.15).

While the main and actual cause of leukemia is unknown, but scientists and researchers believe that a combination of genetic and environmental factors are involved in the disease. Chromosomal translocations are the major genetic alteration in leukemias found in over 50% of leukemic patients in both infants and adults. Translocations can activate proto-oncogenes near breakpoints in two ways: (1) by fusing the coding sequences of two genes that are normally unrelated, or (2) by placing a gene under the transcriptional control of an unrelated gene that is expressed at high levels (Tosi and Reid 2016). We summarized the most reported genes involved in different types of leukemias (Table 5.16).

5.7 Laryngeal Cancer

Laryngeal cancer is, globally, one of the most prevalent cancer of Squamous cell carcinoma of the head and neck (SCCHN). The major risk factors for laryngeal cancer are cigarette smoking and alcohol consumption. Although the involvement of Human Papilomavirus (HPV) is well known in squamous cell carcinoma of the

Table 5.15 Type of hematopoietic malignancy

Leukemias	Percentage	Total (%)
Acute lymphoblastic leukemia (ALL)	4	30.4
Acute myelogenous leukemia (AML)	8.7	
Chronic lymphocytic leukemia (CLL)		
Chronic myelogenous leukemia (CML)	10.2	
Acute monocytic leukemia (AMoL)	0.7	
Other leukemias	3.1	
Lymphomas		
Hodgkin's lymphomas (all four subtypes)	7	55.6
Non-Hodgkin's lymphomas (all subtypes)	48.6	
Myelomas		14

Table 5.16 The most reported genes involved in pathogenesis of different types of lymphoma

Gene	Location	Molecular pathology	Function	Type of leukemia	Other related disease	OMIM	References
AFF1	4q21	t(4;11)(q21;q23)	– This gene encodes a member of the AF4/lymphoid nuclear protein related to AF4/Fragile X E mental retardation syndrome family of proteins, which have been implicated in childhood lymphoblastic leukemia, Fragile X E site mental retardation, and ataxia	Acute lymphoblastic leukemia	Not reported	159557	Wiernik (2003)
MYC	8q24.21	t(8;12)(q24;q22) with BTG1	– Transcription factor that binds DNA in a non-specific manner, yet also specifically recognizes the core sequence 5′-CAC [GA] TG-3′ – Activates the transcription of growth-related genes	Acute lymphoblastic leukemia	Burkitt lymphoma	190080	Handin et al. (2003)
IKZF1	7p12.2	Alterations or deletions	– Transcription regulator of hematopoietic cell differentiation	Acute lymphoblastic leukemia	B-cell non-Hodgkin lymphomas	603023	Mullighan et al. (2009)
AFF3	2q11.2	t(2;11)(p15;p14) ins (11;2)(q23;q11.2q11.2)	– Putative transcription activator that may function in lymphoid development and oncogenesis	Acute lymphoblastic leukemia	Mesomelic dysplasia, MLL	601464	Hoffman et al. (2013)
TCF3	19p13.3	t(1;19)(q23;p13.3) with PBX1 t(17;19)(q22; p13.3) with HLF inv(19) (p13;q13) with TFPT	– Transcriptional regulator. Involved in the initiation of neuronal differentiation.	Acute lymphoblastic leukemia	Autosomal agammaglobulinemia	147141	Coppola (2014)

(continued)

Table 5.16 (continued)

Gene	Location	Molecular pathology	Function	Type of leukemia	Other related disease	OMIM	References
PAX5	9p13.2	t(9;18)(p13;q11.2) t(9;3)(p13;p14.1) t(9;12)(p13;p13)	– May play an important role in B-cell differentiation as well as neural development and spermatogenesis – Involved in the regulation of the CD19 gene, a B-lymphoid-specific target gene	Acute lymphoblastic leukemia	Not reported	167414	Wiernik (2003)
ARNT	1q21.3	t(1;12)(q21;p13)	– This protein is required for the ligand-binding subunit to translocate from the cytosol to the nucleus after ligand binding. The complex then initiates transcription of genes involved in the activation of PAH procarcinogens	Acute myeloblastic leukemia	Not reported	126110	Degos et al. (1999)
IRF1	5q31.1	Deletion in 5q	– Transcriptional regulator which displays a remarkable functional diversity in the regulation of many genes expressed during hematopoiesis, inflammation, immune responses and cell proliferation and differentiation and etc.	Acute myelogenous leukemia	Gastric cancer, preleukemi Myelodysplastic syndrome,	147575	Boultwood et al. (1993)
RGS2	1q31.2	Deletion/duplication differential expression	– Regulates G protein-coupled receptor signaling cascades.	Acute myelogenous leukemia	Hypertension Metabolic syndrome Platelet Gs hypofunction	600861	Carroll et al. (2011)

(continued)

Table 5.16 (continued)

Gene	Location	Molecular pathology	Function	Type of leukemia	Other related disease	OMIM	References
GMPS	3q25.31	t(3;11)(q25;q23)	– Involved in the de novo synthesis of guanine nucleotides which are not only essential for DNA and RNA synthesis, but also provide GTP, which is involved in a number of cellular processes important for cell division	Acute myeloid leukemias	Acute Myelogenous Leukemia	600358	Goardon et al. (2011)
DEK	6p22.3	t(6;9)(p23;q34) with NUP214/CAN	– Involved in chromatin organization	Acute myeloid leukemias		125264	Ohminami et al. (1999)
FUS	16p11.2	t(16;21)(p11;q22) with ERG	– Binds both single-stranded and double-stranded DNA and promotes ATP-independent annealing of complementary single-stranded DNAs and D-loop formation in super helical double-stranded DNA – May play a role in maintenance of genomic integrity	Acute myeloid leukemias	Angiomatoid fibrous histiocytoma (AFH) Amyotrophic lateral sclerosis 6 Tremor, hereditary essential 4	137070	Kong et al. (1997)
HOXA9	7p15.2	t(7;11)(p15;p15) with NUP98	– Sequence-specific transcription factor which is part of a developmental regulatory system that provides cells with specific positional identities on the anterior-posterior axis	Acute myeloid leukemia	Chronic myeloid leukemia	142956	Iwasaki et al. (2005)

(continued)

Table 5.16 (continued)

Gene	Location	Molecular pathology	Function	Type of leukemia	Other related disease	OMIM	References
NUP214	9q34.13	t(6;9)(p23;q34) with DEK (DEK-CAN fusion gene)	– May serve as a docking site in the receptor-mediated import of substrates across the nuclear pore complex	Acute myeloid leukemia	Acute undifferentiated leukemia (AUL)	114350	Hoffman et al. (2012)
NUP98	11p15.4	t(7;11)(p15;p15) with HOXA9 t(11;17)(p15;p13) with PHF23 t(5;11)(q35;p15.5) with NSD1 t(8;11)(p11.2;p15) with WHSC1L1	– Plays a role in the nuclear pore complex (NPC) assembly and/or maintenance – Nup98 and Nup96 are involved in the bidirectional transport across the NPC	Acute myeloid leukemia	Therapy-related myelodysplastic syndrome T-cell acute lymphoblastic leukemia (T-ALL)	601021	Borrow et al. (1996)
CBFB	16q22.1	Pericentric inversion inv(16)(p13;q22)	– CBF binds to the core site, 5′-PYGPYGGT-3′, of a number of enhancers and promoters, including murine leukemia virus, polyomavirus enhancer, T-cell receptor enhancers, LCK, IL3 and GM-CSF promoters – CBFB enhances DNA binding by RUNX1	Acute myeloid leukemia	Delayed skull ossification and cleft palate Various skeletal abnormalities	121360	Byrd Jr (2008)
MLLT10	10p12.31	t(10;11)(p12;q23) with KMT2A/MLL1	– Probably involved in transcriptional regulation – In vitro or as fusion protein with KMT2A/MLL1 has transactivation activity – Binds to cruciform DNA	Acute myeloid leukemia	Diffuse histiocytic lymphomas	602409	Borel et al. (2012)

(continued)

Table 5.16 (continued)

Gene	Location	Molecular pathology	Function	Type of leukemia	Other related disease	OMIM	References
RUNX1		t(8;21)(q22;q22) with RUNX1T1	– The protein encoded by this gene represents the alpha subunit of CBF and is thought to be involved in the development of normal hematopoiesis	Acute myeloid leukemia	Therapy-related myelodysplastic syndrome (T-MDS); CML; childhood acute lymphoblastic leukemia (ALL)	151385	Andreeff (2014)
CEBPA	19q13.11	caused by mutations	– Transcription factor that coordinates proliferation arrest and the differentiation of myeloid progenitors, adipocytes, hepatocytes, and cells of the lung and the placenta	Acute myeloid leukemia	None	116897	Pabst et al. (2001)
LCP1	13q14.13	Translocation and mutation	– Actin-binding protein. Plays a role in the activation of T-cells in response to costimulation through TCR/CD3 and CD2 or CD28. Modulates the cell surface expression of IL2RA/CD25 and CD69	Acute myeloid leukemia	B-cell non-Hodgkin lymphomas (B-cell NHL)	153430	Tsang et al. (2004)
ARHGEF12	11q23.3	t(11;11)(q23;23) with KMT2A/MLL1	– May play a role in the regulation of RhoA GTPase by guanine nucleotide-binding alpha-12 (GNA12) and alpha-13 (GNA13) – Acts as guanine nucleotide exchange factor (GEF) for RhoA GTPase and may act as GTPase-activating protein (GAP) for GNA12 and GNA13	Acute myeloid leukemia	Increased insulin sensitivity	604763	Pui (2012)

(continued)

Table 5.16 (continued)

Gene	Location	Molecular pathology	Function	Type of leukemia	Other related disease	OMIM	References
CREBBP	16p13.3	t(8;16)(p11;p13) with KAT6A t(11;16)(q23;p13.3) with KMT2A/MLL1 t(10;16)(q22;p13) with KAT6B	– Acetylates histones, giving a specific tag for transcriptional activation. Also acetylates non-histone proteins, like NCOA3 and FOXO1 – Acetylates PCNA; acetylation promotes removal of chromatin-bound PCNA and its degradation during nucleotide excision repair (NER)	Acute myeloid leukemia	Rubinstein-Taybi syndrome	600140	Rozman et al. (2004)
NPM1	5q35.1	Translocation t(5;17)(q32;q11) with RARA	– Involved in diverse cellular processes such as ribosome biogenesis, centrosome duplication, protein chaperoning, histone assembly, cell proliferation, and regulation of tumor suppressors p53/TP53 and ARF	Acute promyelocytic leukemia	non-Hodgkin lymphoma myelodysplastic syndrome acute myelogenous leukemia	164040	Ahmad et al. (2009)
THRA	17q21.1	translocation	– Isoform Alpha-1: Nuclear hormone receptor that can act as a repressor or activator of transcription – High affinity receptor for thyroid hormones, including triiodothyronine and thyroxine – Isoform Alpha-2: Does not bind thyroid hormone and functions as a weak dominant negative inhibitor of thyroid hormone action	Acute promyelocytic leukemia	Hypothyroidism, congenital, non-goitrous, 6 (CHNG6)	190120	Borrow et al. (1990)

(continued)

Table 5.16 (continued)

Gene	Location	Molecular pathology	Function	Type of leukemia	Other related disease	OMIM	References
PML	15q24.1	Translocation t(15;17)(q21;q21) with RARA	– Functions via its association with PML-nuclear bodies (PML-NBs) in a wide range of important cellular processes, including tumor suppression, transcriptional regulation, apoptosis, senescence, DNA damage response, and viral defense mechanisms	Acute promyelocytic leukemia	Breast cancer? Colon cancer?	102578	Beez et al. (2012)
SET	9q34.11	Translocation t(6;9)(q21;q34.1) with NUP214/CAN	– Multitasking protein, involved in apoptosis, transcription, nucleosome assembly and histone chaperoning	Acute undifferentiated leukemia	None	600960	Von Lindern et al. (1992)
BTG1	12q21.33	Translocation t(8;12)(q24;q22) with MYC	– Anti-proliferative protein	B-cell chronic lymphocytic leukemia	None	109580	Buhl et al. (2006)
BCL3	19q13.32	Translocation t(14;19)(q32;q13.1) with immunoglobulin gene regions	– Contributes to the regulation of transcriptional activation of NF-kappa-B target genes	B-cell chronic lymphocytic leukemia	None	109560	Ueshima et al. (1985)
PBX1	1q23.3	Translocation t(1;19)(q23;p13.3) with TCF3	– Binds the sequence 5′-ATCAATCAA-3′ – Acts as a transcriptional activator of PF4 in complex with MEIS1 – Converted into a potent transcriptional activator by the (1; 19) translocation. May have a	Pre-B-cell acute lymphoblastic leukemia (B-ALL)	None	176310	Gatter et al. (2011)

(continued)

Table 5.16 (continued)

Gene	Location	Molecular pathology	Function	Type of leukemia	Other related disease	OMIM	References
			role in steroidogenesis and, subsequently, sexual development and differentiation				
DLEU2	13q14.3	Deletion	– DLEU2 (Deleted In Lymphocytic Leukemia 2 (Non-Protein Coding)) is an RNA Gene, and is affiliated with the non-coding RNA class	Chronic lymphocytic leukemia	None	605766	Klein et al. (2010)
DELU1	13q14.2-q14.3	Deletion	– May act as a tumor suppressor.	Chronic lymphocytic leukemia	None	605765	Chung et al. (2013)
AXL	19q13.1	Mutation	– The protein encoded by this gene is a member of the Tyro3-Axl-Mer (TAM) receptor tyrosine kinase subfamily – This gene may be involved in several cellular functions including growth, migration, aggregation and anti-inflammation in multiple cell types	Chronic myelocytic leukemia	Thyroid carcinoma myeloproliferative disorders prostatic carcinoma breast cancer	109135	Dufies et al. (2011)
EVI2A	17q11.2	Mutation	– May complex with itself or/and other proteins within the membrane, to function as part of a cell-surface receptor	Murine myeloid leukemia	None	158380	Buchberg et al. (1990)
EVI2B	17q11.2	Mutation	– Function as part of a cell-surface receptor	Murine Myeloid Leukemia	None	158381	Rücker et al. (2006)

(continued)

Table 5.16 (continued)

Gene	Location	Molecular pathology	Function	Type of leukemia	Other related disease	OMIM	References
PBX1	1q23.3	Translocation t(1;19) (q23;p13.3) with TCF3	– Acts as a transcriptional activator. May have a role in steroidogenesis and, subsequently, sexual development and differentiation	Pre B-cell leukemia	None	176310	Kamps and Baltimore (1993)
PBX2	6p21.32		– Transcriptional activator that binds the sequence 5′-ATCAATCAA-3′ – Activates transcription of PF4 in complex with MEIS1	Pre B-cell leukemia	None	176311	Aguado and Campbell (1995)
PBX3	9q33.3		– Transcriptional activator that binds the sequence 5′-ATCAATCAA-3′	Pre B-cell leukemia	None	176312	Milech et al. (2001)
TCL6	14q32.13	chromosomal rearrangements with T-cell receptor (TCR) loci	– TCL6 (T-cell leukemia/Lymphoma 6 (Non-Protein Coding)) is an RNA Gene, and is affiliated with the non-coding RNA class	T-cell leukemia	None	604412	Saitou et al. (2000)
TCL1B	14q32.13	chromosomal translocations and inversions at 14q32.1	– Among its related pathways are PI3 K-Akt signaling pathway. An important paralog of this gene is MTCP1. Enhances the phosphorylation and activation of AKT1 and AKT2	T-cell leukemia	None	603769	Künstle et al. (2002)

(continued)

Table 5.16 (continued)

Gene	Location	Molecular pathology	Function	Type of leukemia	Other related disease	OMIM	References
TRAC	14q11.2	inv(14)(q11;q32) t (11;14)(p13;q11) t (11;14)(p15;q11)	– Among its related pathways are Immune System and G-protein signaling_Regulation of RAC1 activity	T-cell leukemia	Tcr-alpha-beta-positive t-cell deficiency and immunodeficiency 7, tcr-alpha/beta deficient	186880	Rabbitts et al. (1985)
MTCP-1	Xq28	t(X;14) translocation	– This gene was identified by involvement in some t(X; 14) translocations associated with mature T-cell proliferations – This protein may be involved in leukemogenesis – Among its related pathways are PI3 K-Akt signaling pathway – An important paralog of this gene is TCL1A – Enhances the phosphorylation and activation of AKT1 and AKT2	T-cell leukemia	Queensland tick typhus	300116	Gritti et al. (1998)
LDB1	10q24.32		– Binds to the LIM domain of a wide variety of LIM domain-containing transcription factors – May regulate the transcriptional activity of LIM-containing proteins by determining specific partner interactions – Plays a role in the development of interneurons and motor	T-cell leukemia	None	603451	Valge-Archer et al. (1998)

(continued)

Table 5.16 (continued)

Gene	Location	Molecular pathology	Function	Type of leukemia	Other related disease	OMIM	References
			neurons in cooperation with LHX3 and ISL1 – Acts with LMO2 in the regulation of red blood cell development, maintaining erythroid precursors in an immature state				
NOTCH1	9q34.3	Mutation	– Notch signaling is an evolutionarily conserved intercellular signaling pathway that regulates interactions between physically adjacent cells through binding of Notch family receptors to their cognate ligands – This receptor plays a role in the development of numerous cell and tissue types	T-cell acute lymphoblastic leukemia	Aortic valve disease Adams-Oliver syndrome chronic lymphocytic leukemia head and neck squamous cell carcinoma	190198	Zhu et al. (2006)
NOTCH3	19p13.12	Mutation	– Functions as a receptor for membrane-bound ligands Jagged1, Jagged2 and Delta1 to regulate cell-fate determination – Affects the implementation of differentiation, proliferation and apoptotic programs (By similarity)	T-cell acute lymphoblastic leukemia	Lateral meningocele syndrome Myofibromatosis Cerebral arteriopathy	600276	Xiang et al. (2012)
LYL1	19p13.13	t(7;19)(q35;p13) with TCRB	– The encoded protein may play roles in blood vessel maturation and hematopoeisis	T-cell acute lymphoblastic leukemia	None	151440	Meng et al. (2005)

(continued)

Table 5.16 (continued)

Gene	Location	Molecular pathology	Function	Type of leukemia	Other related disease	OMIM	References
HOX11	10q24.31	t(10;14)(q24;q11) with TCRD	– Controls the genesis of the spleen. Binds to the DNA sequence 5′-GGCGGTAAGTGG-3′ – This protein is also involved in specification of neuronal cell fates	T-cell acute lymphoblastic leukemia	None	186770	Kees et al. (2003)
BAX	19q13.33	Mutation	– Accelerates programmed cell death by binding to, and antagonizing the apoptosis repressor BCL2 or its adenovirus homolog E1B 19 k protein – Under stress conditions, undergoes a conformation change that causes translocation to the mitochondrion membrane, leading to the release of cytochrome c that then triggers apoptosis – Promotes activation of CASP3, and thereby apoptosis	T-cell acute lymphoblastic leukemia	Colorectal cancer	600040	Prokop et al. (2000)
LMO1	11p15.4	t(11,14)(p15;q11) with TCRD	– LIM domains may play a role in protein interactions; thus the encoded protein may regulate transcription by competitively	T-cell acute lymphoblastic leukemia	cd3epsilon deficiency exencephaly	186921	Herblot et al. (2000)

(continued)

Table 5.16 (continued)

Gene	Location	Molecular pathology	Function	Type of leukemia	Other related disease	OMIM	References
			binding to specific DNA-binding transcription factors – May be involved in gene regulation within neural lineage cells potentially by direct DNA binding or by binding to other transcription factors				
LMO2	11p13	t(11,14)(p13;q11) with TCRD	– Acts with TAL1/SCL to regulate red blood cell development – Also acts with LDB1 to maintain erythroid precursors in an immature state The LMO2 protein has a central and crucial role in hematopoietic development and is highly conserved	T-cell acute lymphoblastic leukemia	Norrie disease	180385	Pike-Overzet et al. (2007)
TAL1	1p33	t(1;14)(p32;q11) with T-cell receptor alpha chain (TCRA) genes	– Implicated in the genesis of hemopoietic malignancies – It may play an important role in hemopoietic differentiation – Serves as a positive regulator of erythroid differentiation (By similarity)	T-cell acute lymphoblastic leukemia	Glaucoma	187040	Reynaud et al. (2005)
TAL2	9q31.2	t(7;9)(q34;q32) with TCRB	– This intronless gene encodes a helix-loop-helix protein	T-cell acute lymphoblastic leukemia	None	186855	Xia et al. (1991)

(continued)

Table 5.16 (continued)

Gene	Location	Molecular pathology	Function	Type of leukemia	Other related disease	OMIM	References
CAV1	7q31.2	Mutation	– May act as a scaffolding protein within caveolar membranes. Interacts directly with G-protein alpha subunits and can functionally regulate their activity (By similarity) – Involved in the costimulatory signal essential for T-cell receptor (TCR)-mediated T-cell activation. Its binding to DPP4 induces T-cell proliferation and NF-kappa-B activation in a T-cell receptor/CD3-dependent manner – Recruits CTNNB1 to caveolar membranes and may regulate CTNNB1-mediated signaling through the Wnt pathway	T-cell leukemia (in lung carcinoma)	Congenital generalized lipodystrophy 3 Pulmonary hypertension Partial lipodystrophy, congenital cataracts, and neurodegeneration syndrome	601047	Sasaki et al. (2005)
LAF4	2q11.2	Mutation	– Putative transcription activator that may function in lymphoid development and oncogenesis – Binds, in vitro, to double-stranded DNA	MLL	fibular aplasia and bone inflammation disease	601464	Hiwatari et al. (2003)
NFKB2	10q24.32	chromosomal aberration involving NFKB2	– The NFkB complex is expressed in numerous cell types and functions as a central activator of genes involved in inflammation and immune function	Cutaneous T-cell leukemia	B-cell non Hodgkin lymphoma (B-NHL); Immunodeficiency, common variable, 10 (CVID10)	164012	Neri et al. (1996)

(continued)

Table 5.16 (continued)

Gene	Location	Molecular pathology	Function	Type of leukemia	Other related disease	OMIM	References
			– The protein encoded by this gene can function as both a transcriptional activator and repressor depending on its dimerization partner				
ETS1	11q24.3	Regulatory mutation	– Transcription factor – Directly controls the expression of cytokine and chemokine genes in a wide variety of different cellular contexts – May control the differentiation, survival and proliferation of lymphoid cells – May also regulate angiogenesis through regulation of expression of genes controlling endothelial cell migration and invasion	Human monocytic leukemia	Jacobsen syndrome fuchs' heterochromic uveitis Systemic lupus erythematosus	164720	Akao et al. (1991)
KIT	4q12	Somatic mutations that lead to constitutive activation of KIT	– Tyrosine-protein kinase that acts as cell-surface receptor for the cytokine KITLG/SCF – Plays an essential role in the regulation of cell survival and proliferation, hematopoiesis, stem cell maintenance, gametogenesis, mast cell development, migration and function, and in melanogenesis	Mast cell leukemia	Piebald trait (PBT) Gastrointestinal stromal tumor (GIST) Testicular germ cell tumor (TGCT)	164920	Pietsch et al. (1992)
MLLT3	9p21.3	Translocation t(9;11)(p22;q23) with KMT2A/MLL1	– Component of the super elongation complex (SEC), a complex required to increase the	Mixed linkage leukemia	Acute leukemias	159558	O'Brien et al. (2010)

(continued)

Table 5.16 (continued)

Gene	Location	Molecular pathology	Function	Type of leukemia	Other related disease	OMIM	References
			catalytic rate of RNA polymerase II transcription by suppressing transient pausing by the polymerase at multiple sites along the DNA				
KMT2C	7q36.1	Mutations	– This gene is a member of the myeloid/lymphoid or mixed-lineage leukemia (MLL) family and encodes a nuclear protein with an AT hook DNA-binding domain, a DHHC-type zinc finger, six PHD-type zinc fingers, a SET domain, a post-SET domain and a RING-type zinc finger – This protein is a member of the ASC-2/NCOA6 complex (ASCOM), which possesses histone methylation activity and is involved in transcriptional coactivation	Mixed linkage leukemia	None	606833	Ruault et al. (2002)

oropharynx, its role in laryngeal cancer is not still completely clear. Also, individuals with laryngeal cancer may develop second primary tumor because of chronic aero digestive tract exposure to carcinogen. Up to now, tumor staging is the main prognostic factor for overall survival (OS). However, despite of technological and clinical improvements, OS of patients with laryngeal cancer has remained unsatisfactory (Barnes 2005; Stewart 2005; Naghavi et al. 2015).

Recent advances in cancer genetics suggest that genetic elements could be as the predisposing factors involved in laryngeal cancer development as the predisposing factors. It has been reported that having first degree relatives affected with multiple primary head and neck cancer increases the relative risk (RR) for SSCHN to 7.89 fold. In addition, many cytogenetic alterations including loss of heterozygosity (LOH) in a variety of chromosomes including 3p, 4q, 8p, 9p, 11q, 13q, 17p and amplification in 3q, 3q24-qter, 5p, 8q23-24, 11q13, 11q14-22, 18p, 18q11.2, and 19q have shown in previous publications (Barnes 2005; Pai and Westra 2009).

Furthermore, involvement of MicroRNAs (miRNAs) in the regulation of many crucial cellular and physiological processes such as cell differentiation, proliferation, metabolism, and apoptosis has been reported and confirmed in a large volume of literatures. These micro-molecules is reported to be involved in regulating of about 60–70% of human genes. Therefore, deregulation of these important biomolecules must be disease-causing (Di Leva et al. 2014). The results of miR microarrays and massive sequencing have revealed that a large number of miRNAs are deregulated in laryngeal cancer (Cao et al. 2013a, b).

Hence, identification of molecular biomarkers with prognostic and predictive values could be a crucial step in preventing of laryngeal cancer. On the other hand, tracing the new targets may lead to development of new therapeutics strategy in order to improve survival period and possibly finding a cure for patients affected with laryngeal cancer (Avissar et al. 2009a, b). In Tables 5.17 and 5.18, most reported genes and miRNAs involved in laryngeal cancer are listed.

5.7.1 Genetic Etiology

See Table 5.17.

5.7.2 miRNA Etiology

See Table 5.18.

Table 5.17 Most reported genes related to laryngeal cancer

Gene symbol	Gene locus	Function	Alteration in Cancer	OMIM	References
ABCG2	4q22	– Functions as a xenobiotic transporter which may play a major role in multi-drug resistance	The resistance of cancer stem cells to chemotherapy was correlated with higher expression of ABCG2	603756	Yang et al. (2011)
ADAM12	10q26.3	– Involved in biological processes like cell-cell and cell-matrix interactions, including fertilization, muscle development, and neurogenesis	Metal-proteinaseADAM12 occurred to be good marker of the neoplasm in laryngeal carcinoma	602714	Markowski et al. (2009)
APC	5q21-q22	– A tumor suppressor protein that acts as an antagonist of the Wnt signaling pathway – It is also involved in other processes including cell migration and adhesion, transcriptional activation, and apoptosis	– Consistent aberrant methylation of multiple tumor suppressor genes – Promoter hypermethylation of APC was detected with subsequent progression to SCC	611731	Stephen et al. (2010a, b)
BCCIP	10q26.1	– May be an important cofactor for BRCA2 in tumor suppression, and a modulator of CDK2 kinase activity via p21	– Loss of expression of BCCIP in combination with normal p53 was associated with local recurrence and poor overall survival compared to patients who did express BCCIP – Expression of BCCIP or p53 alone was not found to be independently associated with benefits in local control or overall survival	611883	Rewari et al. (2009)

(continued)

Table 5.17 (continued)

Gene symbol	Gene locus	Function	Alteration in Cancer	OMIM	References
BCL2	18q21.3	– An integral outer mitochondrial membrane protein that blocks the apoptotic death of some cells such as lymphocytes	– Bcl-2 expression was low and showed no significant difference between laryngeal papillomatosis and normal larynxes – By contrast, Bcl-2 was clearly up-regulated in cancer. High bcl-2 expression may be playing an important role to block apoptosis	151430	Manjarrez et al. (2006)
BRCA1	17q21	– A nuclear phosphoprotein that plays a role in maintaining genomic stability, and it also acts as a tumor suppressor	– Promoter region of BRCA1 displayed recurrent alteration in the methylation levels in cancer samples as compared to controls – For BRCA1, cell lines from primary laryngeal cases showed hypomethylation	113705	Szaumkessel et al. (2011)
BRCA2	13q12.3	– BRCA2 is considered a tumor suppressor gene, as tumors with BRCA2 mutations generally exhibit loss of heterozygosis (LOH) of the wild-type allele	– Promoter hypermethylation of BRCA2 was detected with subsequent progression to SCC	600185	Stephen et al. (2010a, b)
BRIP1	17q22.2	– DNA-dependent ATPase and 5′ to 3′ DNA helicase required for the maintenance of chromosomal stability – Involved in the repair of DNA double-strand breaks by homologous recombination in a manner that depends on its association with BRCA1	– Fanconi anemia (FA) associated genes [FANCA, -B, -C, FANCD1 (BRCA2), -D2, -E, -F, -G, -I, -L, -M, FANCN (PALB2), FANCJ (BRIP1) and FA-linked BRCA1] encode proteins of DNA damage response pathways mutated in FA patients	605882	Szaumkessel et al. (2011)

(continued)

Table 5.17 (continued)

Gene symbol	Gene locus	Function	Alteration in Cancer	OMIM	References
			– FA is characterized by congenital malformations, chromosomal instability and high cancer susceptibility – FA patients have a 500-700 times higher risk of head and necksquamous cell carcinoma (HNSCC) compared to the non-FA population		
BRMS1	11q13-q13.2	– Transcriptional repressor. Down-regulates transcription activation by NF-kappa-B by promoting the deacetylation of RELA at 'Lys-310' – Promotes HDAC1 binding to promoter regions – Down-regulates expression of anti-apoptotic genes that are controlled by NF-kappa-B – Promotes apoptosis in cells that have inadequate adherence to a substrate, a process called anoikis, and may thereby inhibit metastasis – May be a mediator of metastasis suppression in breast carcinoma	– The expression of BRMS1 protein in supraglottic cancer is significantly decreased – The decrease of BRMS1 mRNA expression may be related to clinical stage and low differentiation and lymph node metastasis of supraglottic laryngeal cancer	606259	Li et al. (2008)
CAPN10	2q37.3	– Calcium-regulated non-lysosomal thiol-protease which catalyze limited proteolysis of substrates involved in cytoskeletal remodeling and signal transduction – May play a role in insulin-stimulated glucose uptake	– A novel association between calpain 10 (CAPN10) haplotypes and laryngeal cancer has been found recently – CAPN10 seems to be related with a worse prognosis in laryngeal cancer	605286	Moreno-Luna et al. (2011)

(continued)

Table 5.17 (continued)

Gene symbol	Gene locus	Function	Alteration in Cancer	OMIM	References
CASP2	7q34-q35	– The encoded protein may function in stress-induced cell death pathways, cell cycle maintenance, and the suppression of tumorigenesis	– Gain of CASP2 marked early LSCC, whereas loss was associated with late LSCC	600639	Saglam et al. (2007)
CASP3	4q34	– Involved in the activation cascade of caspases responsible for apoptosis execution – This gene encodes a protein which is the predominant caspase involved in the cleavage of amyloid-beta 4A precursor protein, which is associated with neuronal death in Alzheimer's disease	The enhanced apoptotic effect correlates with high expression and activation of Bax, FADD, caspase-8 as well as caspase-3 and decreased protein levels of Bcl(2) and SIRT1, suggesting that both the extrinsic and intrinsic apoptosis pathways are involved in the apoptotic processes. It is the active form of caspase-8 that activates downstream effector caspase-3, resulting in the cleavage of critical cellular proteins and apoptosis	600636	Kang et al. (2010)
CAV1	7q31.1	– The scaffolding protein encoded by this gene is the main component of the caveolae plasma membranes found in most cell types	– Caveolin-1 inhibits the growth of human laryngeal squamous cell carcinoma. Downregulation of EGFR-mitogen-activated protein kinase signaling pathway may be critical for understanding its mechanism of tumor suppression – Caveolin-1, a tumor suppressor gene, inactivated by structural abnormalities or epigenetic changes plays a role in the pathogenesis of oral cancer	601047	Gu et al. (2007)

(continued)

Table 5.17 (continued)

Gene symbol	Gene locus	Function	Alteration in Cancer	OMIM	References
CCND1	11q13	– Cyclin D1 (CCND1) is a set of periodic regulatory proteins that is believed to govern cell cycle transit from G1 into S phase – Overexpression of CCND1 leads to abnormal cellular proliferation which underlies processes of tumorigenesis – Mutations, amplification and overexpression of this gene, which alters cell cycle progression, are observed frequently in a variety of tumors and may contribute to tumorigenesis	– CCND1 can function as a cooperative oncogene in cell transformation. CCND1 expression appears to be an early event in processes of tumorigenesis and tumor progression in some LSCC – CCND1 and MLH1 seem functionally interconnected in regard to chromosomal imbalances in larynx cancer. CCND1 over-expression is accompanied by a decrease in MLH1 expression	168461	Sasiadek et al. (2006)
CD44	11p13	– The protein encoded by this gene is a cell-surface glycoprotein involved in cell-cell interactions, cell adhesion and migration	– The reduced expression of CD44 behaves as a marker of a poor laryngeal cancer prognosis	107269	Esteban et al. (2005)
CDC25B	20p13	– CDC25B activates the cyclin dependent kinase CDC2 by removing two phosphate groups – It is required for entry into mitosis. CDC25B has oncogenic properties	– CDC25B phosphatases expression was higher in LSCC compare to the control group	116949	Fraczek et al. (2006)

(continued)

Table 5.17 (continued)

Gene symbol	Gene locus	Function	Alteration in Cancer	OMIM	References
CDKN2A (p16INK4a)	9p21	– It is capable of inducing cell cycle arrest in G1 and G2 phases – It acts as a tumor suppressor	– Promoter hypermethylation of CDKN2A was detected with subsequent progression to LSCC – Fragile histidine triad and p16INK4a expression are altered in malignant lesions. Most likely, the decreasing levels of fragile histidine triad is directly involved in cancer development, while the accumulation of p16INK4a in head and neck squamous cell carcinoma may be the consequence of loss of functional tumor suppressor retinoblastoma pathway	600160	Stephen et al. (2010a, b) Paradiso et al. (2004)
CHD5	1p36.31	– CHD5 belongs to a group of SWI/SNF proteins called CHD proteins, which contain a SWI/SNF-like helicase/ATPase domain, as well as a DNA-binding domain and a chromodomain that directly modifies chromatin structure	– CHD5 is a tumor suppressor gene that is epigenetically downregulated in LSCC	610771	Wang et al. (2011a, b)
CLDN7	17p13	– It plays a major role in tight junction-specific obliteration of the intercellular space	– Transcriptional activity of claudin 7 gene is lower in laryngeal tumour cells compared to histologically normal tissues – On the other hand, CLDN7expression is elevated in cancers of the thyroid	609131	Kapral et al. (2011) Hewitt et al. (2006)

(continued)

Table 5.17 (continued)

Gene symbol	Gene locus	Function	Alteration in Cancer	OMIM	References
CTGF	6q23.1	– The encoded protein plays a role in chondrocyte proliferation and differentiation, cell adhesion in many cell types, and is related to platelet-derived growth factor	– The expression of CTGF might be important biological markers in reflecting the progression, biological behaviors, metastatic potential and prognosis of the laryngeal squamous cell carcinoma	121009	Li et al. (2007)
DNMT1	19p13.2	– DNA (cytosine-5-)-methyltransferase 1 (DNMT1) has a role in the establishment and regulation of tissue-specific patterns of methylated cytosine residues	– The expression of DNMT1 mRNA in LSCC is up-regulated – DNMT1 may initiate the oncogenesis of LSCC by increasing expression, and smoking habit may induce the expression of DNMT1 gene	126375	Wang et al. (2010a, 2010b)
ERBB4	2q33.3-q34	– The protein encoded by this gene binds to and is activated by neuregulins and other factors – Induces a variety of cellular responses including mitogenesis and differentiation	– Loss of ERBB4 significantly discriminated between early and late stage LSCC	600543	Saglam et al. (2007)
ESR1	6q25.1	– This gene encodes an estrogen receptor, a ligand-activated transcription factor composed of several domains important for hormone binding, DNA binding, and activation of transcription	– Aberrant methylation of ESR1 signified independent markers of poorer outcome in patients with LSCC	133430	Stephen et al. (2010a, b)

(continued)

Table 5.17 (continued)

Gene symbol	Gene locus	Function	Alteration in Cancer	OMIM	References
FADD	11q13.3	– The protein encoded by this gene is an adaptor molecule that interacts with various cell surface receptors and mediates cell apoptotic signals	– The enhanced apoptotic effect correlates with high expression and activation of Bax, FADD, caspase-8 as well as caspase-3 and decreased protein levels of Bcl(2) and SIRT1, suggesting that both the extrinsic and intrinsic apoptosis pathways are involved in the apoptotic processes	602457	Kang et al. (2010), Wachters et al. (2017)
FANCA	16q24.3	– This gene encodes the protein for complementation group A – Alternative splicing results in multiple transcript variants encoding different isoforms – Mutations in this gene are the most common cause of Fanconi anemia	– Fanconi anemia (FA) associated genes [FANCA, -B, -C, FANCD1 (BRCA2), -D2, -E, -F, -G, -I, -L, -M, FANCN (PALB2), FANCJ (BRIP1) and FA-linked BRCA1] encode proteins of DNA damage response pathways mutated in FA patients – FA is characterized by congenital malformations, chromosomal instability and high cancer susceptibility – FA patients have a 500-700 times higher risk of head and neck squamous cell carcinoma (HNSCC) compared to the non-FA population – For FANCA, hypomethylation was observed in cell lines compared to controls	607139	Szaumkessel et al. (2011)

(continued)

Table 5.17 (continued)

Gene symbol	Gene locus	Function	Alteration in Cancer	OMIM	References
FANCB	Xp22.2	– This gene encodes the protein for complementation group B – Alternative splicing results in two transcript variants encoding the same protein – DNA repair protein required for FANCD2 ubiquitination	– Fanconi anemia (FA) associated genes [FANCA, -B, -C, FANCD1 (BRCA2), -D2, -E, -F, -G, -I, -L, -M, FANCN (PALB2), FANCJ (BRIP1) and FA-linked BRCA1] encode proteins of DNA damage response pathways mutated in FA patients – FA is characterized by congenital malformations, chromosomal instability and high cancer susceptibility – FA patients have a 500-700 times higher risk of head and neck squamous cell carcinoma (HNSCC) compared to the non-FA population	300515	Szaumkessel et al. (2011)
FANCD2	3p26	– This gene encodes the protein which is monoubiquinated in response to DNA damage, resulting in its localization to nuclear foci with other proteins (BRCA1 AND BRCA2) involved in homology-directed DNA repair	– Fanconi anemia (FA) associated genes [FANCA, -B, -C, FANCD1 (BRCA2), -D2, -E, -F, -G, -I, -L, -M, FANCN (PALB2), FANCJ (BRIP1) and FA-linked BRCA1] encode proteins of DNA damage response pathways mutated in FA patients – FA is characterized by congenital malformations, chromosomal instability and high cancer susceptibility – FA patients have a 500-700 times higher risk of head and neck squamous cell carcinoma (HNSCC) compared to the non-FA population	613984	Szaumkessel et al. (2011)

(continued)

Table 5.17 (continued)

Gene symbol	Gene locus	Function	Alteration in Cancer	OMIM	References
FANCE	6p22-p21	– This gene encodes the protein for complementation group E – As part of the Fanconi anemia (FA) complex functions in DNA cross-links repair. Required for the nuclear accumulation of FANCC and provides a critical bridge between the FA complex and FANCD2	– Fanconi anemia (FA) associated genes [FANCA, -B, -C, FANCD1 (BRCA2), -D2, -E, -F, -G, -I, -L, -M, FANCN (PALB2), FANCJ (BRIP1) and FA-linked BRCA1] encode proteins of DNA damage response pathways mutated in FA patients – FA is characterized by congenital malformations, chromosomal instability and high cancer susceptibility – FA patients have a 500–700 times higher risk of head and neck squamous cell carcinoma (HNSCC) compared to the non-FA population	613976	Szaumkessel et al. (2011)
FANCL	2p16.1	– This gene encodes the protein for complementation group L – Alternative splicing results in two transcript variants encoding different isoforms – Ubiquitin ligase protein that mediates monoubiquitination of FANCD2, a key step in the DNA damage pathway – Also mediates monoubiquitination of FANCI. May stimulate the ubiquitin release from UBE2 W – May be required for proper primordial germ cell proliferation in the embryonic stage, whereas it is probably not needed	– Fanconi anemia (FA) associated genes [FANCA, -B, -C, FANCD1 (BRCA2), -D2, -E, -F, -G, -I, -L, -M, FANCN (PALB2), FANCJ (BRIP1) and FA-linked BRCA1] encode proteins of DNA damage response pathways mutated in FA patients – FA is characterized by congenital malformations, chromosomal instability and high cancer susceptibility – FA patients have a 500-700 times higher risk of head and neck squamous cell carcinoma (HNSCC) compared to the non-FA population	608111	Szaumkessel et al. (2011)

(continued)

Table 5.17 (continued)

Gene symbol	Gene locus	Function	Alteration in Cancer	OMIM	References
		for spermatogonial proliferation after birth			
FANCM	14q21.2	– This gene encodes the protein for complementation group M – ATPase required for FANCD2 ubiquitination, a key reaction in DNA repair – Binds to ssDNA but not to dsDNA – Recruited to forks stalled by DNA interstrand cross-links, and required for cellular resistance to such lesions	– Fanconi anemia (FA) associated genes [FANCA, -B, -C, FANCD1 (BRCA2), -D2, -E, -F, -G, -I, -L, -M, FANCN (PALB2), FANCJ (BRIP1) and FA-linked BRCA1] encode proteins of DNA damage response pathways mutated in FA patients – FA is characterized by congenital malformations, chromosomal instability and high cancer susceptibility – FA patients have a 500-700 times higher risk of head and neck squamous cell carcinoma (HNSCC) compared to the non-FA population	609644	Szaumkessel et al. (2011)
FAS	10q24.1	– It has been shown to play a central role in the physiological regulation of programmed cell death, – Has been implicated in the pathogenesis of various malignancies and diseases of the immune system	– Fas is expressed in squamous cell laryngeal cancer	134637	Asensio et al. (2007)
HGF	7q21.1	– Hepatocyte growth factor regulates cell growth, cell motility, and morphogenesis by activating a tyrosine kinase signaling cascade after binding to the proto-oncogenic c-Met receptor	– HGF may play an important role in the development and progression of human laryngeal cancer – Elevated serum HGF levels predict a more aggressive biological behavior in laryngeal squamous cell cancer	142409	Liu et al. (2010a, b, c, d)

(continued)

Table 5.17 (continued)

Gene symbol	Gene locus	Function	Alteration in Cancer	OMIM	References
HIC1	17p13.3	– This gene functions as a growth regulatory and tumor repressor gene – Hypermethylation or deletion of the region of this gene have been associated with tumors	– Aberrant methylation of HIC1 signified independent markers of poorer outcome in patients with LSCC	603825	Stephen et al. (2010a, b)
ICAM1	19p13.3-p13.2	– This gene encodes a cell surface glycoprotein which binds to integrins of type CD11a/CD18, or CD11b/CD18 – It is also exploited by Rhinovirus as a receptor	– The development, metastasis and prognosis of laryngeal carcinoma may be correlated to ICAM-1 expression of lymphocyte	147840	Wang et al. (2005)
ILK	11p15.4	– This gene encodes a serine/threonine protein kinase with 4 ankyrin-like repeats – Acts as a proximal receptor kinase regulating integrin-mediated signal transduction – Integrin-linked kinase (ILK) has been implicated in the development and progression of several human malignancies	– Increased cytoplasmic and nuclear expression of ILK was found in human laryngeal carcinoma – Enhanced ILK expression correlates with activation of Akt pathway	602366	Goulioumis et al. (2008)

(continued)

Table 5.17 (continued)

Gene symbol	Gene locus	Function	Alteration in Cancer	OMIM	References
KLK10	19q13	– Its encoded protein is secreted and may play a role in suppression of tumorigenesis	– KLK10 was over-expressed at least 10-fold in tumors over any of the normal tissues – Expression of KLK10 at the protein level was determined by immunohistochemistry in seven supraglottic laryngeal cancer specimens. So it was concluded that this molecules are potential targets for immunotherapy of HNSCC patients	602673	Dasgupta et al. (2006)
KRT16	17q21.2	– The keratins are intermediate filament proteins responsible for the structural integrity of epithelial cells and are subdivided into cytokeratins and hair keratins	– KRT16 is significantly expressed in laryngeal SCC – KRT16 is upregulated in tumour cells sensitive to the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor gefitinib in head and neck cancer. KRT16 is associated with EGFR pathway	148067	Ma et al. (2008) Hickinson et al. (2009)
MLH1	3p21.3	– It heterodimerizes with PMS2 to form MutL alpha, a component of the post-replicative DNA mismatch repair system (MMR)	– MLH1 plays an important role in Laryngeal squamous cell carcinoma (LSCC) development and progression. A correlation of both LOH and hypermethylation with the loss of expression for MLH1 was found. LOH in MLH1 correlates with lower grades of LSCC – Down-regulation of MLH1 in combination with aberrant cell cycle	120436	Sasiadek et al. (2004)

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Table 5.17 (continued)

Gene symbol	Gene locus	Function	Alteration in Cancer	OMIM	References
			control may contribute to chromosomal instability in LSCC. CCND1 and MLH1 seem functionally interconnected in regard to chromosomal imbalances in larynx cancer		
MMP14	14q11-q12	– The protein encoded by this gene is a member of the membrane-type MMP (MT-MMP) subfamily – This protein activates MMP2 protein, and this activity may be involved in tumor invasion	– The over-expression of MMP14 protein may be a marker for lymph node metastasis of laryngeal cancer	600754	Liu et al. (2004)
MMP7	11q21-q22	– The enzyme encoded by this gene degrades proteoglycans, fibronectin, elastin and casein and differs from most MMP family members in that it lacks a conserved C-terminal protein domain	– MMP-7 possesses close relationship with the invasion, metastasis, and prognosis of laryngeal cancer, and it may be served as a marker in estimating the invasive and metastatic potency and prognosis of laryngeal cancer	178990	Sun et al. (2004)
MUC1	1q21	– This gene encodes a membrane-bound protein that is a member of the mucin family – Can act both as an adhesion and an anti-adhesion protein. May provide a protective layer on epithelial cells against bacterial and enzyme attack – The beta subunit contains a C-terminal domain which is involved in cell signaling, through phosphorylations and protein-protein interactions	MUC1 is definitely expressed in SCC larynx. But further study is required to determine its' role	158340	Sipaul et al. (2011)

(continued)

Table 5.17 (continued)

Gene symbol	Gene locus	Function	Alteration in Cancer	OMIM	References
		– Modulates signaling in ERK, SRC and NF-kappa-B pathways – In activated T-cells, influences directly or indirectly the Ras/MAPK pathway – Promotes tumor progression. Regulates TP53-mediated transcription and determines cell fate in the genotoxic stress response – Binds, together with KLF4, the PE21 promoter element of TP53 and represses TP53 activity – This gene is known to contain a highly polymorphic variable number tandem repeats (VNTR) domain			
MUC2	11p15.5	– This gene encodes a member of the mucin protein family – Coats the epithelia of the intestines, airways, and other mucus membrane-containing organs – Thought to provide a protective, lubricating barrier against particles and infectious agents at mucosal surfaces – Major constituent of both the inner and outer mucus layers of the colon and may play a role in excluding bacteria from the inner mucus layer	– This is seen to be expressed in patients suffering from laryngeal carcinoma, but further investigation is needed	158370	Sipaul et al. (2011)

(continued)

Table 5.17 (continued)

Gene symbol	Gene locus	Function	Alteration in Cancer	OMIM	References
MUC4	3q29	– This gene encodes an integral membrane glycoprotein found on the cell surface – May play a role in tumor progression – Has anti-adhesive properties. Seems to alter cellular behavior through both anti-adhesive effects on cell-cell and cell-extracellular matrix interaction – Plays an important role in cell proliferation and differentiation of epithelial cells by inducing specific phosphorylation of ERBB2 – The formation of a MUC4-ERBB2-ERBB3-NRG1 complex leads to down-regulation of CDKN1B, resulting in repression of apoptosis and stimulation of proliferation	There is a survival advantage for patients with advanced-stage nonmetastatic cancer when the MUC 4 gene is expressed	158372	Paleri et al. (2004)
NBN	8q21	– The encoded protein is a member of the MRE11/RAD50 double-strand break repair complex which consists of 5 proteins – This gene product is thought to be involved in DNA double-strand break repair and DNA damage-induced checkpoint activation – Mutations in this gene are associated with Nijmegen breakage syndrome, an autosomal recessive chromosomal	– Specific haplotypes of the NBN gene may be related to increased susceptibility to laryngeal cancer and second primary tumors localized in the head and neck	602667	Ziółkowska–Suchanek et al. (2012)

(continued)

Table 5.17 (continued)

Gene symbol	Gene locus	Function	Alteration in Cancer	OMIM	References
		instability syndrome characterized by microcephaly, growth retardation, immunodeficiency, and cancer predisposition			
PALB2	16p12.2	– This protein binds to and colocalizes with the breast cancer 2 early onset protein (BRCA2) in nuclear foci and likely permits the stable intranuclear localization and accumulation of BRCA2 – Involved in DNA repair and genome stability	– Fanconi anemia (FA) associated genes [FANCA, -B, -C, FANCD1 (BRCA2), -D2, -E, -F, -G, -I, -L, -M, FANCN (PALB2), FANCJ (BRIP1) and FA-linked BRCA1] encode proteins of DNA damage response pathways mutated in FA patients – FA is characterized by congenital malformations, chromosomal instability and high cancer susceptibility – FA patients have a 500–700 times higher risk of head and neck squamous cell carcinoma (HNSCC) compared to the non-FA population	610355	Szaumkessel et al. (2011)
PCNA	20pter-p12	– In response to DNA damage, the protein encoded by this gene is ubiquitinated and is involved in the RAD6-dependent DNA repair pathway	– PCNA play important roles in the progression of hypopharyngeal and laryngeal squamous cell carcinoma and are possible prognostic discriminators in hypopharyngeal and laryngeal squamous cell carcinoma	176740	Li et al. (2005a, b)
PDGFRA	4q12	– This gene encodes a cell surface tyrosine kinase receptor for members of the platelet-derived growth factor family	– PDGFRA protein expression is found in laryngeal cancer cells. High expression of PDGFR is detected in human HNSCC tissues	173490	Ongkeko et al. (2005)

(continued)

Table 5.17 (continued)

Gene symbol	Gene locus	Function	Alteration in Cancer	OMIM	References
		– These growth factors are mitogens for cells of mesenchymal origin – Studies suggest that this gene plays a role in organ development, wound healing, and tumor progression	– PDGFR is tyrosine kinase. This is transmembrane TK receptors that transduce signals from outside and inside the cell and function as relay points for signaling pathways inside the cell – They have a key role in numerous processes that affect tumor development, growth, progression, metastasis, differentiation, and modulation of apoptosis – The presence of these TKs in cancers is important because they can be targets for chemical agents or drugs that inhibit their activity		
PDPN	1p36.21	– It may be involved in cell migration and/or actin cytoskeleton organization	– Podoplanin (PDPN) is involved in tumorigenesis and cancer progression in head and neck malignancies and its expression is not restricted to lymphatic vessel endothelium. Podoplanin expression was extremely low in tumor free resection margins of OSCCs – Podoplanin and ABCG2 in OLP(Oral lichen planus) is a potentially malignant disorder associated with an increased risk for oral cancer. Podoplanin and ABCG2 may be useful as biomarkers for	608863	Margaritescu et al. (2010) Shi et al. (2010a, b) Rodrigo et al. (2010)

(continued)

Table 5.17 (continued)

Gene symbol	Gene locus	Function	Alteration in Cancer	OMIM	References
			risk assessment of oral malignant transformation in patients with OLP – Podoplanin expression increases in the early stages of laryngeal tumourigenesis and it seems to be associated with a higher laryngeal cancer risk. Podoplanin expression in laryngeal squamous cell carcinomas diminishes during tumour progression		
RAD23B	9q31.2	– This protein may be involved in the ubiquitin mediated proteolytic pathway in cells	– Val-carriers of RAD23B Ala249Val had an increased cancer risk in heavy smokers and high alcohol consumers	600062	Abbasi et al. (2009)
RARB	3p24	– It is thought that this protein limits growth of many cell types by regulating gene expression	– RARB is significantly expressed in laryngeal squamous cell carcinoma – RARB is most frequently hypermethylated in primary HNSCC – Overexpression of retinoic acid receptor beta induces growth arrest and apoptosis in oral cancer cell lines	180220	Ma et al. (2008) Chen et al. (2007a, b)
RECQL4	8q24.3	– The protein encoded by this gene is a DNA helicase that belongs to the RecQ helicase family. DNA helicases unwind double-stranded DNA into single-stranded DNAs and may modulate chromosome segregation	– Gain of RECQL4 copy number was associated with late LSCC	603780	Saglam et al. (2007)
RUNX3	1p36	– It functions as a tumor suppressor, and the gene is frequently deleted or transcriptionally silenced in cancer	– Hypermethylation of Runx3 promoter is one of the inactivation re-seasons in laryngeal squamous cell carcinoma, and	600210	Tang et al. (2010)

(continued)

Table 5.17 (continued)

Gene symbol	Gene locus	Function	Alteration in Cancer	OMIM	References
			the decreasing of Runx3 mRNA expression may be related to lymph node metastasis and development of laryngeal squamous cell carcinoma		
S100A8	1q21	– The protein encoded by this gene is a member of the S100 family of proteins – S100 proteins are involved in the regulation of a number of cellular processes such as cell cycle progression and differentiation	– The novel partners of S100A8 identified in the study might be involved in NF-kappa B pathway – The binding ability of MHC class I HLA-B to S100A8 implies that S100A8 might function as a new member with other proteins including HLA-B in NF-kappa B pathway – These findings provide a new clue to further study on the molecular mechanism of S100A8 in the genesis of laryngeal carcinomas	123885	Fu et al. (2007)
SH3GL2	9p22	– A novel tumor suppressor gene in laryngeal squamous cell carcinoma (LSCC) – Induces apoptosis of tumor cells by regulating intra-cellular signal transduction networks	– SH3GL2 a novel tumor suppressor gene in laryngeal squamous cell carcinoma (LSCC), induces apoptosis of tumor cells by regulating intra-cellular signal transduction networks – Down-regulation of SH3GL2 promoted apoptosis while decreasing cell proliferation	604465	Shang et al. (2010)
SKP2	5p13	– This gene encodes a member of the F-box protein family which is an essential element of the cyclin A-CDK2 S-phase kinase	– S-phase kinase-associated protein 2 (Skp2) is related to cellular proliferation and differentiation in laryngeal carcinoma	601436	Liu et al. (2010a, b, c, d)

(continued)

Table 5.17 (continued)

Gene symbol	Gene locus	Function	Alteration in Cancer	OMIM	References
			– It is upregulated in laryngeal carcinoma. Overexpression of Skp2 is frequently found in oral squamous cell carcinoma		Kudo et al. (2005)
SLC2A1	1p34.2	– This gene encodes a major glucose transporter in the mammalian blood-brain barrier – Facilitative glucose transporter. This isoform may be responsible for constitutive or basal glucose uptake – Has a very broad substrate specificity; can transport a wide range of aldoses including both pentoses and hexoses	– Recently, it was shown that the increased expression of SLC2A1 in head and neck carcinomas is correlated with lymph node metastasis, poor survival and clinical stage. Glucose transporter-1 (Glut-1; encoded by the SLC2A1 gene in humans) is the main transporter of glucose in solid carcinomas and has become a focus of cancer research. Thus, it is proposed that the suppression of SLC2A1 expression is a new therapeutic target for laryngeal carcinoma	138140	Luo et al. (2010)
UGT1A	2q37	– This gene represents a complex locus that encodes several UDP-glucuronosyltransferases – Each first exon encodes the substrate binding site, and is regulated by its own promoter – UDPGT is of major importance in the conjugation and subsequent elimination of potentially toxic xenobiotics and endogenous compounds	– High activity UGT1A1 polymorphisms is present significantly in patients with laryngeal cancer, older patients, heavy smokers and heavy drinkers – High activity UGT1A1*1 polymorphism, which results in lower serum levels of the endogenous antioxidant bilirubin, is associated with an increased risk of head and neck cancer	191740	Lacko et al. (2010)

(continued)

Table 5.17 (continued)

Gene symbol	Gene locus	Function	Alteration in Cancer	OMIM	References
XIAP	Xq25	– This gene encodes a protein that belongs to a family of apoptotic suppressor proteins – X-linked inhibitor of apoptosis protein (XIAP) is a novel member of the inhibitors of apoptosis (IAPs) family – The overexpression of XIAP is associated with radioresistance of human malignancies	– XIAP is overexpressed in laryngeal cancer – RNAi-mediated downregulation of XIAP expression can inhibit proliferation, induce apoptosis and diminish the radioresistance of laryngeal carcinoma cells	300079	Wang et al. (2009)

Table 5.18 Most reported miRNAs involved in laryngeal cancer

MiRNA	Function	Alteration in cancer	References
mir-21	– miR-21 functions as an oncogene and modulates tumorigenesis through regulation of several genes – miR-21 regulates cell growth, cytochrome C release, and apoptosis	High expression in HNOC	Ren et al. (2010)
miR-28	– miR-28 targets the 3′untranslated (3′ UTR) region of MPL(myeloproliferative leukemia), inhibiting its translation, as well as other proteins potentially involved in mega karyocyte differentiation, such as E2F6 – Expression of miR-28 in CD34-derivedmegakaryocytes inhibited terminal differentiation	High expression in HNOC	Shiiba et al. (2010)
miR-34c	– Members of the miR-34 family are known to induce a senescent-like growth arrest when overexpressed in cells, with miR-34c being the most potent – miR-34c inhibition of Myc in response to DNA damage prevents S-phase progression	Downregulatedin HNOC	Cai et al. (2010)
miR-137	– miR-137 has been implicated to act as a tumor suppressor in several cancer types via cell cycle control – miR-137 regulates cell growth and CDK6 expression – miR-137 has also been shown to regulate dendritic development and maturation of neurons	Downregulatedin HNOC	Langevin et al. (2010)
miR-378	– MicroRNA-378 promotes cell survival, tumor growth, and angiogenesis by targeting SuFu and Fus-1 expression – It also regulates CYP2E1, one of the pharmacologically and toxicologically important cytochrome P450 isoforms	Expression lowered in HNOC	Wang et al. (2010a, b)
miR-376c	– miR-376c enhances proliferation, survival and chemoresistance by targeting, at least in part, ALK7 (activin receptor-like kinase 7)	Downregulatedin HNOC	Kozaki et al. (2008)
miR-144	– miR-144 that is highly conserved appeared to be associated with the aging progression – miR-144 plays a central role in regulating the expression of ataxin 1 (ATXN1), the	Downregulatedin Head and Neck Cancer	Wang et al. (2010a, b)

(continued)

Table 5.18 (continued)

MiRNA	Function	Alteration in cancer	References
	disease-causing gene for the development spinocerebellar ataxia type 1 (SCA1) – miR-144 directly regulates nuclear factor-erythroid 2-related factor 2, a central regulator of cellular response to oxidative stress, and modulates the oxidative stress response in primary erythroid progenitor cells		

5.8 Oral Cancer

Uncontrollable growth of cells that invade and cause damage in surrounding tissue leads to cancer. Oral cancer is a general word which includes cancer of the tongue, floor of the mouth, throat, palate, and lips. These cancers may be considered as the life-threatening diseases, especially if they are not be diagnosed and treated early (Speight et al. 1996; Walker et al. 2003). Oral cancer is one of the most common cancers in the world and is one of 10 more common causes of cancer related death. Epidemiological studies show that the prevalence of oral cancer is varied in different parts of the world and its frequency differs from less than 0.1% to over 40%. Unfortunately, the majority of oral cancers are diagnosed in advanced stages of the disease (Warnakulasuriya 2009).

Smoking, excessive consumption of alcohol, excessive sun exposure, infection with Human papillomavirus (HPV), and family history of cancer are the more common causes of the oral cancers (Petersen et al. 2005; Ragin et al. 2007). Regardless of the etiology, these factors ultimately cause genomic instability, activation of oncogenes, tumor suppressor inactivation and other molecular dis-regulation. These irregularities lead to uncontrollable cell proliferation and ultimately oral cancer. We summarized an overview to most reported gene involved in oral cancer (Table 5.19).

5.8.1 Genetic Pathology

See Table 5.19

Table 5.19 Summary of reported genes involved in oral cancer pathogenesis through mutation, deregulation, methylation and other related mechanisms

Gene	Location	Function	Alteration in cancer	OMIM	References
ABCB1 (MDR1)	7q21.12	– The membrane-associated protein encoded by this gene is a member of the superfamily of ATP-binding cassette (ABC) transporters – The protein encoded by this gene is an ATP-dependent drug efflux pump for xenobiotic compounds with broad substrate specificity	ABCB1is up-regulated in a CD44 + ve HNSCC stem cells. This gene is related to chemoresistance	171050	Okamoto et al. (2009)
ACE	17q23.3	– This gene encodes an enzyme involved in catalyzing the conversion of angiotensin I into a physiologically active peptide angiotensin II	– The insertion/deletion (I/D) polymorphism, by affecting the ACE gene expression, is associated with the progress of oral oncogenesis – A significant increase of I alleles is observed in patients regardless their smoking or alcohol consumption habits, early or advanced stage of cancer, presence or absence of a family history for cancer or thrombophilia	106180	Vairaktaris et al. (2007a, b)
AKT2	19q13.1-q13.2	– The encoded protein is a general protein kinase capable of phophorylating several known proteins – This gene is a putative oncogene	– It was investigated the overexpression of pan-Akt and its phosphorylated form (p-Akt), Akt1, and Akt2 in oral squamous cell carcinoma (OSCC)cell.p-Akt and Akt2 were overexpressed in all oral cancer cell lines in comparison with	164731	Iamaroon and Krisanaprakornkit (2009)

(continued)

Table 5.19 (continued)

Gene	Location	Function	Alteration in cancer	OMIM	References
			human oral keratinocytes (HOKs), whereas Akt2 mRNA was constitutively expressed, suggesting post-transcriptional regulation		
AREG	4q13-q21	– Bi-functional growth-modulating glycoprotein – Inhibits growth of several human carcinoma cells in culture and stimulates proliferation of human fibroblasts and certain other tumor cells	– Higher mRNA levels of amphiregulin (AREG) is found in epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor gefitinib sensitive versus resistant head and neck cancer cell lines – AREG is over-expressed at least 10-fold in tumors over any of the normal tissues in HNSCC patients. AREG protein is expressed in the tumor samples with high intensity	104640	Hickinson et al. (2009)
ATP2B1 (PMCA1)	12q21.3	– The protein encoded by this gene belongs to the family of P-type primary ion transport ATPases characterized by the formation of an aspartyl phosphate intermediate during the reaction cycle – These enzymes remove bivalent calcium ions from eukaryotic cells against very large concentration gradients and play a critical role in intracellular calcium homeostasis	– Inactivation of the PMCA1 gene is a frequent and early event during oral carcinogenesis, and gene expression may be regulated by an epigenetic mechanism	108731	Saito et al. (2006)

(continued)

Table 5.19 (continued)

Gene	Location	Function	Alteration in cancer	OMIM	References
ATP7B	13q14.3	– This protein functions as a monomer, exporting copper out of the cells, such as the efflux of hepatic copper into the bile	– ATP7B may be a key determinant in the acquired resistance to CDDP in OSCC – Overexpression of ATP7B in esophageal carcinoma could be associated with unfavorable clinical outcome in patients treated with cisplatin-based chemotherapy – Therefore, ATP7B gene expression might be considered as a chemoresistance marker for cisplatin in the patients of esophageal carcinoma and provider of important information on the strategy against esophageal carcinoma – High levels of ATP7B expression in oral SCC are associated with unfavorable clinical outcome in patients with oral SCCs treated with cisplatin-based chemotherapy – ATP7B expression may be a preoperative indicator for a choice of cisplatin in some patients	606882	Yoshizawa et al. (2007)

(continued)

Table 5.19 (continued)

Gene	Location	Function	Alteration in cancer	OMIM	References
AURKB	17p13.1	– The Aurora kinases associate with microtubules during chromosome movement and segregation – Aurora kinase B localizes to microtubules near kinetochores, specifically to the specialized microtubules called K-fibers	– Aurora-B expression is well correlated with cell proliferation, induction of multinuclear cells, histological differentiation, and metastasis in OSCC – Aurora-B may be involved in tumor progression and can be a new diagnostic and therapeutic target for OSCC	604970	Qi et al. (2007)
CAV1	7q31.1	– The scaffolding protein encoded by this gene is the main component of the caveolae plasma membranes found in most cell types – Interacts directly with G-protein alpha subunits and can functionally regulate their activity – Involved in the costimulatory signal essential for T-cell receptor (TCR)-mediated T-cell activation – Recruits CTNNB1 to caveolar membranes and may regulate CTNNB1-mediated signaling through the Wnt pathway	– Caveolin-1 inhibits the growth of human laryngeal squamous cell carcinoma. Downregulation of EGFR-mitogen-activated protein kinase signaling pathway may be critical for understanding its mechanism of tumor suppression – Caveolin-1, a tumor suppressor gene, inactivated by structural abnormalities or epigenetic changes plays a role in the pathogenesis of oral cancer	601047	Han et al. (2004)

(continued)

Table 5.19 (continued)

Gene	Location	Function	Alteration in cancer	OMIM	References
CCNA1	13q12.3-q13	– May be involved in the control of the cell cycle at the G1/S (start) and G2/M (mitosis) transitions – May primarily function in the control of the germline meiotic cell cycle and additionally in the control of mitotic cell cycle in some somatic cells	– Significant tumour specific methylation of cyclin A1 promoter is seen in oral cancer	604036	Shaw et al. (2006)
CCR2	3p21.31	– This gene encodes two isoforms of a receptor for monocyte chemoattractant protein-1, a chemokine which specifically mediates monocyte chemotaxis – Monocyte chemoattractant protein-1 is involved in monocyte infiltration in inflammatory diseases such as rheumatoid arthritis as well as in the inflammatory response against tumors	Genetic polymorphism of CCR2 may contribute to the susceptibility to oral cancer	601267	Chen et al. (2011a, b, c)
CD40	20q12-q13.2	– Protein encoded by this gene is a member of the TNF-receptor superfamily – This receptor has been found to be essential in mediating a broad variety of immune and inflammatory responses including T cell-dependent immunoglobulin class switching, memory B cell development, and germinal center formation	– CD40 is expressed on basal keratinocytes and Squamous Cell Cancer of the Head and Neck (SCCHN) tumor cells in vivo and in vitro – CD40 signaling may enhance the survival of SCCHN and tumor stroma	109535	Cao et al. (2005)

(continued)

Table 5.19 (continued)

Gene	Location	Function	Alteration in cancer	OMIM	References
CDC20	1p34.1	– CDC20 appears to act as a regulatory protein interacting with several other proteins at multiple points in the cell cycle	– It was observed overexpression of CDC20 in several oral squamous cell carcinoma (OSCC) cell lines and primary head and neck tumors – Thus, abnormalities in the cellular level of CDC20 may deregulate the timing of anaphase promoting complex (APC/C) in promoting premature anaphase, which often results in aneuploidy in the tumor cells	603618	Mondal et al. (2006)
CDH1	16q22.1	– CDH1 is involved in mechanisms regulating cell-cell adhesions, mobility and proliferation of epithelial cells – Has a potent invasive suppressor role	– CDH1-347 polymorphisms are associated with increased risks of oral cancer, and may be a predictive factor for tumor lymph node metastasis – CDH1 promoter is often methylated in HNSCC which leads to its epigenetic inactivation – The promoter hypermethylation of CDH1 was significantly related with better survival in HNSCC treated with radiotherapy	192090	Chien et al. (2012)
CDH11	16q22.1	– This gene encodes a type II classical cadherin from the cadherin superfamily, integral membrane proteins that mediate	– CDH11 gene expression was upregulated in OSCC tissue compared with control tissue	600023	Choi et al. (2008)

(continued)

Table 5.19 (continued)

Gene	Location	Function	Alteration in cancer	OMIM	References
		calcium-dependent cell-cell adhesion	– CDH11 encodes an integral membrane protein, cadherin-11, which mediates cell-cell adhesion and is thought to be involved in bone cell differentiation and bone formation		
CDK2	12q13	– The protein encoded by this gene is a member of the Ser/Thr protein kinase family – It is a catalytic subunit of the cyclin-dependent protein kinase complex, whose activity is restricted to the G1-S phase, and essential for cell cycle G1/S phase transition	– CDK2 is a critical factor in oral cancer progression and can be used as a negative predictive marker of the patients' prognosis	116953	Mihara et al. (2001)
CDK4	12q14	– The protein encoded by this gene is a member of the Ser/Thr protein kinase family – It is a catalytic subunit of the protein kinase complex that is important for cell cycle G1 phase progression – This kinase was shown to be responsible for the phosphorylation of retinoblastoma gene product (Rb)	– Cyclin Dependent Kinase 4 (Cdk4), an oncogene, is over-expressed in oral cancer, either by genomic amplification or by c-myc dependent manner – CdK4 Regulating Factor (KRF) maintains the basal level transcription in normal and activates Cdk4 transcription in the initial stage, whereas the same role is carried by c-myc in higher stage of chewing tobacco mediated oral cancer development	123829	Mishra and Das (2003)

(continued)

Table 5.19 (continued)

Gene	Location	Function	Alteration in cancer	OMIM	References
CDKN1A	6p21.2	– It is a regulator of cell cycle progression DNA damage repair	– The tumor suppressor gene p53 and its downstream effector p21 (CDKN1A/WAF1/CIP1) are thought to play major roles in the development of human malignancy – Polymorphic variants of p53, at codon 72, and CDKN1A, at codon 31, have been associated with oral cancer susceptibility	116899	Bau et al. (2007)
CDKN2A (p16INK4a)	9p21	– It is capable of inducing cell cycle arrest in G1 and G2 phases. It acts as a tumor suppressor	– Promoter hypermethylation of CDKN2A was detected with subsequent progression to LSCC – Fragile histidine triad and p16INK4a expression are altered in malignant lesions – Most likely, the decreasing levels of fragile histidine triad is directly involved in cancer development, while the accumulation of p16INK4a in head and neck squamous cell carcinoma may be the consequence of loss of functional tumor suppressor retinoblastoma pathway	600160	Paradiso et al. (2004)

(continued)

Table 5.19 (continued)

Gene	Location	Function	Alteration in cancer	OMIM	References
CHD5	1p36.31	– CHD5 belongs to a group of SWI/SNF proteins called CHD proteins, which contain a SWI/SNF-like helicase/ATPase domain, as well as a DNA-binding domain and a chromodomain that directly modifies chromatin structure	– CHD5 is a tumor suppressor gene that is epigenetically downregulated in LSCC	610771	Wang et al. (2011a, b)
CLDN1	3q28-q29	– Tight junctions represent one mode of cell-to-cell adhesion in epithelial or endothelial cell sheets, forming continuous seals around cells and serving as a physical barrier to prevent solutes and water from passing freely through the paracellular space – The protein encoded by this gene, a member of the claudin family	– CLDN1 over-expression is significantly correlated with more advanced-stage tumors of oral cancer	603718	Warner et al. (2004)
CLEC3B	3p22-p21.3	– Tetranectin binds to plasminogen and to isolated kringle 4 – It may be involved in the packaging of molecules destined for exocytosis	– Tetranectin, a protein encoded by the CLEC3B gene, is found significantly under-expressed in both serum and saliva of metastatic OSCC compared to primary OSCC	187520	Arellano-Garcia et al. (2010)
COL5A2	2q14-q32	– It is a minor connective tissue component of nearly ubiquitous distribution	– COL5A2 is upregulated in the primary OSCC relative to normal tissue	120190	Ziober et al. (2006)

(continued)

Table 5.19 (continued)

Gene	Location	Function	Alteration in cancer	OMIM	References
CTNNAL1	9q31.2	– May modulate the Rho pathway signaling by providing a scaffold for the Lbc Rho guanine nucleotide exchange factor (ARHGEF1)	– Microarray analysis was performed using a panel of anoikis-resistant oral cancer cell lines grown under attached and detached growth conditions – KLK6 gene whose expression levels were differentially regulated in the anoikis-resistant cell lines compared to the anoikis-sensitive cells under detached conditions – The anoikis-resistant phenotype of squamous cell carcinoma has a distinct genetic expression pattern that is marked by chromosomal alterations that may contribute to differential expression of genes involved in diverse cellular functions – Therapies targeting these potential mediators of anoikis resistance may prove to be beneficial in the treatment of metastatic squamous cell carcinoma	604785	Kupferman et al. (2007)
CTSB	8p22	– The protein encoded by this gene is a lysosomal cysteine proteinase – It is also known as amyloid precursor protein secretase and is involved in the proteolytic processing of amyloid precursor protein (APP)	– Lysosomal protease cathepsin B mediates TRAIL-induced cell death in oral squamous cell carcinoma (OSCC) cells	116810	Nagaraj et al. (2006)

(continued)

Table 5.19 (continued)

Gene	Location	Function	Alteration in cancer	OMIM	References
CTSD	11p15.5	– This gene encodes a lysosomal aspartyl protease – Acid protease active in intracellular protein breakdown – Plays a role in APP processing following cleavage and activation by ADAM30 which leads to APP degradation – Involved in the pathogenesis of several diseases such as breast cancer and possibly Alzheimer disease	– Increased expression of cathepsin D correlated significantly with the presence of metastasis, poor histologic malignancy grade, and high proliferation rate in oral carcinomas	116840	Vigneswaran et al. (2000)
CXCL1 (GRO1)	4q21	– Chemokines are a group of small (approximately 8 to 14 kD), mostly basic, structurally related molecules that regulate cell trafficking of various types of leukocytes through interactions with a subset of 7-transmembrane, G protein-coupled receptors – Chemokines also play fundamental roles in the development, homeostasis, and function of the immune system, and they have effects on cells of the central nervous system as well as on endothelial cells involved in angiogenesis or angiostasis	– GRO-1 is upregulated in oral cancer – GRO-1 expression is also associated with leukocyte infiltration, and lymph node metastasis – These suggest a possible relationship between the expression level of GRO-1 and tumor progression	155730	Shintani et al. (2004)

(continued)

Table 5.19 (continued)

Gene	Location	Function	Alteration in cancer	OMIM	References
CXCL12 (SDF1)	10q11.1	– It can activate lymphocytes and have been implicated in the metastasis of some cancers	– The chemokine stromal cell-derived factor (SDF-1/CXCL12) and its specific receptor, CXCR4, have been implicated in the regulation of tumor growth and organ-specific spread – The expression of CXCL12 and CXCR4 has been determined in samples obtained from primary squamous cell carcinoma (SCC) of the oral cavity (OCSCC) and of the lip (LSCC) and in metastatic and non-metastatic lymph node tissues – The G801A polymorphism of the SDF-1 gene is associated with advanced stages of oral cancer, especially in alcohol abusers	600835	Oliveira-Neto et al. (2008)
CYB5R3 (DIA1)	22q13.2-q13.31	– This gene encodes cytochrome b5 reductase, which includes a membrane-bound form in somatic cells (anchored in the endoplasmic reticulum, mitochondrial and other membranes) and a soluble form in erythrocytes – The membrane-bound form exists mainly on the cytoplasmic side of the endoplasmic reticulum and	– Relative copy number loss involving the DIA1 gene is correlated to family history of oral cancer, death, and consumption of alcohol	613213	Reis et al. (2002)

(continued)

Table 5.19 (continued)

Gene	Location	Function	Alteration in cancer	OMIM	References
		functions in desaturation and elongation of fatty acids, in cholesterol biosynthesis, and in drug metabolism			
CYGB	17q25.3	– Cytoglobin is a ubiquitously expressed hexacoordinate hemoglobin that may facilitate diffusion of oxygen through tissues, scavenge nitric oxide or other reactive oxygen species, or serve a protective function during oxidative stress	– Cytoglobin is a novel candidate tumour suppressor gene highly methylated in upper aero-digestive tract squamous cancer – In clinically derived HNSCC samples, CYGB mRNA expression shows a striking correlation with tumour hypoxia and consistent associations with histopathological measures of tumour aggression – CYGB expression also shows a marked negative correlation with promoter methylation – In the HNSCC cell lines cultured under hypoxic conditions, a trend of increasing expression of both CYGB and HIF1A with progressive hypoxia is observed	608759	Shaw et al. (2006)
CYP1B1	2p21	– The enzyme encoded by this gene localizes to the endoplasmic reticulum and metabolizes procarcinogens such as polycyclic aromatic hydrocarbons and 17beta-estradiol	– Interindividual variation in CYP1B1 expression may account in part for variation in tobacco-related oral SCC risk – CYP1B1 may be an important early biomarker for risk of tobacco-induced oral SCC development	601771	Chi et al. (2009)

(continued)

Table 5.19 (continued)

Gene	Location	Function	Alteration in cancer	OMIM	References
CYP2A13	19q13.2	– This gene encodes a member of the cytochrome P450 superfamily of enzymes – The cytochrome P450 proteins are monooxygenases which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids	– The aberrant hypermethylation of CYP2A13 gene is found in head and neck cancer tissues – Significant interaction between smoking and methylation status of CYP2A13 is observed in head and neck cancer	608055	Sharma et al. (2010)
CYP2E1	10q24.3-qter	– This gene encodes a member of the cytochrome P450 superfamily of enzymes – The enzyme encoded by this gene may be involved in such varied processes as gluconeogenesis, hepatic cirrhosis, diabetes, and cancer	– CYP2E1 Rsa I/Pst I c2 allele may be a biomarker for oral cancer, especially among Asian populations	124040	Niu et al. (2012)
CYR61	1p22.3	– CYR61 is a secreted, cysteine-rich, heparin-binding protein encoded by a growth factor-inducible immediate-early gene	– Cyr61 expression is significantly correlated with Ki-67 expression and may have potential value in screening high-risk cases for recurrence and metastasis, as well as identifying poor prognosis in SACC patients	602369	Tang et al. (2011a, b)
DKK1	10q11.2	– DKKs play an important role in vertebrate development, where they locally inhibit Wnt regulated processes such as antero-posterior	– Dkk1 plays an important role in regulating cellular migration and invasiveness, making Dkk1 a potential biomarker for early	605189	Ogoshi et al. (2011)

(continued)

Table 5.19 (continued)

Gene	Location	Function	Alteration in cancer	OMIM	References
		axial patterning, limb development, somitogenesis and eye formation – In the adult, Dkks are implicated in bone formation and bone disease, cancer and Alzheimer disease – Dickkopf-1 (Dkk1), a negative regulator of the Wnt signaling pathway, is implicated in tumorigenesis in several types of cancer	detection of lymph node metastasis in OSCCs.		
DPAGT1	11q23.3	– The protein encoded by this gene is an enzyme that catalyzes the first step in the dolichol-linked oligosaccharide pathway for glycoprotein biosynthesis – This enzyme belongs to the glycosyltransferase family 4	– Oral squamous cellcarcinomas displays over-expression of DPAGT1 that correlated with diminished localization of E-cadherin and alpha-catenin at the sites of adherens junctions – The inverse relationship between DPAGT1 expression and intercellular adhesion is a feature of oral squamous cell carcinoma – DPAGT1 is an upstream regulator of E-cadherin N-glycosylation status and adherens junction composition and suggest that dysregulation of DPAGT1 causes disturbances in intercellular adhesion in oral cancer	191350	Nita-Lazar et al. (2009)

(continued)

Table 5.19 (continued)

Gene	Location	Function	Alteration in cancer	OMIM	References
DSPP	4q21.3	– This gene encodes two principal proteins of the dentin extracellular matrix of the tooth – The preproprotein is secreted by odontoblasts and cleaved into dentin sialoprotein and dentin phosphoprotein – Dentin phosphoprotein is thought to be involved in the biomineralization process of dentin	– DSPP-silencing in OSC2 cell decreased salient hallmarks of oral tumorigenesis and provides the first functional evidence of a potential key role for DSPP in oral cancer biology – The down-regulation of MMP-2, MMP-3, MMP-9, p53 and VEGF in DSPP-silenced OSC2 cells provides a significant functional/molecular framework for deciphering the mechanisms of DSPP activities in oral cancer biology	125485	Joshi et al. (2010)
ECT2	3q26.1-q26.2	– The protein encoded by this gene is a transforming protein that is related to Rho-specific exchange factors and yeast cell cycle regulators	– ECT2 is an indicator of cellular proliferation in OSCCs and ECT2 is a potential therapeutic target for the development of new treatments for OSCCs	600586	Iyoda et al. (2010)
EDN1 (ET1)	6p24.1	– The protein encoded by this gene is proteolytically processed to release a secreted peptide termed endothelin 1 – This peptide is a potent vasoconstrictor and is produced by vascular endothelial cells – Endothelin 1 also can affect the central nervous system	– ET-1 mRNA in significantly overexpressed in the oral SCC patients	131240	Pickering et al. (2007)

(continued)

Table 5.19 (continued)

Gene	Location	Function	Alteration in cancer	OMIM	References
EGFR	7p12	– The protein encoded by this gene is a transmembrane glycoprotein and leads to cell proliferation	– Overexpression of EGFR is a common event in many human solid tumors. Elevated levels of EGFR mRNA in human cancer occur with and without gene rearrangement – Structural alterations in the receptor can also result in the dysregulation of the EGFR pathway – EGFR overexpression without gene re-arrangement is frequently observed in human oral cancers	131550	Hiraishi et al. (2008)
ELAVL1 (HUR)	19p13.2	– The protein encoded by this gene is a member of the ELAVL protein family – This encoded protein contains 3 RNA-binding domains and binds cis-acting AU-rich elements – It destabilizes mRNAs and thereby regulates gene expression	– HuR binds to AU-rich element-containing mRNA to protect them from rapid degradation – HuR knockdown changes the oncogenic properties of oral cancer cells, at least in part, by affecting their cell cycle and shows potential as an effective therapeutic approach	603466	Kakuguchi et al. (2010)
ENG (CD105)	9q33-q34.1	– This gene encodes a homodimeric transmembrane protein which is a major glycoprotein of the vascular endothelium – This protein is a component of the transforming growth factor beta receptor complex and it binds TGFB1 and TGFB3 with high affinity	– High expressions of CD105 is significantly correlated with positive nodal metastasis – The expressions of both CD105 and VEGF can be useful to guide the elective treatment for clinical N0 neck in early oral cancer	131195	Chien et al. (2006)

(continued)

Table 5.19 (continued)

Gene	Location	Function	Alteration in cancer	OMIM	References
ERBB2	17q21.1	– This gene encodes a member of the epidermal growth factor (EGF) receptor family of receptor tyrosine kinases	– Amplification and/or overexpression ErbB2 regulates FAS expression in HNSCC and point out Ki-67 as a useful prognostic marker for these tumors	164870	Silva et al. (2004)
ERCC6	10q11.23	– This gene encodes a DNA-binding protein that is important in transcription-coupled excision repair – The protein has ATP-stimulated ATPase activity; there are contradictory publications reporting presence or absence of helicase activity	– A allele of the ERCC6 codon 399 is associated with the development of oral cancer and may be a novel useful marker for primary prevention and anticancer intervention	609413	Chiu et al. (2008)
ETS1	11q23.3	– ETS transcriptions factors, such as ETS1, regulate numerous genes and are involved in stem cell development, cell senescence and death, and tumorigenesis – The conserved ETS domain within these proteins is a winged helix-turn-helix DNA-binding domain that recognizes the core consensus DNA sequence GGAA/T of target genes	– Over-expression of Ets-1 protein is a risk factor for lymph node metastasis in oral cancer	164720	Pande et al. (2002)

(continued)

Table 5.19 (continued)

Gene	Location	Function	Alteration in cancer	OMIM	References
FAT1	4q35	– This gene is an ortholog of the Drosophila fat gene, which encodes a tumor suppressor essential for controlling cell proliferation during Drosophila development – The gene product is a member of the cadherin superfamily, a group of integral membrane proteins characterized by the presence of cadherin-type repeats	– Mutations in FAT is an important factor in the development of oral cancer	600976	Nakaya et al. (2007)
FGF2	4q26	– This protein has been implicated in diverse biological processes, such as limb and nervous system development, wound healing, and tumor growth	– Significantly higher levels of expression of FGF2 exist in OSCC tissue compared to normal oral mucosal tissue. In OSCC cells, significant positive correlations exist between the lymphatic microvessel density and FGF2 expression which suggests it may promote lymph angiogenesis in OSCC – The co-expression of angiogenic VEGF and bFGF is positively correlated with increased microvessel density underlines the importance of both factors for tumor angiogenesis in HNSCC. VEGF and bFGF might act cooperatively in the process of neovascularization in human head and neck cancer	134920	Riedel et al. (2000)

(continued)

Table 5.19 (continued)

Gene	Location	Function	Alteration in cancer	OMIM	References
FGFR3	4p16.3	– This gene encodes a member of the fibroblast growth factor receptor (FGFR) family, with its amino acid sequence being highly conserved between members and among divergent species – This particular family member binds acidic and basic fibroblast growth hormone and plays a role in bone development and maintenance	– FGFR3 contributes to malignancy in oral cancer cells and may be useful biomarkers for early detection and possible targets for therapy	134934	Henson and Gollin (2010)
FGFR4	5q35.1-qter	– The protein encoded by this gene is a member of the fibroblast growth factor receptor family, where amino acid sequence is highly conserved between members and throughout evolution – A full-length representative protein would consist of an extracellular region, composed of three immunoglobulin-like domains, a single hydrophobic membrane-spanning segment and a cytoplasmic tyrosine kinase domain – The extracellular portion of the protein interacts with fibroblast growth factors, setting in motion a cascade of downstream signals, ultimately influencing mitogenesis and differentiation	– FGFR4 Arg (388) allele can be associated with increased risk of developing HNSCC and increased cisplatin sensitivity	134935	Ansell et al. (2009)

(continued)

Table 5.19 (continued)

Gene	Location	Function	Alteration in cancer	OMIM	References
GPX1	3p21.3	– This gene encodes a member of the glutathione peroxidase family – Glutathione peroxidase functions in the detoxification of hydrogen peroxide, and is one of the most important antioxidant enzymes in humans	– Polymorphisms in the GPX1 gene may be a marker for second primary tumour development after a primary HNSCC development	138320	Jefferies et al. (2005)
HDAC2	6q21	– This gene product belongs to the histone deacetylase family – This protein forms transcriptional repressor complexes by associating with many different proteins, including YY1, a mammalian zinc-finger transcription factor – Thus, it plays an important role in transcriptional regulation, cell cycle progression and developmental events	– Oral cancer with advanced stage, larger tumor size, or positive lymph node metastasis had higher level of HDAC2 protein expression	605164	Chang et al. (2009)
HOXB7	17q21.3	– This gene is a member of the Antp homeobox family and encodes a protein with a homeobox DNA-binding domain – The encoded nuclear protein functions as a sequence-specific transcription factor that is involved in cell proliferation and differentiation	– HOXB7 contributes to oral carcinogenesis by increasing tumor cell proliferation, and this implies that HOXB7 may be an important determinant of OSCC patient prognosis	142962	Destro et al. (2010)

(continued)

Table 5.19 (continued)

Gene	Location	Function	Alteration in cancer	OMIM	References
HSPA5	9q33.3	– It is involved in the folding and assembly of proteins in the ER	– GRP78 is a putative candidate on mediating the stemness and tumorigenic properties of head and neck cancer initiating cells (HN-CICs) by differential systemic analyses – Subsequently, cells with GRP78 anchored at the plasma membrane (memGRP78 +) exerted cancer stemness properties of self-renewal, differentiation and radio resistance. High levels of Grp78 are significantly associated with advanced cancer and poor survival in head and neck cancer patients – Acquisition of the oncogenic function of Grp78 contributes to aggressive head and neck cancers – Hyper expression of GRP78 is found to be correlated with increasing malignant potential of oral lesions	138120	Wu et al. (2010a, b)
HTR7	10q21-q24	– This is one of the several different receptors for 5-hydroxytryptamine (serotonin), a biogenic hormone that functions as a neurotransmitter, a hormone, and a mitogen – The activity of this receptor is mediated by G proteins that stimulate adenylate cyclase	– Weaker signals of hTR and hTRT gene expression were observed in normal oral epithelia and hyperplasia lesions; hTR and hTRT were positive and the expression of telomerase genes in the precancerous lesions became stronger due to phenotypic	182137	Zhang and Zhang (1999)

(continued)

Table 5.19 (continued)

Gene	Location	Function	Alteration in cancer	OMIM	References
			progression and the increasing degree of dysplasia – Stronger hTR and hTRT expressions were observed in squamous cell carcinoma, with an equal positivity of 81.6% (31 of 38) – Telomerase gene expression is closely related to the malignant degree of oral mucosa – Telomerase is reactivated frequently during late stages of oral precancerous lesions and may play a crucial role in progression of oral cancer		
IFI6	1p35	– The encoded protein may play a critical role in the regulation of apoptosis	– The oral tongue squamous cell carcinoma (OTSCC) is one of the most common types of HNOSCC – It is significantly more aggressive than other forms of HNOSCC, in terms of local invasion and spread – IFI6 is up-regulated control biological processes in OTSCCs	147572	Ye et al. (2008)
IFITM3	11p15.5	– IFN-induced antiviral protein that mediates cellular innate immunity to at least three major human pathogens, namely influenza A H1N1 virus, West Nile virus (WNV), and dengue virus (WNV),	– Smoking and alcohol consumption are considered to be the main risk factors for Oral squamous cell carcinoma (OSCC)	605579	Ye et al. (2008)

(continued)

Table 5.19 (continued)

Gene	Location	Function	Alteration in cancer	OMIM	References
		by inhibiting the early step(s) of replication	– Down regulation of IFITM3 inhibit specific pathways during cancer development and progression		
IFNA2 (IFNA)	9p22	– This gene is a member of the alpha interferon gene cluster on chromosome 9 – Use of the recombinant form of this protein has been shown to be effective in reducing the symptoms and duration of the common cold	– Allelic imbalance or loss of heterozygosity (LOH) on chromosome region 9p22, containing tumor suppressor interferon a cluster (IFNA) gene is associated with the tumorigenesis of oral SCC	147562	Nakanishi et al. (1998)
IGF2	11p15.5	– The major role of IGF2 is as a growth promoting hormone during gestation	– It was analysed in head and neck squamous carcinoma for the loss of imprinting in the IGF2 that determine the implications of this alteration in the development and progression of tumors – Amplification & Overexpression of IGF2 play a role in the oncogenesis of head and neck carcinoma – IGF-2 gene is used as a marker for prediction of the risk of oral carcinogenesis	147470	El-Naggar et al. (1999)

(continued)

Table 5.19 (continued)

Gene	Location	Function	Alteration in cancer	OMIM	References
IGF2R	6q26	– The protein encoded by this gene is a receptor for both, insulin-like growth factor 2 (IGF2) and mannose 6-phosphate (M6P) – This receptor functions in the intracellular trafficking of lysosomal enzymes, the activation of transforming growth factor beta, and the degradation of IGF2	– IGF2R genetic polymorphism may be associated with decreased function of IGF2 receptor there by contributing to the advancement and distant metastasis of localized oral cancer	147280	Yoon et al. (2012)
IGFBP5	2q33-q36	– IGF-binding proteins prolong the half-life of the IGFs and have been shown to either inhibit or stimulate the growth promoting effects of the IGFs on cell culture – They alter the interaction of IGFs with their cell surface receptors	– The IGFBP5 -1195T > C polymorphism is functional and may potentially be a biomarker for susceptibility to late-stage SCCHN – Insulin-like growth factor binding protein-5 (IGFBP-5) is suppressors of head and neck carcinogenesis. Curcumin is an inducer of IGFBP-5 expression in multiple types of oral keratinocytes; it induces IGFBP-5 promoter activity in SAS oral cancer cells	146734	Chang et al. (2010)
IL10	1q31-q32	– The protein encoded by this gene is a cytokine produced primarily by monocytes and to a lesser extent by lymphocytes – This cytokine has pleiotropic effects in immunoregulation and inflammation	– IL-10 gene promoter -1082 A/G (rs1800870) polymorphism, and its haplotype are significantly associated with the risk of oral cancer – IL-10 gene plays an important role in the development of oral cancer	124092	Yao et al. (2008)

(continued)

Table 5.19 (continued)

Gene	Location	Function	Alteration in cancer	OMIM	References
IL4	5q31.1	– The protein encoded by this gene is a pleiotropic cytokine produced by activated T cells – This cytokine is a ligand for interleukin 4 receptor. The interleukin 4 receptor also binds to IL13, which may contribute to many overlapping functions of this cytokine and IL13 – STAT6, a signal transducer and activator of transcription, has been shown to play a central role in mediating the immune regulatory signal of this cytokine	– The IL-4 gene -590 C/T polymorphism is associated with oral cancer and is a suitable genetic marker for screening for oral cancer – However, whether the -590 C/T polymorphism of the IL4 gene plays a role in oral cancer remains unclear	147780	Tsai et al. (2005a, b)
IL6	7p21	– This gene encodes a cytokine that functions in inflammation and the maturation of B cells – In addition, the encoded protein has been shown to be an endogenous pyrogen capable of inducing fever in people with autoimmune diseases or infections	– Increased salivary IL-6 might play a certain role in oral leukoplakia – Elevations in serum level of interleukin 6 (IL-6) in tumor samples were found to be associated with poor clinical outcomes in multiple types of cancer, including SCCHN	147620	Brailo et al. (2006)
ITGA2 (GPIa)	5q11.2	– This gene product belongs to the integrin alpha chain family – Integrins are involved in cell adhesion and also participate in cell-surface mediated signalling	– C807/T807 polymorphism in platelet glycoprotein Ia (GPIa) gene is indeed a genetic predisposing factor which contributes to increased risk for oral cancer	192974	Vairaktaris et al. (2006)

(continued)

Table 5.19 (continued)

Gene	Location	Function	Alteration in cancer	OMIM	References
ITGB6	2q24.2	– Integrin alpha-V/beta-6 is a receptor for fibronectin and cytotactin. It recognizes the sequence R-G-D in its ligands – Internalisation of integrin alpha-V/beta-6 via clathrin-mediated endocytosis promotes carcinoma cell invasion	– Integrin beta6 signaling activates Fyn and thus promotes oral cancer progression	147558	Li et al. (2003)
JUP	17q21	– The membrane-associated plaques are architectural elements in an important strategic position to influence the arrangement and function of both the cytoskeleton and the cells within the tissue	– Catenins and E-cadherin are important epithelial adhesion molecules in normal epithelium. Loss of E-cadherin-catenin adhesion is an important step in the progression of many epithelial cancers. Catenins were highly under-expressed in oral tongue carcinoma, metastatic lymph node, and recurrent tumour. Gamma-catenin had predictive value for nodal metastasis – JUP over expression is a significant factor for cervical lymph node metastasis of tongue squamous cell carcinoma. High ITGB4/JUP levels are associated with a significantly high death rate in TSCC patients	173325	Chow et al. (2001)

(continued)

Table 5.19 (continued)

Gene	Location	Function	Alteration in cancer	OMIM	References
KAL1	Xp22.32	– The encoded protein is similar in sequence to proteins known to function in neural cell adhesion and axonal migration – In addition, this cell surface protein is N-glycosylated and may have anti-protease activity	– Down-regulation of KAI1 protein expression in oral cancer correlates with reduced disease free survival and overall patient survival	300836	Farhadieh et al. (2004)
KLF13	15q12	– KLF13 belongs to a family of transcription factors that contain 3 classical zinc finger DNA-binding domains consisting of a zinc atom tetrahedrally coordinated by 2 cysteines and 2 histidines (C2H2 motif) – These transcription factors bind to GC-rich sequences and related GT and CACCC boxes	– KLF13 contributes to malignancy in oral cancer cells and may be useful biomarkers for early detection and possible targets for therapy	605328	Henson and Gollin (2010)
KLK10	19q13	– Its encoded protein is secreted and may play a role in suppression of tumorigenesis	– KLK10 was over-expressed at least 10-fold in tumors over any of the normal tissues – Expression of KLK10 at the protein level was determined by immunohistochemistry in seven supraglottic laryngeal cancer specimens – So it was concluded that this molecules are potential targets for immunotherapy of HNSCC patients	602673	Dasgupta et al. (2006)

(continued)

Table 5.19 (continued)

Gene	Location	Function	Alteration in cancer	OMIM	References
KRT13 (K13)	17q12-q21.2	– The protein encoded by this gene is a member of the keratin gene family – The keratins are intermediate filament proteins responsible for the structural integrity of epithelial cells	– K13 is a promising tumor marker gene for detecting the micrometastases in oral cancer	148065	Hamakawa et al. (2000)
LAMA3	18q11.2	– Binding to cells via a high affinity receptor, laminin is thought to mediate the attachment, migration and organization of cells into tissues during embryonic development by interacting with other extracellular matrix components	– LAMA3 is amplified and overexpressed in oral SCC. It is associated with cell adhesion and migration – A splice variant of LAMA3 (Laminin alpha 3), LAMA3-A, known to be involved in tumour cell invasion and progression is identified to be expressed in head and neck tumours and associated with hypoxia – The full-length transcript of the gene (LAMA3-B) did not appear to be hypoxia-associated – In head and neck tumours, expression of LAMA3-A has prognostic significance	600805	Snijders et al. (2005)
MAGE A (1-6)	Xq28	– The MAGE genes encode certain tumor-associated antigens recognized by cytotoxic T lymphocytes	– Oral squamous cell carcinoma develops continuously out of predamaged oral mucosa – MAGE-A antigens are tumor antigens that are found solely in malignant transformed cells	300097	Krauss et al. (2011)

(continued)

Table 5.19 (continued)

Gene	Location	Function	Alteration in cancer	OMIM	References
			– These antigens might be useful in distinguishing precancerous from cancerous lesions – The MAGE-A1 gene is expressed in cancer cell lines of salivary glands. It is highly expressed		
MMP13	11q22.3	– It degrades collagen type I and does not act on gelatin or casein – It can have a role in tumoral process	– MMP-13 gene expression-related polymorphism is associated with risk for the highly aggressive form of oral cancer – The high expression A allele of the −77A/G polymorphism seems to be a prognostic factor for tumor progression	600108	Vairaktaris et al. (2007a, b)
MMP2	16q13-q21	– It is involved in diverse functions such as remodeling of the vasculature, angiogenesis, tissue repair, tumor invasion, inflammation, and atherosclerotic plaque rupture	– MMP-2 was implied to contribute to the invasiveness and metastatization of various malignancies because of the degradation of type IV collagen – Active MMP-2 in the serum was found elevated during oral cancer progression – In the metastatic stage, total MMP-2 level and active MMP-2 level is higher than it in the expansive stage – MMP-2 protein and MMP-2 mRNA was found to be increased during oral cancer development	120360	Zhang et al. (2006)

(continued)

Table 5.19 (continued)

Gene	Location	Function	Alteration in cancer	OMIM	References
			– The increase of MMP-2 expression and activation starts prior to the metastasis occurrence, indicating the important role of MMP-2 in the early phase of oral cancer progression – Active MMP-2 level in serum may be a useful indicator for monitoring oral cancer progression		
MYB	6q22-q23	– This gene encodes a transcription factor that is a member of the MYB family of transcription factor genes – This protein plays an essential role in the regulation of hematopoiesis and may play a role in tumorigenesis	– The transcription factor MYB was recently proposed to be a promising oncogene candidate in salivary gland adenoid cystic carcinoma (ACC) – However, the up-regulation of MYB in ACC could not be explained solely by deletion of its 3′ end – An alternative mechanism needs to be proposed for the transcriptional control of MYB in ACC	189990	Shao et al. (2011)
MYC	8q24.21	– Participates in the regulation of gene transcription – Binds DNA in a non-specific manner, yet also specifically recognizes the core sequence 5′-CAC [GA] TG-3′ – Seems to activate the transcription of growth-related genes	– Pathway analysis revealed proteasome machinery, MYC, and ribosomal components as the top gene sets associated with oral cancer risk – In multiple independent data sets, the expression profiles of the genes	190080	Popović et al. (2010)

(continued)

Table 5.19 (continued)

Gene	Location	Function	Alteration in cancer	OMIM	References
			can differentiate head and neck cancer from normal mucosa – Various results showed that gene expression profiles may improve the prediction of oral cancer risk in OPL patients and the significant genes identified may serve as potential targets for oral cancer chemoprevention		
NANOG	12p13.31	– It is a transcription factor critically involved with self-renewal of undifferentiated embryonic stem cells	– Nanog expression is increased in HNC tissues as compared to adjacent normal cells – Overexpression of NANOG is associated with increased metastasis in HNC cells and poor survival of head and neck cancer patients – mRNA expression of the stemness-related gene Nanog is significantly increased in cancer stem cells within HNSCC cell lines – Initiation, growth, recurrence, and metastasis of head and neck squamous cell carcinomas (HNSCC) have been related to the behavior of cancer stem cells (CSC) – Clinically, co-expression of GRP78 and Nanog predicted the worse survival prognosis of HNSCC patients	607937	Yu et al. (2011)

(continued)

Table 5.19 (continued)

Gene	Location	Function	Alteration in cancer	OMIM	References
NES	1q23.1	– Nestin is an intermediate filament protein that was first identified with a monoclonal antibody	– Nestin,a stem cell marker, is up-regulated in HNSCC	600915	Lim et al. (2011)
NOS2 (INOS)	17q11.2-q12	– This gene encodes a nitric oxide synthase which is expressed in liver and is inducible by a combination of lipopolysaccharide and certain cytokines	– INOS gene suppresses the tumorigenicity and metastasis of oral cancer cells	163730	Harada et al. (2004)
ORAOV1 (TAOS1)	11q13.3	– ORAOV1, an oncogene, is overexpressed in various cancers	– TAOS1 (tumor amplified and overexpressed sequence 1) is both amplified and overexpressed in oral cancer cells – ORAOV1 plays pivotal roles in the growth and angiogenesis of OSCC	607224	Huang et al. (2002)
PCBP2 (hnRNP-E2)	12q13.12-q13.13	– The protein encoded by this gene appears to be multifunctional – Along with PCBP-1 and hnRNPK, it is one of the major cellularpoly(rC)-binding proteins	– Human hnRNP E2 is significantly downregulated in oral cancer tissues compared to normal one – Downregulation of hnRNP E2 is a novel mechanism to enhance the resistance of cancer cells to apoptosis	601210	Roychoudhury et al. (2007)
RAD21	8q24	– The protein encoded by this gene is highly similar to the gene product of Schizosaccharomyces pombe rad21, a gene involved in the repair of DNA double-strand breaks, as well as in chromatid cohesion during mitosis	– RAD21 gene is closely related to the invasion and metastasis of oral cancer cells	606462	Yamamoto et al. (2006)

(continued)

Table 5.19 (continued)

Gene	Location	Function	Alteration in cancer	OMIM	References
		– The highly regulated association of this protein with mitotic chromatin specifically at the centromere region suggests its role in sister chromatid cohesion in mitotic cells			
RARB	3p24	– It is thought that this protein limits growth of many cell types by regulating gene expression	– RARB is significantly expressed in laryngeal squamous cell carcinoma – RARB is most frequently hypermethylated in primary HNSCC – Overexpression of retinoic acid receptor beta induces growth arrest and apoptosis in oral cancer cell lines	180220	Hayashi et al. (2001)
RRAS2 (TC21)	11p15.2	– This gene encodes a member of the R-Ras subfamily of Ras-like small GTPases – The encoded protein associates with the plasma membrane and may function as a signal transducer – This protein may play an important role in activating signal transduction pathways that control cell proliferation	– TC21 expression is an early event in oral cancer and correlates with poor prognosis of OSCCs – TC21 interactions with Erk2, PI3-K, 14-3-3zeta and 14-3-3sigma proteins in oral cancer cells and tissues suggests the involvement of TC21 in signaling pathways in oral cancer	600098	Macha et al. (2010)
S100A2	1q21	– The protein encoded by this gene is a member of the S100 family of proteins containing 2 EF-hand calcium-binding motifs	– Loss of nuclear S100A2 serves as an independent prognostic marker for early-stage oral cancer patients at high risk of recurrence	176993	Tsai et al. (2005a, b)

(continued)

Table 5.19 (continued)

Gene	Location	Function	Alteration in cancer	OMIM	References
		– S100 proteins are localized in the cytoplasm and/or nucleus of a wide range of cells, and involved in the regulation of a number of cellular processes such as cell cycle progression and differentiation			
SIRT3	11p15.5	– This gene encodes a member of the sirtuin family of proteins, homologs to the yeast Sir2 protein – Members of the sirtuin family are characterized by a sirtuin core domain and grouped into four classes	– A novel role has been revealed for SIRT3 in oral cancer carcinogenesis as a promoter of cell proliferation and survival, thus implicating SIRT3 as a new potential therapeutic target to treat oral cancer	604481	Alhazzazi et al. (2011)
SKP2	5p13	– This gene encodes a member of the F-box protein family which is an essential element of the cyclin A-CDK2 S-phase kinase	– S-phase kinase-associated protein 2 (Skp2) is related to cellular proliferation and differentiation in laryngeal carcinoma – It is upregulated in laryngeal carcinoma – Overexpression of Skp2 is frequently found in oral squamous cell carcinoma	601436	Liu et al. (2010a, b, c, d)

(continued)

Table 5.19 (continued)

Gene	Location	Function	Alteration in cancer	OMIM	References
SP1	12q13.1	– It preferentially catalyzes the dephosphorylation of 'Ser-5' within the tandem 7 residues repeats in the C-terminal domain (CTD) of the largest RNA polymerase II subunit POLR2A	– Up-regulation of Sp1 is observed in oral cancer cells – MEPC decreases an apoptosis-related downstream target of Sp1 protein, survivin – Emodin can inhibit oral cancer cell growth and induced caspase-dependent apoptosis by decreasing Sp1	189906	Shin et al. (2011)
TP53	17p13.1	– It acts as a tumor suppressor in many tumor types; induces growth arrest or apoptosis depending on the physiological circumstances and cell type	– TP53 is a key gene in cellular homeostasis and is frequently mutated in head and neck squamous cell carcinoma (HNSCC). In HNSCCs, a truncating TP53 mutation is associated with a poor prognosis – TP53 genetic aberration and loss of heterozygosity is significant prognostic factors for OSCC – HNSCC patients with tumours containing TP53 mutations in DNA-binding regions (L2, L3 and LSH motif) had a significantly poorer prognosis and response to radiotherapy	191170	Wang et al. (2011a, b)

5.8.2 *miRNA and Oral Cancer*

Micro RNAs are a large subgroup of non-coding RNAs with length of 18–25 bp which are, evolutionary, conserved. This micro molecules control gene expression through either inhibition of translation or induction of mRNA degradation. The miRNAs are involved in a variety of developmental and physiological processes. In addition, they can act as oncogene and tumor suppressor gene by suppressing the gene expression of targets involved in carcinogenesis. Mechanisms of major alterations in miRNome, can be ectopic expression, single nucleotide polymorphisms and mutations in the precursor sequence of pri-miRNA. Hence, oncomiRs may be used as biomarkers in diagnosis, prediction, and treatment of cancers (Pham 2012; Yoshizawa and Wong 2013). There are many reports on the involvement of miRNAs in development of oral cancer, where more reported ones are listed in Table 5.20.

Table 5.20 miRNA deregulation involved in pathogenesis of oral cancer

MiRNAN	Function	Alteration in cancer	References
mir-21	– miR-21 functions as anoncogene and modulates tumorigenesis through regulation of several genes – miR-21 regulates cell growth, cytochrome C release, and apoptosis	High expression in HNOC	Reis et al. (2010), Chen et al. (2017)
miR-23a	– miR-23a is a novel microRNA normalizer – miR-23a represses Runx2 in the terminally differentiated osteocyte, representing a feedback mechanism to attenuate osteoblastmaturation – A regulatory network have been established with a central role for the miR cluster 23a-27a-24-2 in both progression and maintenance of the osteocyte phenotype	High èxpression in HNOC	Scapoli et al. (2010)
miR-31	– miR-31 acts as a master regulator of metastasis – miR-31 activation in established metastases elicits metastatic regression & prolongs survival – Acute miR-31 expression fails to affect primary tumor growth in many cases	High expression in HNOC	Liu et al. (2010a, b, c, d)

(continued)

Table 5.20 (continued)

MiRNAN	Function	Alteration in cancer	References
miR-320a	– miR-320a as a potential modulator of aquaporin 1 & 4.	High expression in HNOC	Shiiba et al. (2010)
miR-23b	– miR-23b repress bile duct gene expression in fetal hepatocytes while promoting their growth by down-regulating Smads and consequently TGF beta signalling – Low levels of the miR-23b is needed in cholangiocytes to allow TGFbeta signaling and bile duct formation	High expression in HNOC	Scapoli et al. (2010)
miR-24	– miR-24, a ubiquitously expressed miRNA, has an anti-proliferative effect	High expression in HNOC	Lin et al. (2010a, b, c)
let-7c	– let7c may be involved in the process of metastasization – let7c is functionally involved in the pathogenesis of nevi and possibly malignant melanoma	High expression in HNOC	Shiiba et al. (2010)
miR-29b	– miR-29b is a key regulator of development of the osteoblast phenotype by targeting anti-osteogenic factors and modulating bone extracellular matrix proteins	High expression in HNOC	Shiiba et al. (2010)
miR-30b	– It may play role in tumorigenesis.	High expression in HNOC	Shiiba et al. (2010)
miR-107	– This miRNAs regulate gene expression involved in cell division, metabolism, stress response, and angiogenesis in vertebrate species	Downregulated in HNOC	Liu et al. (2009a, b)
miR-200b	– It play an essential role intumour suppression by inhibiting epithelial-mesenchymal transition (EMT), the initiating step of metastasis	High expression in HNOC	Yu et al. (2009)
mir-15b	– It has a critical role in apoptosis	High expression in HNOC	Shiiba et al. (2010)
miR-200a	– It regulates regulates epithelial-mesenchymal transition	Lower expression in HNOC	Xia et al. (2010)
let-7b	– let-7b acts as a tumor suppressor that represses cancer cell proliferation and migration as well as tumor metastasis – let-7b can suppress the expression of Bsg(Basiginalso called extracellular matrix metalloproteinase inducer,	Low expression in HNOC	Jakymiw et al. (2010)

(continued)

Table 5.20 (continued)

MiRNAN	Function	Alteration in cancer	References
	EMMPRIN, is highly expressed on the surface of tumor cells and stimulates adjacent fibroblasts or tumor cells to produce matrix metalloproteinases (mmps)) and also it is evident that this suppression could result in the indirect suppression of mmp-9 – let-7b regulates neural stem cell proliferation and differentiation by targeting the stem cell regulator TLX and the cell cycle regulator cyclinD1		
miR-28	– miR-28 targets the 3'untranslated (3'UTR) region of MPL (myeloproliferative leukemia), inhibiting its translation, as well as other proteins potentially involved in mega karyocyte differentiation, such as E2F6 – Expression of miR-28 in CD34-derivedmegakaryocytes inhibited terminal differentiation	High expression in HNOC	Shiiba et al. (2010)
miR-100	– It regulates the expression of Ataxia telangiectasia-mutated gene (ATM)	Low expression in HNOC	Henson et al. (2009)
miR-361	– It functions as an oncogenic RNA – miR-361 were predicted to target Vascular endothelial growth factor (VEGF)	High expression in HNOC	Shiiba et al. (2010)
miR-137	– miR-137 has been implicated to act as a tumor suppressor in several cancer types via cell cycle control – miR-137 regulates cell growth and CDK6 expression – miR-137 has also been shown to regulate dendritic development and maturation of neurons	Downregulated in HNOC	Kozaki et al. (2008)
miR-124	– miR-124 is almost exclusively expressed in the central nervous system and neuronal cells, suggesting that it might be useful as a potential biomarker for neurological diseases	Downregulated in HNOC	(Hunt et al. 2011)
miR-154	– miR-154 directly targets CCND2, which is essential for the control of cell cycle	Upregulated in HNOC	Shiiba et al. (2010)

(continued)

Table 5.20 (continued)

MiRNAN	Function	Alteration in cancer	References
	progression. MiR-154 inhibited tumor cell malignancy and the G1/S transition – mir-154 enriches in embryonic tissues and is related to embryonic development		
miR-132	– miR-132 has role in neurological development, synaptic transmission, inflammation and angiogenesis	Upregulated in HNOC	Kozaki et al. (2008)
miR-198	– miR-198 inhibits HIV-1 gene expression and replication in monocytes and its mechanism of action appears to involve repression of cyclin T1	Upregulated in HNOC	Shiiba et al. (2010)
miR-30a	– miR-30a is important for the development of the prefrontal cortex via the regulation of brain-derived neurotrophic factor (BDNF)and in vertebrate hepatobiliary development – Other experimentally confirmed targets of miR-30a are adenylate kinase (Ak1) and the GW182 protein (Tnrc6a), a component of the RNA interference silencing complex	Upregulated in HNOC	Shiiba et al. (2010)
miR-340	– miR-340 directly targets c-Met to mediate cell migration and invasion through regulation of MMP-2 and MMP-9 expression	Upregulated in HNOC	Shiiba et al. (2010)
miR-224	– miR-224, is known to have a dual function, conditionally inducing both apoptosis and cell proliferation, and it was found to be either over or under expressed in several tumor types	Upregulated in HNOC	Kozaki et al. (2008)
miR-10a	– miR-10a is an essential mediator of RA-induced neuroblastoma differentiation and of the associated changes in migration, invasion, and in vivo metastasis	Upregulated in HNOC	Kozaki et al. (2008)
miR-140	– miR-140 plays dual roles in both cartilage development and homeostasis – miR-140 is involved in the pathogenesis of osteoarthritis by regulating, at least in part, ADAMTS5	Upregulated in HNOC	Kozaki et al. (2008)
miR-126	– miR-126 is a novel physiological regulator of the proto-oncogene c-myb during definitive hematopoiesis – A major role of miR-126 has been suggested in angiogenesis and vascular	Upregulated in HNOC	Kozaki et al. (2008)

(continued)

Table 5.20 (continued)

MiRNAN	Function	Alteration in cancer	References
	integrity, which was mediated by the repression of inhibitors of VEGF-induced proliferation in endothelial cells		
miR-302b	– miR-302b maintains "stemness" of human embryonal carcinoma cells by post-transcriptional regulation of Cyclin D2 expression – miR-302b indirectly regulates expression of the pluripotentstem cell marker Oct4, and it directly regulates expression of Cyclin D2 protein, a developmental regulator during gastrulation	Expression lowered in HNOC	Kozaki et al. (2008)
miR-382	– miR-382 targeted SOD2 and contributed to TGF-Beta1-induced loss of epithelial characteristics in human renal epithelial cells – miR-382 is involved in the inhibition of HIV-1 protein contributing to viral latency, as an unstable miRNA in cells	Expression lowered in HNOC	Shiiba et al. (2010)
miR-378	– MicroRNA-378 promotes cell survival, tumor growth, and angiogenesis by targeting SuFu and Fus-1 expression – It also regulates CYP2E1, one of the pharmacologically and toxicologically important cytochrome P450 isoforms	Expression lowered in HNOC	Scapoli et al. (2010)
miR-302c	– The mir-302 microRNA (miRNA) family (mir-302 s) is expressed most abundantly in slow-growing human embryonic stem (ES) cells, and quickly decreases after cell differentiation and proliferation – Therefore, mir-302 s was investigated as one of the key factors essential for maintenance of ES cell renewal and pluripotency	Expression lowered in HNOC	Kozaki et al. (2008)
miR-371	– It promotes cellular transformation in cooperation with oncogenes – miRNAs of the miR-372/373 cluster contribute to tumorigenesis from cells that retain wt-p53	Expression lowered in HNOC	Kozaki et al. (2008)
miR-127	– miR-127 functions to regulate the expression levels of genes involved in lung development, placental formation and apoptosis – MicroRNA-127 mainly modulates fetal lung development	Expression lowered in HNOC	Kozaki et al. (2008)

(continued)

Table 5.20 (continued)

MiRNAN	Function	Alteration in cancer	References
miR-302d	miR-302d is a hES cell-specific miRNA. The TRPS1, KLF13, and MBNL2 genes are very likely to be the common targets of miR-302d in hES cells	Expression lowered in HNOC	Shiiba et al. (2010)

5.9 Pancreatic Cancer

Cancer is a collection of many related diseases. All types of cancers initiate from cells which are the fundamental component of the body tissues. Controlled growth and proliferation of cells sometimes do not progress correctly. This irregularity results in cancer (Harris and McCormick 2010). Pancreas is an organ of digestive system located near the stomach and small intestine. Cancers of the exocrine pancreas are a very serious health threat in the United States where approximately 27,000 patients are, annually, diagnosed with this cancer and about the same number are annually deceased from this disease (Yadav and Lowenfels 2013). Due to difficulties in diagnosis, the intrinsic aggressive nature of pancreatic cancers, and the unavailable systemic treatment options, approximately 4% of patients only diagnosed with pancreatic adenocarcinoma will be alive five years after diagnosis. Pancreatic cancer is the fifth leading cause of cancer related deaths following the cancers of breast; lung, colon, and prostate (Maisonneuve and Lowenfels 2010; Olson and Kurtz 2013).

Neither the presence of a risk factor necessarily means that an individual will develop cancer, nor the absence of risk factors is indicative of the fact that an individual will not develop. The major risk factors of pancreatic cancer include **Age** (the age group 65–79 has the highest incidence of pancreatic cancer), **Smoking** (smokers develop pancreatic cancer more than twice compared with nonsmokers), **Diet** (frequency of pancreatic cancer may be associated with high intakes of meat and fat), **Medical** complications (such as a chronic liver disease, cirrhosis, chronic pancreatitis, diabetes and a history of surgery in upper digestive tract), **Environmental factors** (including gasoline and related compounds, insecticides), **Genetic predisposition** (Possibly in 3% of cases) (Lowenfels et al. 1997; Lowenfels and Maisonneuve 2006; Maisonneuve and Lowenfels 2015).

5.9.1 Genetic Etiology

Several genetic alterations have been found in very high frequencies in pancreatic carcinomas. A family history of pancreatic cancer has also been associated with increased risk of the disease, suggesting that the inherited genetic trait (s) play also

an important role. While the genetic basis for the majority of the familial clustering of pancreatic cancer remains unclear, several important pancreatic cancer genes have been identified. These include K-ras, BRCA2 or PALB2, and ABO blood group locus. Recent advances in genotyping and genetic sequencing have accelerated the rate at which novel pancreatic cancer susceptibility genes have been identified within the past few years (Yadav and Lowenfels 2013; Rustgi 2014; Grant et al. 2015). In Table 5.21, we address some information about pancreatic cancer susceptibility genes.

5.9.2 Epigenetic Abnormalities

For a long time, it was believed that cancer is a genetic disease, but in recent decades, scientists have come to believe that epigenetic changes, that do not alter the sequence of DNA, change the genome stability and gene expression and play an important role in cancer (Maitra and Hruban 2008a, b). It is now clear that epigenetic abnormalities are extremely common in cancers and provide an alternative mechanism of transcriptional silencing. Epigenetic abnormalities in cancer predominantly include methylation of CG dinucleotides (CpG islands) in the 5′ regulatory region of tumor suppressor genes, which keep RNA polymerase from binding and initiating transcription (Fukushige and Horii 2014). In cancers, there is preferential methylation of the gene promoter in the neoplastic cells, but not in the corresponding normal cells within the tissue of origin. Alternatively, hypomethylation of oncogene promoter have been observed as an approach of oncogene overexpression and consequently cell over-growth (Neureiter et al. 2014). Epigenetic silencing is frequently observed in pancreatic cancers and tends to involve genes that function in tumor suppression and/or critical homeostatic pathways (Fukushige and Horii 2014). The involved genes in pancreatic cancers through methylation deregulation are summarized in Table 5.22.

5.9.3 MicroRNA Expression in Pancreatic Cancer

Global expression platforms, such as cDNA and oligonucleotide microarrays, and serial analysis of gene expression has helped to elucidate large numbers of differentially expressed transcripts in pancreatic cancer and their precursor lesions. Multiple insights have been gained into the biology of pancreatic cancer and translational applications can be applied from these global expression studies. Micro RNAs are the important micromolecules which its role has been clarified in normal development and cell function. In cancer research field, deregulation of miRNAs is revealed to be related to initiation and progression of cancers. It is believed that they are, directly or indirectly, involved in carcinogenesis

Table 5.21 The most important genes which involved in the pathogenesis of pancreatic cancer

Gene	Gene locus	Molecular pathology	Gene function	OMIM	References
KRAS	12p12.1	point mutation	– Ras proteins bind GDP/GTP and possess intrinsic GTPase activity – Plays an important role in the regulation of cell proliferation	190070	Bournet et al. (2016)
BRAF	7q34	point mutation	– Protein kinase involved in the transduction of mitogenic signals from the cell membrane to the nucleus – May play a role in the postsynaptic responses of hippocampal neuron. Phosphorylates MAP2K1, and thereby contributes to the MAP kinase signal transduction pathway	164757	Calhoun et al. (2003)
TP53	17p13.1	Mutation	– Acts as a tumor suppressor in many tumor types; induces growth arrest or apoptosis depending on the physiological circumstances and cell type – Involved in cell cycle regulation as a trans-activator that acts to negatively regulate cell division by controlling a set of genes required for this process	191170	Barton et al. (1991)
SMAD4	18q21.2	Deletion & point mutation	– The product of this gene forms homomeric complexes and heteromeric complexes with other activated Smad proteins, which then accumulate in the nucleus and regulate the transcription of target genes – This protein binds to DNA and recognizes an 8-bp palindromic sequence (GTCTAGAC) called the Smad-binding element (SBE)	600993	Hao et al. (2011)
STK11	19p13.3	Indels & point mutations	– Tumor suppressor serine/threonine-protein kinase – Controls the activity of AMP-activated protein kinase (AMPK) family members – Playing a role in various processes such as cell metabolism, cell polarity, apoptosis and DNA damage response	602216	Su et al. (1999)

(continued)

Table 5.21 (continued)

Gene	Gene locus	Molecular pathology	Gene function	OMIM	References
CDKN2A	9p21.3	Inactivating mutations Deletion & point mutation	– Capable of inducing cell cycle arrest in G1 and G2 phases – Acts as a tumor suppressor – Binds to MDM2 and blocks its nucleocytoplasmic shuttling by sequestering it in the nucleolus. This inhibits the oncogenic action of MDM2 by blocking MDM2-induced degradation of p53 and enhancing p53-dependent transactivation and apoptosis – Also induces G2 arrest and apoptosis in a p53-independent manner by preventing the activation of cyclin B1/CDC2 complexes	600160	Bartsch et al. (2002)
RB1	13q14.2	Deletion & point mutation	– The protein encoded by this gene is a negative regulator of the cell cycle and was the first tumor suppressor gene found – The encoded protein also stabilizes constitutive heterochromatin to maintain the overall chromatin structure – The active, hypophosphorylated form of the protein binds transcription factor E2F1	614041	Park et al. (2011a, b)
AKT2	19q13.2	Amplification & overexpression	– AKT2 is one of 3 closely related serine/threonine-protein kinases (AKT1, AKT2 and AKT3) called the AKT kinase – Regulate many processes including metabolism, proliferation, cell survival, growth and angiogenesis	164731	Ruggeri et al. (1998)
TGFBR2	3p24.1	Deletion & point mutation	– This gene encodes a member of the Ser/Thr protein kinase family and the TGFB receptor subfamily – The encoded protein is a transmembrane protein that has a protein kinase domain, forms a heterodimeric complex with another receptor protein, and binds TGF-beta – This receptor/ligand complex phosphorylates proteins, which then enter the nucleus and regulate the transcription of a subset of genes related to cell proliferation	190182	Goggins et al. (1998)

(continued)

Table 5.21 (continued)

Gene	Gene locus	Molecular pathology	Gene function	OMIM	References
MAP2K4	17p12	Point mutation & copy number variation	– This gene encodes a member of the mitogen-activated protein kinase (MAPK) family – Members of this family act as an integration point for multiple biochemical signals and – Involved in a wide variety of cellular processes such as proliferation, differentiation, transcription regulation, and development	601335	Xin et al. (2004)
BRCA2	13q13.1	loss of heterozygosity (LOH)	– Both BRCA1 and BRCA2 are involved in maintenance of genome stability, specifically the homologous recombination pathway for double-strand DNA repair – The BRCA2 protein contains several copies of a 70 aa motif called the BRC motif, and these motifs mediate binding to the RAD51 recombinase which functions in DNA repair – BRCA2 is considered a tumor suppressor gene	600185	Naderi and Couch (2002)
hMLH1	3p22.2	Epigenetic abnormalities	– Heterodimerizes with PMS2 to form MutL alpha, a component of the post-replicative DNA mismatch repair system (MMR)	120436	(Goggins and Sato 2014)
hMSH2	2p21	Deletion & point mutation	– Component of the post-replicative DNA mismatch repair system (MMR)	609309	Wolfgang et al. (2013)
FANCC	9q22.32	Deletion & point mutation	– DNA repair protein that may operate in a postreplication repair or a cell cycle checkpoint function – May be implicated in interstrand DNA cross-link repair and in the maintenance of normal chromosome stability – Upon IFNG induction, may facilitate STAT1 activation by recruiting STAT1 to IFNGR1	613899	Rogers et al. (2004)

(continued)

Table 5.21 (continued)

Gene	Gene locus	Molecular pathology	Gene function	OMIM	References
FANCG	9p13.3	Deletion & point mutation	– DNA repair protein that may operate in a postreplication repair or a cell cycle checkpoint function – May be implicated in interstrand DNA cross-link repair and in the maintenance of normal chromosome stability – Candidate tumor suppressor gene	602956	Rogers et al. (2004)
PRSS1	7q34	Point mutation	– This gene encodes a trypsinogen, which is a member of the trypsin family of serine proteases – This enzyme is secreted by the pancreas and cleaved to its active form in the small intestine – It is active on peptide linkages involving the carboxyl group of lysine or arginine – This gene and several other trypsinogen genes are localized to the T cell receptor beta locus on chromosome 7	276000	Yi et al. (2016)
MYC	8q24.21	Mutations, overexpression, rearrangement and amplification	– The protein encoded by this gene is a multifunctional, nuclear phosphoprotein that plays a role in cell cycle progression, apoptosis and cellular transformation – It functions as a transcription factor that regulates transcription of specific target genes	190080	Wirth et al. (2016)
MYB	6q23.3	rearrangement and amplification	– Transcriptional activator; DNA-binding protein that specifically recognize the sequence 5-YAAC [GT]G-3 – Plays an important role in the control of proliferation and differentiation of hematopoietic progenitor cells	189990	Srivastava et al. (2013)
EGFR	7p11.2	Amplification	– Receptor tyrosine kinase binding ligands of the EGF family and activating several signaling cascades to convert extracellular cues into appropriate cellular responses – Binding of the protein to a ligand induces receptor dimerization and tyrosine autophosphorylation and leads to cell proliferation	131550	Frolov et al. (2007)

(continued)

Table 5.21 (continued)

Gene	Gene locus	Molecular pathology	Gene function	OMIM	References
AIB1/NCOA3	20q12	Amplification	– Nuclear receptor coactivator that directly binds nuclear receptors and stimulates the transcriptional activities in a hormone-dependent fashion – Plays a central role in creating a multisubunit coactivator complex, which probably acts via remodeling of chromatin – Involved in the coactivation of different nuclear receptors, such as for steroids (GR and ER), retinoids (RARs and RXRs), thyroid hormone (TRs), vitamin D3 (VDR) and prostanoids (PPARs) – Displays histone acetyltransferase activity – Also involved in the coactivation of the NF-kappa-B pathway via its interaction with the NFKB1 subunit	601937	Simeone and Maitra (2013)
MEN1	11q13.1	LOH	– This gene encodes menin, a putative tumor suppressor associated with a syndrome known as multiple endocrine neoplasia type 1 – In vitro studies have shown menin is localized to the nucleus, possesses two functional nuclear localization signals, and inhibits transcriptional activation by JunD, however, the function of this protein is not known	613733	Jiao et al. (2011)
TGFBR1	9q22.33	Deletion & LOH	– Involved in the regulation of cellular processes, including division, differentiation, motility, adhesion and death	190181	Hansel et al. (2003)
TGFBR2	3p24.1	Deletion & LOH	– Transduces the TGFB1, TGFB2 and TGFB3 signal from the cell surface to the cytoplasm – Regulating a plethora of physiological and pathological processes including cell cycle arrest in epithelial and hematopoietic cells, control of mesenchymal cell proliferation and differentiation, wound healing, extracellular matrix production, immunosuppression and carcinogenesis	190182	Goggins et al. (1998)

(continued)

Table 5.21 (continued)

Gene	Gene locus	Molecular pathology	Gene function	OMIM	References
ABCB1	7q21.12	Mutation & deregulation	– Energy-dependent efflux pump responsible for decreased drug accumulation in multidrug-resistant cells	171050	Zhou et al. (2008)
ACVR1B	12q13	homozygous deletions & LOH	– Transduces the activin signal from the cell surface to the cytoplasm and – Thus regulating a many physiological and pathological processes including neuronal differentiation and neuronal survival, hair follicle development and cycling, FSH production by the pituitary gland, wound healing, extracellular matrix production, immunosuppression and carcinogenesis	601300	Murphy et al. (2002)
ANG	14q11.2	Up-regulation	– Binds to actin on the surface of endothelial cells; once bound, angiogenin is endocytosed and translocated to the nucleus – Stimulates ribosomal RNA synthesis including that containing the initiation site sequences of 45S rRNA – Angiogenin induces vascularization of normal and malignant tissues. Angiogenic activity is regulated by interaction with RNH1 in vivo	105850	Shimoyama et al. (1996)
BAG3	10q26.11	Oncogenic Activation & overexpression	– Inhibits the chaperone activity of HSP70/HSC70 by promoting substrate release – Has anti-apoptotic activity	603883	Liao et al. (2001)
BIRC5	17q25.3	abundant expression	– Multitasking protein that has dual roles in promoting cell proliferation and preventing apoptosis – Component of a chromosome passage protein complex (CPC) which is essential for chromosome alignment and segregation during mitosis and cytokinesis	603352	Glienke et al. (2009)

Table 5.22 The epigenetically deregulated gene involved in pancreatic

Gene	Locus	Molecular pathology	Gene function	OMIM	References
CDKN2A	9p21.3	Hyper-methylation	– Capable of inducing cell cycle arrest in G1 and G2 phases – Acts as a tumor suppressor – Binds to MDM2 and blocks its nucleocytoplasmic shuttling by sequestering it in the nucleolus. This inhibits the oncogenic action of MDM2 – Also induces G2 arrest and apoptosis in a p53-independent manner by preventing the activation of cyclin B1/CDC2 complexes	600160	Sato and Goggins (2006)
CDHE	16q22.1	Hyper-methylation	– Cadherins may thus contribute to the sorting of heterogeneous cell types – CDH1 is involved in mechanisms regulating cell-cell adhesions, mobility and proliferation of epithelial cells. Has a potent invasive suppressor role	192090	Sato and Goggins (2006)
RARB	3p24.2	Hyper-methylation	– Receptor for retinoic acid and regulate gene expression in various biological processes – In concert with RARG, required for skeletal growth, matrix homeostasis and growth plate function	180220	Maitra and Hruban (2008a)
SOCS-1	16p13.13	Hyper-methylation	– OCS1 is involved in negative regulation of cytokines that signal through the JAK/STAT3 pathway	603597	Sato and Goggins (2006)
Reprimo	2q23.3	Hyper-methylation	– May be involved in the regulation of p53-dependent G2 arrest of the cell cycle – Seems to induce cell cycle arrest by inhibiting CDK1 activity and nuclear translocation of the CDC2 cyclin B1 complex (By similarity)	612171	Maitra and Hruban (2008a, b)

(continued)

Table 5.22 (continued)

Gene	Locus	Molecular pathology	Gene function	OMIM	References
TSLC1	11q23.3	Hyper-methylation	– May be involved in neuronal migration, axon growth, pathfinding, and fasciculation on the axons of differentiating neurons – May play diverse roles in the spermatogenesis including in the adhesion of spermatocytes and spermatids to Sertoli cells and for their normal differentiation into mature spermatozoa – May contribute to the less invasive phenotypes of lepidic growth tumor cells	605686	Sato and Goggins (2006)
RELN	7q22.1	Hyper-methylation	– Extracellular matrix serine protease that plays a role in layering of neurons in the cerebral cortex and cerebellum – Regulates microtubule function in neurons and neuronal migration – Affects migration of sympathetic preganglionic neurons in the spinal cord, where it seems to act as a barrier to neuronal migration	600514	Sato et al. (2006)
SERPINB5	18q21.33	Hypo-methylation	– Tumor suppressor. It blocks the growth, invasion, and metastatic properties of mammary tumors – As it does not undergo the S (stressed) to R (relaxed) conformational transition characteristic of active serpins, it exhibits no serine protease inhibitory activity	154790	Sato et al. (2003)
S100A4	1q21.3	Hypo-methylation	– S100 proteins are involved in the regulation of a number of cellular processes such as cell cycle progression and differentiation – This protein may function in motility, invasion, and tubulin polymerization – Chromosomal rearrangements and altered expression of this gene have been implicated in tumor metastasis	114210	Sato et al. (2003)

(continued)

Table 5.22 (continued)

Gene	Locus	Molecular pathology	Gene function	OMIM	References
MSLN	16p13.3	Hypo-methylation	– Membrane-anchored forms may play a role in cellular adhesion	601051	Sato et al. (2003)
PSCA	8q24.3	Hypo-methylation	– May be involved in the regulation of cell proliferation – Has a cell-proliferation inhibition activity in vitro	602470	Sato et al. (2003)
CLDN4	7q11.23	Hypo-methylation	– Plays a major role in tight junction-specific obliteration of the intercellular space	602909	Sato et al. (2003)
VAV1	19p13.3	Hypo-methylation	– Couples tyrosine kinase signals with the activation of the Rho/Rac GTPases, thus leading to cell differentiation and/or proliferation	164875	Fernandez-Zapico et al. (2005)

Table 5.23 Summary of miRNAs deregulated in pancreatic adenocarcinoma

miRNA	Expression status	Target genes	Potential clinical value	References
miR-21	Upregulation	PTEN, PDCD4, TPM1, TIMP3	– Biomarker for diagnosis – Predictive value for prognosis – Indicator for chemosensitivity – Potential target for treatment	Bloomston et al. (2007)
miR-221/222	Upregulation	CDKN1B (p27), PUMA, PTEN	– Biomarker for diagnosis – Predictive value for prognosis – Potential target for treatment	Bloomston et al. (2007)
miR-155	Upregulation	TP53INP1, SEL1L	– Biomarker for diagnosis – Predictive value for prognosis	Bloomston et al. (2007)
miR-196a	Upregulation	HOXB8, ANXA1, HMGA2	– Biomarker for diagnosis – Predictive value for prognosis	Bloomston et al. (2007)
miR-424-5p	Upregulation	SOCS6	– Predictive value for prognosis	Lee et al. (2007)
miR-10a	Upregulation	HOXA1	– Predictive value for prognosis – Potential target for treatment	Ohuchida et al. (2012)
miR-373	Upregulation	TP53INP1, LATS2, CD44	– Biomarker for diagnosis	Zhang et al. (2013a, b, c, d)
miR-27a	Upregulation	Spry2	– Predictive value for prognosis – Potential target for treatment	Ma et al. (2010)
miR-210	Upregulation	HOXA1, FGFRL1, HOXA9	– Predictive value for prognosis	Takikawa et al. (2013)
miR-15b	Upregulation	CCNE1	– Predictive value for prognosis	Zhang et al. (2009a, b, c)
miR-181	Upregulation	TIMP3, TCL1	– Indicator for chemosensitivity	Panarelli et al. (2012)
miR-148a, b	Downregulation	DNMT3b, Mtif, CCKBR, BCL2	– Biomarker for diagnosis	Zhang et al. (2014a, b, c, d, e, f)
miR-198	Downregulation	MSLN, PBX-1, VCP	– Predictive value for prognosis – Potential target for treatment	Marin-Muller et al. (2013)
miR-146a	Downregulation	TRAF6, IRAK1, Stat1	– Potential target for treatment	Li et al. (2010a, b, c, d)

(continued)

Table 5.23 (continued)

miRNA	Expression status	Target genes	Potential clinical value	References
miR-20a	Downregulation	Stat3	– Potential target for treatment	Yan et al. (2010)
miR-96	Downregulation	KRAS	– Potential target for treatment	Yu et al. (2010a, b, c)
miR-375	Downregulation	PDK1, 14-3-3zeta	– Biomarker for diagnosis	Szafranska et al. (2007)
miR-200c	Downregulation	MUC4, MUC16	– Predictive value for prognosis – Indicator for chemosensitivity – Potential target for treatment	Yu et al. (2010a, b, c))
Let-7	Downregulation	KRAS, MAPK	– Potential target for treatment	Torrisani et al. (2009)

(Papaconstantinou et al. 2013; Sun et al. 2014). Here, most reported miRNAs deregulated in pancreatic adenocarcinoma have been highlighted (Table 5.23).

5.9.4 Chromosomal Alterations

Karyotyping (G-banding), comparative genomic hybridization (CGH), and allelotyping are the more common techniques used to study structural and numerical chromosomal abberations. Chromosome losses are mostly observed than chromosome gains in pancreatic cancer. According to data, DNA loss ranges from 1.6 to 32% of the genome. Nonetheless, frequent gains of DNA, secondary to unbalanced chromosome rearrangements, have been traced in primary pancreatic cancers (Winter et al. 2006) (Table 5.24).

5.10 Pharyngeal Cancer

Pharyngeal cancer is a cancer of the throat. This cancer is common among men than women (Torre et al. 2015). The pharynx is located between the nose and the throat. The cancer may occur in this area and three types of cancer could be developed including nasopharynx, oropharynx, and hypopharynx. The risk of this cancer increases with age and usually occurs in individuals aged over 65 years (Warnakulasuriya 2009).

The more common risk factors for this cancer include Epstein-Barr virus, high salt foods, smoking, alcohol consumption, poor nutrition, human papilloma virus, vitamins A and E insufficiency, Plummer-Vinson syndrome, and exposure to

Table 5.24 Most reported chromosomal alterations in pancreatic cancer

Chromosom arm involvement	Type of alteration	Possible involved gene	References
9p	Genomic loss	CDKN2A/P16/MTS1	Maitra et al. (2006)
17p	Genomic loss	p53	Iacobuzio-Donahue et al. (2004)
18q	Genomic loss	MADH4/SMAD4/DPC4	Mahlamäki et al. (et al. 1997)
3p	Genomic loss	Unknown	Curtis et al. (1998)
8p	Genomic loss	Unknown	Winter et al. (2006)
6q	Genomic loss	Unknown	
19q	Genomic gain/amplification	AKT2	Curtis et al. (1998)
12p	Genomic gain/amplification	KRAS2	Winter et al. (2006)
12q	Genomic gain/amplification	MDM2	Winter et al. (2006)
17q	Genomic gain/amplification	ERBB2	Winter et al. (2006)
20q	Genomic gain/amplification	AIB1	Ghadimi et al. (1999)

asbestos (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans and International Agency for Research on Cancer 2007; Johnson et al. 2017). These factors finally affect the genome of normal cells and disturb the cell regulation which result in cell death and survival imbalance. Consequently, these uncontrollable cells divide regardless to the essential requirements of cells (Weinberg 2013). Besides, many important coding and non-coding genes are involved in regulating the cell death and survival.

With an aim to enhance early determination and focusing on the therapeutics advancement, extensive number of studies have been and are being completed to figure out the molecular characteristics of pharyngeal cancer (Pai and Westra 2009; Worsham et al. 2012). Albeit the most advances in the treatment of pharyngeal cancer were fundamentally achieved from the clinical trials. Now, how much these attempts have changed the applicable strategy in clinical practice—and at what pace—is to a great extent obscure. The need to sort this puzzle out is a noteworthy main impetus behind the gathering of the most reported **coding** and **non-coding genes** which are listed in Tables 5.25 and 5.26.

5.10.1 Genetic Etiology

See Table 5.25.

Table 5.25 Most reported genes which are involved in development of pharyngeal cancer

Gene symbol	Gene locous	Function	Alteration in cancer	OMIM	References
ADAMTS9	3p14.1	– A disintegrin and metalloproteinase with thrombospondin motifs protein – Have been implicated in the cleavage of proteoglycans – The control of organ shape during development – The inhibition of angiogenesis	– ADAMTS9 maps to 3p14.2 and shows significant associations with the aerodigestive tract cancers esophageal squamous cell carcinoma (ESCC) and nasopharyngeal carcinoma (NPC) – ADAMTS9 contributes an important function in the tumor microenvironment that acts to inhibit angiogenesis and tumor growth in both ESCC and NPC	605421	Lo et al. (2010)
ANXA1	9q21.13	– Annexin I protein has a phospholipase A2 inhibitory activity – Annexin I may have potential anti-inflammatory activity	– Increased expression of ANX-I was significantly associated with disease relapse in NPC	151690	Chow et al. (2009), Huang et al. (2016)
ANXA2	15q22.2	– Play a role in the regulation of cellular growth and in signal transduction pathways	– Annexin II down-regulation was positively associated with lymph node metastasis, suggesting that it may be a prognostic factor in NPC	151740	Chan et al. (2008)
AQP1	7p14	– Aquaporins are a family of small integral membrane proteins related to the major intrinsic protein (MIP or AQP0) – This gene encodes an aquaporin which functions as a molecular water channel protein	– Overexpression of AQP1 in NPC has been confirmed	107776	Li and Zhang (2010)
ATM	11q22-q23	– The protein encoded by this gene belongs to the PI3/PI4-kinase family – This protein is an important cell cycle checkpoint kinase that phosphorylates;	– In NPC cell lines ATM is down-regulated only in the EBV-positive line	607585	Bose et al. (2009)

(continued)

Table 5.25 (continued)

Gene symbol	Gene locous	Function	Alteration in cancer	OMIM	References
		– It functions as a regulator of a wide variety of downstream proteins, including tumor suppressor proteins p53 and BRCA1, checkpoint kinase CHK2, checkpoint proteins RAD17 and RAD9, and DNA repair protein NBS1	– The loss of ATM function could be an important step in the pathogenesis of NPC, and may have implications for the treatment of this disease		
BECN1	17q21	– Beclin-1 participates in the regulation of autophagy – Has an important role in development, tumorigenesis, and neurodegeneration	– HI F-1alpha-associated Beclin 1 high expression might facilitate NPC cells surviving from chemoradiotherapy, suggesting a novel therapeutic molecular target for NPC	604378	Wan et al. (2010)
CCR7	17q12-q21.2	– A member of the G protein-coupled receptor family – It has been shown to control the migration of memory T cells to inflamed tissues, as well as stimulate dendritic cell maturation	– CCR7 is expressed and active in human NPC metastases	600242	Ou et al. (2006)
CDC42	1p36.1	– A small GTPase of the Rho-subfamily – Regulates signaling pathways that control diverse cellular functions including cell morphology, migration, endocytosis and cell cycle progression	– Cdc42/Rac1 participates in the posttranscriptional control of telomerase activity in NPC cells	116952	Yeh et al. (2005)
CTDSP1 (SCP-1)	2q35	– It preferentially catalyzes the dephosphorylation of 'Ser-5' within the tandem 7 residues repeats in the C-terminal domain (CTD) of the largest RNA polymerase II subunit POLR2A	SCP-1 mRNA is expressed in some nasopharyngeal carcinoma, suggesting that SCP-1 might be a new tumor target antigen of nasopharyngeal carcinoma	605323	Yi et al. (2007)

(continued)

Table 5.25 (continued)

Gene symbol	Gene locous	Function	Alteration in cancer	OMIM	References
CXCR4	2q21	– A CXC chemokine receptor specific for stromal cell-derived factor-1 – It acts with the CD4 protein to support HIV entry into cells	– The relative expression level of CXCR4 mRNA in NPC is significantly higher than that of normal nasopharynx tissues – CXCR4 is closely correlated to metastasis of nasopharyngeal carcinoma	162643	Luo et al. (2009)
CXCR6	3p21	– Chemokine C-X-C motif receptor 6 contributes to cell migration during hypoxia – Moreover, CXCR6 and CXCR3 act coordinately with respective ligands and are involved in the pathophysiology of Juvenile Idiopathic Arthritis-associated inflammatory processes	– CXCR6 is expressed and active in human NPC metastases	605163	Ou et al. (2006)
DAB2	5p13	– Adapter protein that functions as clathrin-associated sorting protein (CLASP) required for clathrin-mediated endocytosis of selected cargo proteins	– Frequent down regulation of DAB2 has been reported in NPC – The promoter hypermethylation contributes to the loss of expression of DAB2	601236	Tong et al. (2010)
DDR1	6p21.33	– May be involved in cell-cell interactions and recognition – Discoidin domain receptors (DDR1, DDR2) are receptor-type tyrosine kinases activated by collagen	– Their ability to induce expression of matrix metalloproteinase is related with tumor invasion – DDR2 was differentially upregulated in NPC and modulated by EBV Zta protein – DDR2 may play a role in NPC invasion and serve as a diagnostic and therapeutic target	600408	Chua et al. (2008)

(continued)

Table 5.25 (continued)

Gene symbol	Gene locous	Function	Alteration in cancer	OMIM	References
DLEC1	3p21.3	– May act as a tumor suppressor by inhibiting cell proliferation – This gene is located in the 3p22-p21.3 chromosomal segment that is commonly deleted in various carcinomas	– Down-regulation of DLEC1 and promoter hypermethylation were observed in all NPC cell lines but not in normal nasopharyngeal epithelial cells – Deletion of the short arm of chromosome 3 is a common event in nasopharyngeal carcinoma	604050	Kwong et al. (2007)
DSP	6p24	– Major high molecular weight protein of desmosomes – Involved in the organization of the desmosomal cadherin-plakoglobin complexes into discrete plasma membrane domains and in the anchoring of intermediate filaments to the desmosomes	– Down-regulation of desmoplakin expression represents an useful marker for evaluating the risk of distant metastasis formation in oropharyngeal squamous cell carcinomas	125647	Papagerakis et al. (2009)
DVL3	3q27	– May play a role in the signal transduction pathway mediated by multiple Wnt genes	– Gene expression analysis of a unique HNSCC location, the hypopharynx, revealed that DVL3 is overexpressed due to gene amplification	601368	Cromer et al. (2004)
EIF4G1	3q27-qter	– Component of the protein complex eIF4F, which is involved in the recognition of the mRNA cap, ATP-dependent unwinding of 5′-terminal secondary structure and recruitment of mRNA to the ribosome	– Gene expression analysis of a unique HNSCC location, the hypopharynx, revealed that EIF4G1 is overexpressed due to gene amplification	600495	Cromer et al. (2004)

(continued)

Table 5.25 (continued)

Gene symbol	Gene locous	Function	Alteration in cancer	OMIM	References
EPHB4	7q22	– Ephrin receptors and their ligands, the ephrins, mediate numerous developmental processes, particularly in the nervous system – The protein encoded by this gene binds to ephrin-B2 and plays an essential role in vascular development	– Gene expression analysis of a unique HNSCC location, the hypopharynx, revealed that EPHB4 is overexpressed due to gene amplification	600011	Cromer et al. (2004)
ERCC1	19q13.32	– Participates in the processing of anaphase bridge-generating DNA structures, which consist in incompletely processed DNA lesions arising during S or G2 phase, and can result in cytokinesis failure – Also required for homology-directed repair (HDR) of DNA double-strand breaks, in conjunction with SLX4	– ERCC1 expression might be a useful predictive marker in patients with locally advanced nasopharyngeal cancer who are under consideration for cisplatin-based concurrent chemoradiotherapy	126380	Sun et al. (2011a, b)
ERCC4	16p13.12	– The protein encoded by this gene forms a complex with ERCC1 and is involved in the 5′ incision made during nucleotide excision repair – This complex is a structure specific DNA repair endonuclease that interacts with EME1	– ERCC4 T2505C polymorphism may be associated with improved recovery from radiation treatment toxicity in patients with oropharyngeal squamous cell carcinoma (OPSCC)	133520	Kornguth et al. (2005)

(continued)

Table 5.25 (continued)

Gene symbol	Gene locous	Function	Alteration in cancer	OMIM	References
EZH2	7q35-q36	– Enhancer of zeste homolog 2 (EZH2),as a key component of polycomb-repressive complex 2 – Represses target genes through histone methylation and is frequently overexpressed and associated with poor prognosis in common carcinomas	– Expression levels of EZH2 transcript and protein were apparently upregulated in NPC specimens – At least partly through promoting cell growth, EZH2 implicates disease progression, confers tumor aggressiveness, and represents an independent adverse prognosticator in patients with NPC	601573	Hwang et al. (2012)
FGF8b	10q24	– Plays an important role in the regulation of embryonic development, cell proliferation, cell differentiation and cell migration	– FGF8b is overexpressed in the EBV-associated non-hormone-related cancer of the head and neck, NPC – An important EBV oncoprotein, the latent membrane protein 1 (LMP1), was found to be a direct inducer of FGF8b overexpression in NPC cells	600483	Lui et al. (2011)
FOLR1	11q13.3-q14.1	– The protein encoded by this gene is a member of the folate receptor family – This gene product is a secreted protein that either anchors to membranes via a glycosyl-phosphatidylinositol linkage or exists in a soluble form	– The expression of FOLR1 is closely related to the occurrence of NPC and Taxol resistance – FOLR1 gene may be one of the important target molecules in NPC treatment and reversion of the paclitaxel-resistance in NPC	136430	Li et al. (2010a, b, c, d)

(continued)

Table 5.25 (continued)

Gene symbol	Gene locous	Function	Alteration in cancer	OMIM	References
GABBR1	6p21.31	– Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the mammalian central nervous system – The GABA (B) receptor consists of a heterodimer of two related 7-transmembrane receptors, GABA (B) receptor 1 and GABA (B) receptor 2	– Multiple chromosome 6p susceptibility loci contribute to the risk of NPC, possibly though GABBR1 loss of function	603540	Li et al. (2011a, b, c, d, e)
GJC1 (CX45)	17q21.31	– This gene is a member of the connexin gene family – The encoded protein is a component of gap junctions, which are composed of arrays of intercellular channels – Provide a route for the diffusion of low molecular weight materials from cell to cell	– The abnormal expression of CX45 in nasopharynx tissues may be associated with cancerization and squamatization of human nasopharynx tissue	608655	Xiang et al. (2002)
HHATL (KIAA1173)	3p22.1	– This gene negatively regulates N-terminal palmitoylation of SHH by HHAT/SKN	– KIAA1173 gene is strongly expressed in normal nasopharyngeal mucosa epithelia, but down-regulated in NPC – It may be associated with the tumorigenesis of NPC	608116	San et al. (2005)
HLA-E	6p213	– HLA-E belongs to the HLA class I heavy chain paralogues – HLA-E binds a restricted subset of peptides derived from the leader peptides of other class I molecules	– A potential implication of HLA-E gene polymorphisms is suggested in the susceptibility to NPC among populations with high-risk incidence	143010	Hassen et al. (2011)

(continued)

Table 5.25 (continued)

Gene symbol	Gene locous	Function	Alteration in cancer	OMIM	References
HPSE	4q21.3	– Heparanases are endoglycosidases that cleave the heparan sulfate side chain of HSPGs – Permit the remodeling of the extracellular matrix for cell movement or the release of bioactive molecules from the extracellular matrix or cell surface	– The expression of HPA(heparanase) was associated with invasion and metastasis and prognosis of nasopharyngeal cancer, – It may be a new target for the anti-treatment of nasopharyngeal cancer	604724	Zhu et al. (2008)
KLF6	10p15	– This gene encodes a member of the Kruppel-like family of transcription factors – The zinc finger protein is a transcriptional activator, and functions as a tumor suppressor	– KLF6 gene may be involved in carcinogenesis of sporadic NPC	602053	Chen et al. (2002)
LATS2	13q11-q12	– LATS2, which encodes a novel serine/threonine kinase, is known to be important in centrosome duplication and in the maintenance of genomic stability	– LATS2 overexpression was a significant, independent prognosis predictor in nasopharyngeal carcinoma patients – LATS2 might play a role in the tumorigenesis of nasopharyngeal carcinoma by promoting the growth of nasopharyngeal carcinoma cells	604861	Zhang et al. (2010a, b, c, d)
LTF	3p21.31	– This gene is a member of the transferrin family of genes – Its protein product is found in the secondary granules of neutrophils – The protein is a major iron-binding protein in milk and body secretions with	– There is an inactivation of expression of LTF gene in the NPC cell lines – Its molecular mechanism may be related with methylation of promoter region and deletion mutation	150210	Yi et al. (2010)

(continued)

Table 5.25 (continued)

Gene symbol	Gene locous	Function	Alteration in cancer	OMIM	References
		an antimicrobial activity, making it an important component of the non-specific immune system			
MAD2L2	1p36	– The protein encoded by this gene is a component of the mitotic spindle assembly checkpoint – Prevents the onset of anaphase until all chromosomes are properly aligned at the metaphase plate	– MAD2B is a key factor in regulating cellular response to DNA damage in cancer cells – Inactivation of human MAD2B in nasopharyngeal carcinoma cells leads to chemosensitization to DNA-damaging agents	604094	Cheung et al. (2006)
MCM7	7q21.3-q22.1	– The protein encoded by this gene is one of the highly conserved mini-chromosome maintenance proteins (MCM) – Is essential for the initiation of eukaryotic genome replication – The MCM complex consisting of this protein and MCM2, 4 and 6 proteins possesses DNA helicase activity, and may act as a DNA unwinding enzyme	– Gene expression analysis of a unique HNSCC location, the hypopharynx, revealed that MCM7 is overexpressed due to gene amplification	600592	Cromer et al. (2004)
MDM2	12q14.3-q15	– This gene is a target gene of the transcription factor tumor protein p53 – The encoded protein is a nuclear phosphoprotein that binds and inhibits transactivation by tumor protein p53, as part of an autoregulatory negative feedback loop	– MDM2 SNP309 T/G can be considered a risk marker for the development of NPC mainly in early ages probably as an initiation marker for potential cancer development	164785	Sousa et al. (2011)

(continued)

Table 5.25 (continued)

Gene symbol	Gene locous	Function	Alteration in cancer	OMIM	References
MYCL1	1p34.2	– L-myc-1 proto-oncogene protein is a protein that in humans is encoded by the MYCL1 MYCL1 is a bHLH (basic helix-loop-helix) transcription factor implicated in lung cancer	– Non-random gene amplifications were identified for the first time in NPC on MYCL1 in 1p34.3 – Highest frequencies of gain of novel oncogene is detected on MYCL1	164850	Hui et al. (2002)
NCOA3 (AIB1)	20q12	– The protein encoded by this gene is a nuclear receptor co-activator – Interacts with nuclear hormone receptors to enhance their transcriptional activator functions	– Overexpression of AIB1 was observed more frequently in NPCs in late T stages (T3/T4, 24/35 [69%]) than in earlier stages – Overexpression of AIB1 in NPCs may be important in the acquisition of an invasive and/or metastatic phenotype	601937	Liu et al. (2008)
NEDD9	6p25-p24	– It is a docking protein which plays a central coordinating role for tyrosine-kinase-based signaling related to cell adhesion	– Multiple chromosome 6p susceptibility loci contribute to the risk of NPC, possibly though NEDD9 loss of function	602265	Li et al. (2011a, b, c, d, e)
PCNA	20pter-p12	– In response to DNA damage, the protein encoded by this gene is ubiquitinated and is involved in the RAD6-dependent DNA repair pathway	– PCNA play important roles in the progression of hypopharyngeal and laryngeal squamous cell carcinoma and – It is possibly a prognostic discriminator in hypopharyngeal and laryngeal squamous cell carcinoma	176740	Li et al. (2005a, b)

(continued)

Table 5.25 (continued)

Gene symbol	Gene locous	Function	Alteration in cancer	OMIM	References
PDLIM7 (LMP1)	5q35.3	– May function as a scaffold on which the coordinated assembly of proteins can occur – May play a role as an adapter that, via its PDZ domain, localizes LIM-binding proteins to actin filaments of both skeletal muscle and nonmuscle tissues – Involved in both of the two fundamental mechanisms of bone formation	– The LMP-1 induced down-regulation of the CD99 pathway is important in nasopharyngeal carcinogenesis, and that the expression of CD99 in lymphoid stroma may regulate immune response to NPC	605903	Kim et al. (2006)
PHACTR2	6q24.2	– PHACTR2 a protein containing 4 RPEL repeats may be associated with DNA binding – It binds PPP1CA and actin	– PHACTR2 is downregulated in HPV +ve Oropharyngeal Cancers Compared With HPV -ve Oropharyngeal Controls	608724	Schlecht et al. (2007)
PIGR	1q31-q41	– This gene is a member of the immunoglobulin superfamily – The encoded poly-Ig receptor binds polymeric immunoglobulin molecules at the basolateral surface of epithelial cells; the complex is then transported across the cell to be secreted at the apical surface	– PIGR is an NPC susceptibility gene	173880	Hirunsatit et al. (2003)
PIK3CA	3q26.3	– PI 3-Kinases (phosphoinositide 3-kinases, PI 3-Ks) are a family of lipid kinases capable of phosphorylating the 3′OH of the inositol ring of phosphoinositides – They are responsible for coordinating a diverse range of cell functions including proliferation and survival	– Oncogenic PIK3CA may play a critical role in pharyngeal carcinogenesis	171834	Qiu et al. (2008)

(continued)

Table 5.25 (continued)

Gene symbol	Gene locous	Function	Alteration in cancer	OMIM	References
PLK1	16p12.2	– It is required for recovery after DNA damage checkpoint and entry into mitosis and also for kinetochore localization of BUB1B	– Plk1 is overexpressed in 70% of human NPC – This increase in Plk1 expression was observed at both the transcriptional and protein level, for both primary human samples and cancer cell lines, – Indicating that Plk1 is an important mediator of NPC proliferation and progression	602098	Shi et al. (2010a, b)
PML	15q22	– The protein encoded by this gene is a member of the tripartite motif (TRIM) family – This phosphoprotein localizes to nuclear bodies where it functions as a transcription factor and tumor suppressor	– The close relationship between PML expression and the malignant parameters such as pathological grade and T grade of laryngocarcinoma and nasopharyngeal carcinoma – PML may play a role in cancer repression but not in cancer metastasis	102578	Chen et al. (2007a, b)
PTGS2 (COX2)	1q25.2-q25.3	– Prostaglandin-endoperoxide synthase (PTGS), also known as cyclooxygenase, is the key enzyme in prostaglandin biosynthesis – Acts both as a dioxygenase and as a peroxidase	– COX-2 is overexpressed and is associated with increased lymphatic invasion in NPC	600262	Pan et al. (2008)

(continued)

Table 5.25 (continued)

Gene symbol	Gene locous	Function	Alteration in cancer	OMIM	References
PTHLH (PTHRP)	12p12.1-p11.2	– The protein encoded by this gene is a member of the parathyroid hormone family – This hormone regulates endochondral bone development and epithelial-mesenchymal interactions during the formation of the mammary glands and teeth	– PTHLH gene may affect metastasis and apoptosis of NPC – PTHRP contributes to the malignancy of oral cancers downstream of EGFR signaling, and may thus provide a therapeutic target for oral cancer	168470	Li et al. (2010a, b, c, d)
RAGE	14q32	– RAGE gene encodes an enzyme, MAPK/MAK/MRK overlapping kinase – It is able to phosphorylate several exogenous substrates and to undergo auto-phosphorylation	– The expression of RAGE in NPC is down-regulated RAGE may be involved in the invasion and metastasis of NPC by regulating the expression of MMP-9	605762	Yanfei (2010)
NCOA3 (AIB1)	20q12	– The protein encoded by this gene is a nuclear receptor coactivator – Interacts with nuclear hormone receptors to enhance their transcriptional activator functions	– Overexpression of AIB1 was observed more frequently in NPCs in late T stages (T3/T4, 24/35 [69%]) than in earlier stages – Overexpression of AIB1 in NPCs may be important in the acquisition of an invasive and/or metastatic phenotype	601937	Liu et al. (2008)
RARRES1	3q25.32	– This gene was identified as a retinoid acid (RA) receptor-responsive gene – It encodes a type 1 membrane protein. The expression of this gene is upregulated by tazarotene as well as by retinoic acid receptors – Inhibitor of the cytoplasmic carboxypeptidase AGBL2, may regulate the alpha-tubulin tyrosination cycle	– The retinoic acid receptor responder (tazarotene induced) 1 gene (RARRES1; alias TIG1) is transcriptionally silenced by promoter hypermethylation in approximately 90% of NPC cases, – So, its inactivation may be important in NPC formation	605090	Kwok et al. (2009)

(continued)

Table 5.25 (continued)

Gene symbol	Gene locous	Function	Alteration in cancer	OMIM	References
RECK	9p13.3	– Negatively regulates matrix metalloproteinase-9 (MMP-9) by suppressing MMP-9 secretion and by direct inhibition of its enzymatic activity – RECK down-regulation by oncogenic signals may facilitate tumor invasion and metastasis – Appears to also regulate MMP-2 and MT1-MMP, which are involved in cancer progression	– The expression of RECK in NPC is down-regulated. RECK may be involved in the invasion and metastasis of NPC by regulating the expression of MMP-9	605227	Li and Deng (2010)
SART1	11q13.1	– This gene encodes two proteins, the SART1 (800) protein expressed in the nucleus of the majority of proliferating cells, and the SART1 (259) protein expressed in the cytosol of epithelial cancers – The two encoded proteins are thought to be involved in the regulation of proliferation. Both proteins have tumor-rejection antigens – Plays a role in mRNA splicing as a component of the U4/U6-U5 tri-snRNP, one of the building blocks of the spliceosome – May also bind to DNA	– Gene expression analysis of a unique HNSCC location, the hypopharynx, revealed that SART1 is overexpressed due to gene amplification	605941	Cromer et al. (2004)

(continued)

Table 5.25 (continued)

Gene symbol	Gene locous	Function	Alteration in cancer	OMIM	References
SCGB3A1	5q353	– It is a potential growth inhibitory cytokine	– SCGB3A1 or HIN-1 (High in normal-1) is a tumor suppressor gene that is highly expressed in many epithelial tissues, including breast lung, trachea, pancreas, prostrate and salivary gland – Inactivation of HIN-1 expression by promoter methylation is frequent in many epithelial carcinomas and carcinoma-in situ, including breast, lung and nasopharyngeal – Because HIN-1 silencing commences at an early stage of malignant transformation in these tissues, it may be a useful marker for tumor presence. HIN-1 silencing is associated with dense promoter region hypermethylation in esophageal cancer and methylation of HIN-1 is an early event in dysplastic transformation – HIN-1 promoter hypermethylation is common in NPC. Methylated promoter DNA in nasopharyngeal swab, throat-rinsing fluid, and peripheral blood might be potentially useful as tumor marker for screening of NPC – Promoter methylation and epigenetic silencing of SCGB3A1 is found in OSCC cells	606500	Guo et al. (2008)

(continued)

Table 5.25 (continued)

Gene symbol	Gene locous	Function	Alteration in cancer	OMIM	References
TERC	3q26	– Telomerase RNA component, also known as TERC, is an ncRNA found in eukaryotes – It is a component of telomerase – The enzyme used to extend telomeres – TERC serves as a template for telomere replication (reverse transcription) by telomerase	– Non-random gene amplifications were identified for the first time in NPC on TERC at 3q26.3 – Highest frequencies of gain of novel oncogene is detected in case of TERC	602322	Hui et al. (2002)
TKTL1	Xq28	– Catalyzes the transfer of a two-carbon ketol group from a ketose donor to an aldose acceptor, via a covalent intermediate with the cofactor thiamine pyrophosphate	– TKTL1 may play an important role in the occurrence and metastasis of human NPC and become a potential target for novel anti-cancer therapy	300044	Zhang et al. (2008)
TLR3	4q35	– The protein encoded by this gene is a member of the Toll-like receptor (TLR) family – Plays a fundamental role in pathogen recognition and activation of innate immunity	– TLR3 activation inhibits nasopharyngeal carcinoma metastasis via downregulation of chemokine receptor CXCR4	603029	Zhang et al. (2009a, b, c)
TLR4	9q33.1	– The protein encoded by this gene is a member of the Toll-like receptor (TLR) family – Plays a fundamental role in pathogen recognition and activation of innate immunity	– TLR4 3′-UTR is a potent regulator of gene expression, as the mutated TLR4 3′-UTR was associated with decreased mRNA stability, and may down-regulate TLR4 expression resulting in EBV	603030	Song et al. (2006)

(continued)

Table 5.25 (continued)

Gene symbol	Gene locous	Function	Alteration in cancer	OMIM	References
		– They recognize pathogen-associated molecular patterns (PAMPs) that are expressed on infectious agents, and mediate the production of cytokines necessary for the development of effective immunity	meta-infective antiviral immunologic deficits and a high risk of NPC		
VEGFC	4q34.3	– It is active in angiogenesis and endothelial cell growth, and can also affect the permeability of blood vessels	– VEGF-C facilitates tumor cell growth and motility in HNSCC cells. VEGF-C is overexpressed in head and neck squamous carcinoma cell lines compared to normal keratinocytes – VEGF-C overexpression in OSCC cells is associated with lymph node metastasis – In nasopharyngeal carcinoma, the expression rate of VEGF-C is high. VEGF-C expression is positively correlated with lymph node metastasis and tumor staging. VEGF-C attaches itself to the emergence and infiltration and transfer of NPC. The expression of VEGF-C in NPC is correlated to neoplasm lymph angiogenesis and VEGF-C plays a vital role in the progression of NPC – In papillary thyroid carcinoma, HIF-1 alpha, VEGF and VEGF-C expression	601528	Benke et al. (2010)

(continued)

Table 5.25 (continued)

Gene symbol	Gene locous	Function	Alteration in cancer	OMIM	References
			are significantly increased. In carcinoma patients with lymph node metastasis, the higher expressions of VEGF-C are observed as compared with those without metastasis – VEGF-C and its receptor FLT-4 play an important role in the lymphatic metastasis of laryngeal and hypopharyngeal squamous cell carcinoma		
XPC	3p25	– This gene encodes a component of the nucleotide excision repair (NER) pathway – This component, XPC, plays an important role in the early steps of global genome NER, especially in damage recognition, open complex formation, and repair protein complex formation	– XPC 499Val allele and its haplotype were strongly associated with NPC, which indicated that Val499Ala polymorphism may be a contributing factor in the NPC development	613208	Yang et al. (2008)
ZMYND10 (BLU)	3p21.3	– It is a candidate tumor suppressor gene – Required for motile ciliary function – Probably involved in axonemal assembly of inner and outer dynein arms (IDA and ODA, respectively) for proper axoneme building for cilia motility – May act by indirectly regulating transcription of dynein proteins	– In the NPC cell lines, loss of BLU expression correlated with hypermethylation of the CpG island promoter sequence, and expression was restored after treatment with 5′-aza-2′-deoxycytidine – The high incidence of BLU alterations suggests that it may be one of the critical tumor suppressor genes on chromosome 3p21.3 involved in the development of NPC	607070	Liu et al. (2003)

5.10.2 miRNA Etiology

See Table 5.26.

5.11 Thyroid Cancer

Cancer initiates in a cell that is an independent unit to form group of cells and tissues. Tissues also make up the organs of the body. Cell division is normally based on the actual requirement of tissue or organ to grow. Aging and damaging of normal cells are the main cause of the cell death which will be replaced by new cells. Sometimes, this orderly process may be disturbed. However, cell death and formation of new cells are controlled by the programming systems which depends on the requirements of the body. Sometimes, cells' growth and division are, uncontrollably, under the environmental and genetics disruptive pressure which facilitate the cancer initiation (Harris and McCormick 2010).

Thyroid cancer is a cancer originating from follicular or parafollicular thyroid cells. Papillary thyroid cancer (PTC), follicular thyroid cancer (FTC)—and anaplastic thyroid cancer (ATC) are originated from these cells. Surgical removal of thyroid gland (thyroidectomy) followed by radioactive iodine treatment and thyroid stimulating hormone (TSH)-suppression therapy which are revealed to be the most effective management of aggressive thyroid cancers. Thyroid cancer is the most common endocrine malignancy and the survival rate is approximately five years in over 97% of patients (Figge 2016). Thyroid cancers are thought to be related to a number of environmental and genetic predisposing factors, but significant uncertainty remains regarding its causes. Exposure to ionizing radiation is the most important environmental predisposing factor. Multiple endocrine neoplasia type 2 is particularly one of the effective genetic causes of the rare medullary form of the disease (Khaziev et al. 2008). Along with the progress of science and methods of cancer research, a large number of genes that are disrupted in thyroid cancer have been discovered which can help to understand the genetic basis of this cancer and tracing the therapeutic targets. In Table 5.27, some of the reported genes involved in thyroid cancer are summarized.

5.11.1 Genetic Etiology

See Table 5.27.

Table 5.26 Most reported miRNAs which are involved in development of pharyngeal cancer

miRNA-name	Function	Alteration in cancer	References
miR-15a	– MiR-15a can target oncogenes as well as tumor suppressors, depending on individual tissues	High expression in HNOC	Zhu et al. (2009)
mir-15b	– It has a critical role in apoptosis	High expression in HNOC	Zhao et al. (2011a, b, c)
miR-200a	– It regulates regulates epithelial-mesenchymal transition	Lower expression in HNOC	Xia et al. (2010)
miR-100	– It regulates the expression of Ataxia telangiectasia-mutated gene (ATM)	Low expression in HNOC	Henson et al. (2009)
miR-98	– miR-98 regulated Fas expression and the sensitivity of Fas-mediated apoptosis – miR-98 regulates HMGA2 expression and chemosensitivity to doxorubicin and cisplatin	Variable expression in HNOC	Hebert et al. (2007)
miR-19a	– miR-19a was predicted to target the 3'untranslated region of TNF-alpha mRNA, and this was confirmed by luciferase reporter assay – miR-19a also plays an important role in the flow regulation of cyclinD1 expression	High expression in HNOC	Takakura et al. (2008)
miR-29c	– miR-29c induces cell apoptosis and increases extracellular matrix protein accumulation	Downregulated in nasopharyngeal carcinoma	Sengupta et al. (2008)
miR-155	– miR-155 functions as an oncomiR and has a role in metastasis	Upregulated in HNOC	Du et al. (2011), Hess et al. (2017)
miR-34c	– Members of the miR-34 family are known to induce a senescent-like growth arrest when overexpressed in cells, with miR-34c being the most potent – miR-34c inhibition of Myc in response to DNA damage prevents S-phase progression	Downregulated in HNOC	Cai et al. (2010)
miR-34b	– miR-34b belongs to the evolutionary conserved miRNA family miR-34s known for its role in the p53 tumor suppressor network	Downregulated in HNOC	Chen et al. (2009a, b)

(continued)

Table 5.26 (continued)

miRNA-name	Function	Alteration in cancer	References
miR-139	– miR-139 regulates FoxO1 (a master regulator of signaling pathways used by growth factors and hormones) and maintains the protein level of FoxO1 to preserve homeostatic regulation of its transcriptional activity in response to environmental stimuli	Downregulated in HNOC	Chen et al. (2009a, b)
miR-203	– MicroRNA-203 (miR-203) is a tumor suppressor microRNAoften silenced in different malignancies	Downregulated in HNOC	Lena et al. (2008)
let-7g	– Let-7g targets collagen type I alpha2 and inhibits cell migration in hepatocellular carcinoma – let-7g also inhibits proliferation of hepatocellular carcinoma cells by downregulation of c-Myc and upregulation of p16 (INK4A) – let-7g along with miR-9 enhance the sensitivity to ionizing radiation by suppression of NFKB1	Downregulated in HNOC	Wong et al. (2011)
miR-148a	– miR-148a improves response to chemotherapy in sensitive and resistant oesophageal adenocarcinoma and squamous cell carcinoma cells – miR-148a sensitized chemotherapy-sensitive oesophageal cancer cell lines to cisplatin and, to a lesser extent, to 5-flurouracil and attenuated resistance in chemotherapy—resistant variants	Downregulated in HNOC	Chen et al. (2009a, b)
let-7i	– let-7i, regulates Toll-like receptor 4 expression and contributes to cholangiocyte immune responses against *Cryptosporidiumparvum* infection	Variable expression in different tissues of head and neck	(Wong et al. 2011)
miR-18a	– miR-18a can repress ER alpha (Estrogen receptor alpha) translation by binding to its mRNA at the 3′ untranslated region	Upregulated in Head and Neck cancer	Avissar et al. (2009a, b)

(continued)

Table 5.26 (continued)

miRNA-name	Function	Alteration in cancer	References
miR-152	– miR-152 may function as an immune system enhancer through up-regulating NK cell-mediated cytolysis of host cells	Downregulated in nasopharyngeal carcinoma	Chen et al. (2009a, b)
miR-192	– miR-192 has a role in the regulation of sodium and potassium balance in the kidney – MiR-192 may be a critical downstream mediator of TGF-beta/Smad3 signaling in the development of renal fibrosis	Upregulated in head and neck cancer	(2008)
miR-10b	– miR-10b, can contribute to the development of metastasis	Overexpressed in metastatic nasopharyngeal carcinoma (NPC).	Li et al. (2010a, b, c, d)
miR-17	– The STAT3-regulatedmicroRNA, miR-17 played a critical role in MEK (MAP/ERK kinase) inhibitor resistance, such that miR-17 inhibition sensitized resistant cells to AZD6244 (a small molecule inhibitor of the MEK) by inducing BIM and PARP (poly (ADP-ribose) polymerase) cleavage	Upregulated in NPC (Nasopharyngeal cancer	Chen et al. (2009a, b)

5.11.2 Micro RNA and Thyroid Cancer

miRNAs are the non-coding small RNAs ranging from 18 to 25 nucleotides in length that regulate target messenger RNA (mRNA) often negatively. After processing, a mature miRNA binds to the target and induces cleavage or translational repression depending on the degree of complementarity with the target. In a large volume of literatures, miRNAs have reported to be involved in cell proliferation or apoptosis in various types of cancers. In thyroid cancer, deregulation of several miRNAs has been reported, so it sounds that this novel class of small molecules are involved in pathogenesis of thyroid cancer (TRANSLATIONALHIGHLIGH, de la Chapelle and Jazdzewski 2011). Some of the most reported and studied miRNAs in this cancer are shown in Table 5.28.

Table 5.27 Summary of cancer genes involved in thyroid cancer pathogenesis

Gen	Gene locus	Function	Alteration in cancer	OMIM	References
AFAP1L2	10q25.3	– It may play a role in a signaling cascade by enhancing the kinase activity of SRC – Contributes to SRC-regulated transcription activation	– A human thyroid tissue microarray study identified expression of AFAP1l2 (XB130) in normal thyroid tissue as well as in human thyroid carcinomas – In WRO thyroid cancer cells, knockdown of XB130 using small interfering RNA inhibited G (1)-S phase progression, induced spontaneous apoptosis, and enhanced intrinsic and extrinsic apoptotic stimulus-induced cell death – Growth of tumors in nude mice formed from XB130 shRNA stably transfected WRO cells were significantly reduced, with decreased cell proliferation and increased apoptosis – This observations suggest that the expression of XB130 in these cancer cells may affect cell proliferation and survival by controlling the expression of multiple genes, especially transcription regulators	612420	Shiozaki et al. (2011)

(continued)

Table 5.27 (continued)

Gen	Gene locus	Function	Alteration in cancer	OMIM	References
ALCAM	3q13.1	– It is a cell adhesion molecule that binds to CD6 – Involved in neurite extension by neurons via heterophilic and hemophilic interactions – May play a role in the binding of T- and B-cells to activated leukocytes, as well as in interactions between cells of the nervous system	– Activated leukocyte cell adhesion molecule (ALCAM, CD166) is expressed in various tissues, cancers, and cancer-initiating cells – It was documented high levels of ALCAM expression in human thyroid tumors and cell lines	601662	Miccichè et al. (2011)
ANXA3	4q21.21	– This gene encodes a member of the annexin family – This protein functions in the inhibition of phospholipase A2 and cleavage of inositol1, 2-cyclic phosphate to form inositol 1-phosphate – This protein may also play a role in anti-coagulation	– Decreased expression of ANXA3 in papillary thyroid cancer supports the idea that ANXA3 may be an effective marker of microcarcinoma, and a negative predictor of papillary thyroid cancer progression	106490	Jung et al. (2010)
ARG2	14q24.1	– Arginase catalyzes the hydrolysis of arginine to ornithine and urea – The type II isoform encoded by this gene, is located in the mitochondria and expressed in extra-hepatic tissues – The physiologic role of this isoform is poorly understood; it is thought to play a role in nitric oxide and polyamine metabolism	– ARG2 knockdown decreased eNOS expression as well as the expression of eNOS-related genes (p21, Akt1, HIF-1, VEGF, and CAV1) – ARG2 silencing changed tumor properties of thyroid cancer cells promoting apoptosis and reduced expression of cell proliferation markers	107830	Sousa et al. (2010)

(continued)

Table 5.27 (continued)

Gen	Gene locus	Function	Alteration in cancer	OMIM	References
BRAF	7q34	– It is involved in the transduction of mitogenic signals from the cell membrane to the nucleus – May play a role in the postsynaptic responses of hippocampal neuron	– BRAF mutations are common in melanomas and thyroid cancers – The T1799A BRAF mutation occurs exclusively in PTC and PTC-derived anaplastic thyroid cancer and is a specific diagnostic marker for this cancer when identified in cytological and histological specimens	164757	Kimura et al. (2003), Xing (2005)
CDKN2C	1p32	– It functions as a cell growth regulator that controls cell cycle G1 progression	– DNA damage is an important mechanism in carcinogenesis, so genes related to maintaining genomic integrity may influence papillary thyroid cancer (PTC) risk – CDKN2C was associated with PTC risk at $P < 0.01$, suggest a possible role of genes involved in maintenance of genomic integrity in relation to risk of PTC	603369	Neta et al. (2011)
CEACAM1	19q13.2	– This gene encodes a member of the carcino-embryonic antigen (CEA) gene family – The encoded protein mediates cell adhesion via homophilic as well as heterophilic binding to other proteins of the subgroup – Multiple cellular activities have been attributed to the encoded protein, including roles in the differentiation	– Osteopontin (OPN) and its interacting partner CEA-cell adhesion molecule (CEACAM1) mediate similar biological functions and have been expressed in several types of cancer – It was investigated the prognostic significance of OPN in thyroid tumors and correlated to findings with the expression of CEACAM1	109770	(Briese et al. 2010)

(continued)

Table 5.27 (continued)

Gen	Gene locus	Function	Alteration in cancer	OMIM	References
		and arrangement of tissue three-dimensional structure, angiogenesis, apoptosis, tumor suppression, metastasis, and the modulation of innate and adaptive immune responses	– Nearly all normal samples were negative for OPN. Some thyroid adenomas were weakly OPN positive whereas many carcinomas were strongly positive – In contrast to CEACAM1, which was preferentially expressed in metastatic papillary carcinomas, no associations were found between OPN expression and patient age, gender and tumor size		
CITED1	Xq13.1	– This gene encodes a member of the CREB-binding protein/p300-interacting transactivator with Asp/Glu-rich C-terminal domain (CITED) family of proteins – The encoded protein, also known as melanocyte-specific gene 1, may function as a transcriptional co-activator and may play a role in pigmentation of melanocytes	– More than 80% of all thyroid cancers, the most common endocrine malignancy, are papillary thyroid cancer (PTC) – It is well established that CITED1 (Cbp/p300 Interacting Trans activators with glutamic acid [E] and aspartic acid [D]-rich C-terminal domain) mRNA is characteristically overexpressed in PTC – BRAF mutation and aberrant methylation of CITED1 gene promotes regulates CITED1overexpression in PTC	300149	Sassa et al. (2011)
CLDN7	17p13	– It plays a major role in tight junction-specific obliteration of the intercellular space	– Transcriptional activity of claudin 7 gene is lower in laryngeal tumor cells compared to histologically normal tissues – On the other hand CLDN7expression is elevated in cancers of the thyroid	609131	(Kapral et al. 2011)

(continued)

Table 5.27 (continued)

Gen	Gene locus	Function	Alteration in cancer	OMIM	References
CTSH	15q25.1	– The protein encoded by this gene is a lysosomal cysteine proteinase important in the overall degradation of lysosomal proteins – The encoded protein, which belongs to the peptidase C1 protein family, can act both as an aminopeptidase and as an endopeptidase	– An in vitro functional assay showed that CTSH can increase metastasis – J7 cells overexpressing CTSH were inoculated into severe combined immune-deficient mice and these J7-CTSH mice displayed a greater metastatic potential than did J7-control mice	116820	Wu et al. (2011a, b)
DUOX2	15q15.3	– The protein encoded by this gene is a glycoprotein and a member of the NADPH oxidase family – The synthesis of thyroid hormone is catalyzed by a protein complex located at the apical membrane of thyroid follicular cells – This complex contains an i0odide transporter, thyroperoxidase, and a peroxide generating system that includes this encoded protein and DUOX1	– The thyroid gland is a unique endocrine organ that requires hydrogen peroxide (H (2) O (2)) for thyroid hormone formation – The molecule for H (2) O (2) production in the thyroid gland has been known as dual oxidase2 (DUOX2) – Mutation of either DUOX2 or DUOXA2 gene is a newly recognized cause of hypothyroidism due to insufficient H (2) O (2) production – Papillary thyroid carcinoma, the most common thyroid cancer, is closely linked to the increased ROS production by NOX4 – This phenomenon may be explained by the abnormality of iodide-induced H(2) O(2) or other ROS in susceptible individuals	606759	Ohye and Sugawara (2010)

(continued)

Table 5.27 (continued)

Gen	Gene locus	Function	Alteration in cancer	OMIM	References
IGFBP7	4q12	– It binds IGF-I and IGF-II with a relatively low affinity – It stimulates prostacyclin (PGI2) production and also stimulates cell adhesion	– Insulin-like growth factor-binding protein 7 (IGFBP7) is a secreted protein involved in several cellular processes, including proliferation, senescence and apoptosis – Loss of IGFBP7 expression is a critical step in the development of human tumors, including melanoma and cancer – Downregulation of IGFBP7 gene expression has been recorded in follicular and papillary thyroid tumors in comparison with normal thyroid tissue – The functional consequence of IGFBP7downregulation was addressed in the PTC-derived NIM1 cell line in which IGFBP7 expression is repressed by promoter hypermethylation	602867	Vizioli et al. (2010)
IL13RA1	Xq24	– Binds with low affinity to interleukin-13 (IL13) – Together with IL4RA can form a functional receptor for IL13 – Also serves as an alternate accessory protein to the common cytokine receptor gamma chain for interleukin-4	– In case of papillary thyroid cancer, IL13RA1 expression is increased compared to normal thyroid samples	300119	Jarząb et al. (2005)

(continued)

Table 5.27 (continued)

Gen	Gene locus	Function	Alteration in cancer	OMIM	References
		(IL4) signaling, but cannot replace the function of IL2RG in allowing enhanced interleukin-2 (IL2) binding activity			
IQGAP1	15q26.1	– This gene encodes a member of the IQGAP family – The protein contains four IQ domains, one calponin homology domain, one Ras-GAP domain and one WW domain – It interacts with components of the cytoskeleton, with cell adhesion molecules, and with several signaling molecules to regulate cell morphology and motility	– IQGAP1, plays an important role in the invasiveness of thyroid cancer and may represent a novel prognostic marker and therapeutic target for this cancer	603379	Liu et al. (2010a, b, c, d)
LIG4	13q33-q34	– The protein encoded by this gene is a DNA ligase that joins single-strand breaks in a double-stranded poly deoxynucleotide in an ATP-dependent reaction	– Role of polymorphic variants in LIG4 gene found in thyroid cancer – LIG4 (T9I) polymorphisms in the individual introduce susceptibility for this disease	601837	Gomes et al. (2010)
MMP8	11q22.3	– Proteins of the matrix metalloproteinase (MMP) family are involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling, as well as in	– Frequent SNP A259G (K87E) in the MMP8 gene, was observed in various types of thyroid cancer samples. This gene might be a cause of thyroid cancer risk	120355	Murugan et al. (2011)

(continued)

Table 5.27 (continued)

Gen	Gene locus	Function	Alteration in cancer	OMIM	References
		disease processes, such as arthritis and metastasis – However, the enzyme encoded by this gene is stored in secondary granules within neutrophils and is activated by autolytic cleavage			
NDRG2	14q11.2	– This gene is a member of the N-myc downregulated gene family which belongs to the alpha/beta hydrolase superfamily – The protein encoded by this gene is a cytoplasmic protein that may play a role in neurite outgrowth	– NDRG2 mRNA expression in the primary tumor tissues were statistically significantly lower vs control – The levels of NDRG2 mRNA expression in macroscopically unchanged thyroid tissue ($p < 0.0001$) – The results demonstrates decreased NDRG2 mRNA expression levels in PTC	605272	Mordalska et al. (2010)
NRAS	1p13.2	– This is an N-ras oncogene encoding a membrane protein that shuttles between the Golgi apparatus and the plasma membrane – This shuttling is regulated through palmitoylation and depalmitoylation by the ZDHHC9-GOLGA7 complex – The encoded protein, which has intrinsic GTPase activity, is activated by a guanine nucleotide-exchange factor and inactivated by a GTPase activating protein	– Oncogene mutations and translocations are linked to thyroid cancer – NRAS gene mutation have been observed with the development of thyroid cancer	164790	Tobias et al. (2011)

(continued)

Table 5.27 (continued)

Gen	Gene locus	Function	Alteration in cancer	OMIM	References
PITX2	4q25	– This gene encodes a member of the RIEG/PITX home box family, which is in the bicoid class of home domain proteins – The encoded protein acts as a transcription factor and regulates procollagen lysyl hydroxylase gene expression – This protein plays a role in the terminal differentiation of somatotroph and lactotroph cell phenotypes	– PITX2 was frequently expressed in human follicular cell-derived (papillary, follicular and anaplastic) thyroid cancer tissues but not in normal thyroids, indicating for the first time that over-activated PITX2 may contribute to thyroid cancer – Knockdown of PITX2 gene expression in human thyroid cancer cells significantly reduced cell proliferation	601542	Huang et al. (2010)
PROK1	1p21	– Endocrine gland-derived vascular endothelial growth factor (EG-VEGF) induces proliferation, migration, and fenestration in capillary endothelial cells derived from endocrine glands	– Endocrine gland-derived vascular endothelial growth factor (Prok1) and prokineticin 2 (Prok2) are involved in the organ-specific regulation of angiogenesis, which is a crucial step toward cancer progression in most tumors, including those of thyroid gland – Prok1 mRNA levels were very low in normal thyroid (NT) tissues and thyroid multinodular goiter (TMNG) tissues but significantly higher in papillary thyroid cancer, PTC	606233	Pasquali et al. (2011)

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Table 5.27 (continued)

Gen	Gene locus	Function	Alteration in cancer	OMIM	References
PTPRJ	11p11.2	– The protein encoded by this gene is a member of the protein tyrosine phosphatase (PTP) family – PTPs are known to be signaling molecules that regulate a variety of cellular processes, including cell growth, differentiation, mitotic cycle, and oncogenic transformation	– The strong genetic predisposition to papillary thyroid carcinoma (PTC) might be due to a combination of low-penetrance susceptibility variants – The gene coding for the receptor-type tyrosine phosphatase PTPRJ has been proposed as a cancer susceptibility gene, and its role as a tumor suppressor gene is well established in thyroid carcinogenesis	600925	Iuliano et al. (2010)
PYCARD	16p11.2	– This gene encodes an adaptor protein that is composed of two protein-protein interaction domains: a N-terminal PYRIN-PAAD-DAPIN domain (PYD) and a C-terminal caspase-recruitment domain (CARD) – The PYD and CARD domains mediates assembly of large signaling complexes in the inflammatory and apoptotic signaling pathways via the activation of caspase	– PYCARD (TMS1) is a tumor suppressor gene that encodes for caspase recruitment domain containing regulatory protein and has been shown to be hypermethylated in various cancers – A quarter percentage of TMSI gene is methylated in thyroid cancer cells and repression of methylation by 5-aza-2′-deoxycytidine restored expression of the TMS1 gene and sensitized cells to TRAIL-induced apoptosis	606838	Siraj et al. (2011)
RAP1GAP	1p36.1-p35	– It is a GTPase activator for the nuclear Ras-related regulatory protein RAP-1A (KREV-1), converting it to the putatively inactive GDP-bound state	– Increases in Rap activity have been associated with tumor progression and effectively downregulation of Rap1GAP is frequent in human tumors associated with thyroid carcinomas	600278	Dong et al. (2011)

(continued)

Table 5.27 (continued)

Gen	Gene locus	Function	Alteration in cancer	OMIM	References
RASSF2	20p13	– It is a potential tumor suppressor. Acts as a KRAS-specific effector protein – May promote apoptosis and cell cycle arrest	– RASSF2 methylationwas significantly increased in primary thyroid carcinoma compared to normal thyroid – RASSF2 promoter hypermethylation causes its reduced expression. Overexpression of RASSF2 induces apoptosis in thyroid cancer cell lines. RASSF2 acts as a proapoptotic tumor suppressor in thyroid carcinogenesis	609492	Schagdarsurengin et al. (2010) Mohammadi-asl et al. (2011)
RET	10q11.2	– This gene, a member of the cadherin superfamily, encodes one of the receptor tyrosine kinases, which are cell-surface molecules that transduce signals for cell growth and differentiation – This gene plays a crucial role in neural crest development, and it can undergo oncogenic activation in vivo and in vitro by cytogenetic rearrangement	– The most common molecular alterations in thyroid cancer include RET/PTC rearrangement.	164761	Nikiforov (2011)
SLC34A2	4p15.2	– The protein encoded by this gene is a pH-sensitive sodium-dependent phosphate transporter – Phosphate uptake is increased at lower pH – Defects in this gene are a cause of pulmonary alveolar microlithiasis	– Transcriptional profiles of PTC samples showed an overexpression of SLC34A2 in compared to normal thyroid tissues	604217	Kim et al. (2010a, b)

(continued)

Table 5.27 (continued)

Gen	Gene locus	Function	Alteration in cancer	OMIM	References
SNRPE	1q32	– It appears to function in the U7 snRNP complex that is involved in histone3'-end processing – It is associated with snRNP U1, U2, U4/U6 and U5	– The incidence of thyroid cancer continues to increase and this neoplasia remains the most common endocrine malignancy – SNRPE (B-Raf) gene mutations, and vascular endothelial growth factor receptor 2 (VEGFR-2) angiogenesis pathways are some of the known genetic alterations playing a crucial role in the development of thyroid cancer	128260	Perez et al. (2012)
SOD3	4p15.3-p15.1	– This gene encodes a member of the superoxide dismutase (SOD) protein family – Protect the extracellular space from toxic effect of reactive oxygen intermediates by converting superoxide radicals into hydrogen peroxide and oxygen	– Sod3 is highly expressed in normal thyroid, and becomes even more abundant in rat goiter models – It was shown TSH-stimulated expression of Sod3 via phospholipase C-Ca(2 +) and cAMP-protein kinase A, a pathway that might be disrupted in thyroid cancer – In line with this finding, we demonstrated an oncogene-dependent decrease in Sod3 mRNA expression synthesis in thyroid cancer cell models	185490	Laatikainen et al. (2010)
SPRY2	13q31.1	– This gene encodes a protein belonging to the sprouty family – This protein is indirectly involved in the non-cell autonomous inhibitory	– BRAF mutations activate the mitogen-activated protein kinase pathway and often confer an	602466	Xu et al. (2010)

(continued)

Table 5.27 (continued)

Gen	Gene locus	Function	Alteration in cancer	OMIM	References
		effect on fibroblast growth factor two signaling – The protein interacts with Cas-Br-M (murine) ectropic retroviral transforming sequence, and can function as a bimodal regulator of epidermal growth factor receptor/mitogen-activated protein kinase signaling	aggressive thyroid cancer (TC) phenotype – Spry2 expression correlates with BRAF status in vitro and in human tissue		
SSTR1	14q13	– Receptor for somatostatin with higher affinity for somatostatin-14 than -28 – This receptor is coupled via pertussis toxin sensitive G proteins to inhibition of adenylyl cyclase – In addition it stimulates phosphotyrosine phosphatase and Na +/H+ exchanger via pertussis toxin insensitive G proteins	– Somatostatin receptors (SSTR) are expressed in various endocrine tumors – The expression of SSTR at the tumor cell surface confers the possibility for diagnostic imaging and therapy of tumours using radiolabeled somatostatin analogues – Weak expression of SSTR1 was deleted in PTCs	182451	Klagge et al. (2010)
SSTR2	17q24	– Somatostatin acts at many sites to inhibit the release of many hormones and othersecretoryproteins – SSTR2 is a member of the superfamily of receptors having seven transmembrane segments	– Somatostatin receptors (SSTR) are expressed in various endocrine tumors – The expression of SSTR at the tumor cell surface confers the possibility for diagnostic imaging and therapy of tumors using radiolabeled somatostatin analogues	182452	Klagge et al. (2010)

(continued)

Table 5.27 (continued)

Gen	Gene locus	Function	Alteration in cancer	OMIM	References
			– SSTR2 was significantly upregulated in PTC and ATC – SSTR2 is the predominant subtype in thyroid epithelial tumors with a high expression pattern, in particular, in PTC		
SSTR3	22q13.1	– Somatostatin acts at many sites to inhibit the release of many hormones and other secretory proteins – SSTR3 is a member of the superfamily of receptors having seven transmembrane segments	– Somatostatin receptors (SSTR) are expressed in various endocrine tumors – The expression of SSTR at the tumor cell surface confers the possibility for diagnostic imaging and therapy of tumoursusing radiolabeled somatostatin analogues – Significantupregulation of SSTR3 was found in PTC	182453	Klagge et al. (2010)
SSTR4	20p11.2	– Receptor for somatostatin-14. The activity of this receptor is mediated by G proteins which inhibits adenylyl cyclase – It is functionally coupled not only to inhibition of adenylate cyclase, but also to activation of both arachidonate release and mitogen-activated protein (MAP) kinase cascade – Mediates antiproliferative action of somatostatin in tumor cells	– Somatostatin receptors (SSTR) are expressed in various endocrine tumors – The expression of SSTR at the tumor cell surface confers the possibility for diagnostic imaging and therapy of tumors using radiolabeled somatostatin analogues – Weak SSTR4 mRNA expression was detected in some PTCs	182454	Klagge et al. (2010)

(continued)

Table 5.27 (continued)

Gen	Gene locus	Function	Alteration in cancer	OMIM	References
SSTR5	16p13.3	– Receptor for somatostatin 28 and to a lesser extent for somatostatin-14 – The activity of this receptor is mediated by G proteins which inhibit adenylyl cyclase – Increases cell growth inhibition activity of SSTR2 following heterodimerization	– Somatostatin receptors (SSTR) are expressed in various endocrine tumors – The expression of SSTR at the tumor cell surface confers the possibility for diagnostic imaging and therapy of tumors using radiolabeled somatostatin analogues – Predominant expression of SSTR5 has been observed	182455	Klagge et al. (2010)
TP53INP1	8q22	– In response to double-strand DNA breaks, this gene product promotes p53/TP53 phosphorylation on 'Ser-46' and subsequent apoptosis.	– TP53INP1immunoexpression appears to be a clinical predictor of lymph node metastasis in MTC – The evaluation of TP53INP1 expression may guide the extent of lymph node dissection in the clinically node-negative neck – These findings require prospective validation	606185	Taieb et al. (2010)
VEGFC	4q34.3	– It is active in angiogenesis and endothelial cell growth, and can also affect the permeability of blood vessels.	– VEGF-C facilitates tumor cell growth and motility in HNSCC cells. VEGF-C is overexpressed in head and neck squamous carcinoma cell lines compared to normal keratinocytes – VEGF-Coverexpression in OSCC cells is associated with lymph node metastasis – In nasopharyngeal carcinoma, the expression rate of VEGF-C is high.	601528	Benke et al. (2010) Zhao et al. (2011a, b, c) Hunter et al. (2007)

(continued)

Table 5.27 (continued)

Gen	Gene locus	Function	Alteration in cancer	OMIM	References
			VEGF-C expression is positively correlated with lymph node metastasis and tumor staging. VEGF-C attaches itself to the emergence and infiltration and transfer of NPC. The expression of VEGF-C in NPC is correlated to neoplasm lymph angiogenesis and VEGF-C plays a vital role in the progression of NPC – In papillary thyroid carcinoma, HIF-1 alpha, VEGF and VEGF-C expression are significantly increased. In carcinoma patients with lymph node metastasis, the higher expressions of VEGF-C are observed as compared with those without metastasis – VEGF-C and its receptor FLT-4 play an important role in the lymphatic metastasis of laryngeal and hypopharyngeal squamous cell carcinoma		
FOXE1	9q22.33	– Transcription factor that binds consensus sites on a variety of gene promoters and activate their transcription – Involved in proper palate formation, most probably through the expression of MSX1 and TGFB3 genes which are	– FOXE1 is involved in the morphogenesis and differentiation of the thyroid – FOXE1 germ line variants have been reported in the familial forms of non-medullary thyroid carcinoma (FNMTC)	602617	Pereira et al. (2015)

(continued)

Table 5.27 (continued)

Gen	Gene locus	Function	Alteration in cancer	OMIM	References
		direct targets of this transcription factor – Also implicated in thyroid gland morphogenesis – May indirectly play a role in cell growth and migration through the regulation of WNT5A expression			
NKX2-1	14q13.3	– Transcription factor that binds and activates the promoter of thyroid specific genes such as thyroglobulin, thyroperoxidase, and thyrotropin receptor – Crucial in the maintenance of the thyroid differentiation phenotype – May play a role in lung development and surfactant homeostasis.	– Germ line mutation have been identified in papillary thyroid carcinoma (PTC) among patients with multinodular goiter (MNG) (MNG/PTC) patients	600635	Ngan et al. (2009)
XRCC4	5q14.2	– The protein encoded by this gene functions together with DNA ligase IV and the DNA-dependent protein kinase in the repair of DNA double-strand break by non-homologous end joining and the completion of V (D) J recombination events	– Variations, such as single nucleotide polymorphisms (SNPs) in DNA damage repair genes have been pointed out as possible factors to cancer predisposition – The role of polymorphic variants in XRCC4 (N298S and T134I) gene have been studied on the role of these genes in thyroid cancer susceptibility	194363	Gomes et al. (2010)

(continued)

Table 5.27 (continued)

Gen	Gene locus	Function	Alteration in cancer	OMIM	References
XRCC5	2q35	– The protein encoded by this gene is the 80-kilodalton subunit of the Ku heterodimer protein which is also known as ATP-dependant DNA helicase II or DNA repair protein XRCC5 – Ku is the DNA-binding component of the DNA-dependent protein kinase, and it functions together with the DNA ligase IV-XRCC4 complex in the repair of DNA double-strand break by non-homologous end joining and the completion of V (D) J recombination events	– The role of polymorphic variants in XRCC5 (Ku80) gene have been studied on the role of these genes in thyroid cancer susceptibility	194364	Gomes et al. (2010)

Table 5.28 Most reported and studied miRNAs in thyroid cancer

MiRNA	Function	Alteration in Cancer	References
miR-30b	– It may play role intumorigenesis	High Expression in HNOC	Rahbari et al. (2011)
miR-19a	– miR-19a was predicted to target the 3′ untranslated region of TNF-alpha mRNA, and this was confirmed by luciferase reporter assay – miR-19a also plays an important role in the flow regulation of cyclin D1 expression	High Expression in HNOC	Takakura et al. (2008)
miR-146b	– It is involved in immune response and tragetsIRAK1, TRAF6	Upregulated in HNOC	Chou et al. (2010)
miR-345	– miR-345 target multidrug resistance protein 1 (MRP1)	Overexpressed in progressiveleukoplakia and OSCCs	Marini et al. (2011)
miR-139	– miR-139 regulates FoxO1(a master regulator of signaling pathways used by growth factors and hormones) and maintains the protein level of FoxO1 to preserve homeostatic regulation of its transcriptional activity in response to environmental stimuli	Downregulated in HNOC	Corbetta et al. (2010)
miR-96	– miR-96 is a sensory organ-specific miRNA expressed in the mammalian cochlea during development – miR-96 regulates the progression of differentiation in mammalian cochlear inner and outer hair cells – miR-96 also decreased cancer cell invasion and migration and slowed tumor growth in a manner associated with KRAS downregulation	Downregulated in HNOC	Marini et al. (2011)
miR-130b	– miR-130b promotes CD133 (+) liver tumor-initiating cell growth and self-renewal via tumor protein 53-induced nuclear protein 1	Upregulated in head and neck cancer	Marini et al. (2011)

(continued)

Table 5.28 (continued)

MiRNA	Function	Alteration in Cancer	References
miR-491	– miR-491 markedly decreased cell viability by inducing apoptosis. Bcl-X(L) was a direct target of miR-491, and its silencing contributed to miR-491-induced apoptosis	Upregulated in head and neck cancer	Mitomo et al. (2008)
miR-138	– miR-138 has been thought to play a role in the development of mammary gland, regulating dendritic spine morphogenesis, and modulating cardiac patterning during embryonic development – The precise regulation of these diversified biological processes is dependent on the ability of miR-138 to regulate multiple target genes in the specific physiological/pathological settings	Downregulated	Mitomo et al. (2008)
miR-221	– miR-221 acts as a potent posttranscriptional regulator of FAS-induced apoptosis. miR-221 regulates hepatic expression of p53 up-regulated modulator of apoptosis, a well-known pro-apoptotic member of the Bcl2 protein family – miR-221may serve as a potential therapeutic target for the treatment of hepatitis and liver failure	Upregulated in HNSCC.	Mitomo et al. (2008), Dai et al. (2017)

5.12 Tongue Cancer

Cancer is a genetic disease and the gene variations are the only factors that finally cause cancer. All inherited disorders are classified as genetic diseases but all genetic diseases are not inherited. Cancers are developed in different tissues of the body and every cell may have the potential to become a cancer cell. These cells are mostly somatic cells and not germ line cells (Kleinsmith 2006; Stewart and Wild 2014).

Cancer of soft tissue such as tongue, lips, cheeks and gums consists of over 90% of all the oral cavity cancers from which tongue cancer (TC) is revealed to be the most common. Due to inclusion of tongue in the oral cavity and its visibility, detection of changes related to the oral cavity is possible by the patients themselves, but occurrence of any changes may not be considered as cancer (Walker et al. 2003; de Castro Junior et al. 2016). The most common type of TC is squamous cell carcinoma (SCCA). Squamous cells are flat, skin like cells that cover the lining of the mouth, nose, larynx, thyroid and throat. Squamous cell carcinoma is the given name to a cancer that miss-behave in these cells (Warnakulasuriya 2009). Smoking tobacco (cigarettes, cigars and pipes) and an excess alcohol drinking are the main risk factors for cancers of the head and neck in the western world. The HPV virus transmitted through sexual contact is another risk factor (Petti 2009). The tongue is the most common intraoral site for cancer in most countries, however its global epidemiology shows significant geographic variation and remains a serious health problem in many countries (Moore et al. 2000; Naghavi et al. 2015). In Table 5.29, the most important and reported genes in TC are listed.

5.12.1 Genetic Pathology

See Table 5.29.

5.12.2 How miRNAs Deregulation Can Be Involved in Tongue Cancer?

Oral cancer is the sixth most common malignancy globally. TC is the most common one of the oral cancer. As like as any cancer, its development and progression require tumour suppressor genes to be inactivated and proto-oncogenes to be activated. It is clear that the expression of these genes is relatively associated to RNA and microRNA based mechanisms. Proliferation, differentiation, apoptosis, survival, motility, invasion and morphogenesis are the variety of cellular process under control of microRNAs (Gomes and Gomez 2008, Kolokythas et al. 2011, Courthod et al. 2014). Based on this knowledge, microRNAs are expected to be involved in pathogenesis of cancers, especially tongue cancer. Hence, the most reported miRNAs deregulated in tongue cancer are summarized in Table 5.30.

Table 5.29 Genetic etiology of Tongue cancer. Most reported gene involved in pathogenesis of TC

Gene	Cytogenetic location	Function	Alteration in cancer	OMIM number	References
KIAA1524 (CIP2A)	3q13.13	– Cancerous inhibitor of PP2A (CIP2A) – oncoprotein – Acts as a prognostic marker in gastric and non-small cell lung cancers	– High CIP2A expression leads to poor survival in tongue cancer patients – Cytoplasmic CIP2A expression has been shown to be higher in severe dysplasia than in mild dysplasia	610643	Böckelman et al. (2011)
PTK2 (FAK)	8q24.3	– This gene encodes acytoplasmic protein tyrosine kinase – Activation of this gene may be an important early step in cell growth and intracellular signal transduction pathways triggered in response to certain neural peptides or to cell interactions with the extracellular matrix	– Primary tongue cancers show high expression of FAK – RNAi-mediated FAK reduction decreased tongue cancer cell migration, invasion and anoikis resistance	600758	Jiang et al. (2010)
FANCC	9q22.3	– The protein encoded by this gene delays the onset of apoptosis and promotes homologous recombination repair of damaged DNA – DNA repair protein that may operate in a postreplication repair or a cell cycle checkpoint function – May be implicated in interstrand DNA cross-link repair and in the maintenance of normal chromosome stability – Upon IFNG induction, may facilitate STAT1 activation by recruiting STAT1 to IFNGR1	– The expression level have been evaluated for the Fanconi anemia (FA) genes FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCJ, FANCL and FANCM in HNSCC cell lines and tongue carcinoma samples – Down-regulation of at least one FA gene was observed in HNSCC cell lines and tongue carcinoma samples – FANCB, FANCC, FANCF, FANCJ and FANCM were most commonly affected by down-regulation, whereas down-regulation of FANCA, FANCE and FANCD2 was rare	613899	Wreesmann et al. (2007)

(continued)

Table 5.29 (continued)

Gene	Cytogenetic location	Function	Alteration in cancer	OMIM number	References
FHIT	3p14.2	– This gene, a member of the histidine triad gene family, encodes a adenosine 5′, 5‴-P1, P3-triphosphate hydrolase involved in purine metabolism	– Loss of FHIT expression predicts significantly poor overall survival in patients with HNSCC and an increased rate of distant metastasis – Loss of FHIT is frequent in SCC of the tongue. FHIT plays an important role in tongue tumorigenesis and may be an independent negative prognostic indicator for clinical outcome – The FHIT gene is disrupted in HNSCCs and hence, loss of FHIT function may be important in the development and/or progression of head and neck cancers	601153	Tai et al. (2004)
IFI6	1p35	– The encoded protein may play a critical role in the regulation of apoptosis.	– The oral tongue squamous cell carcinoma (OTSCC) is one of the most common types of HNOSCC – It is significantly more aggressive than other forms of HNOSCC, in terms of local invasion and spread – IFI6 is up-regulated control biological processes in OTSCCs	147572	Ye et al. (2008)
ITGA3	17q21.33	– Integrin alpha-3/beta-1 is a receptor for fibronectin, laminin, collagen, epiligrin, thrombospondin and CSPG4 – Integrin Alpha-3/beta-1 may mediate with LGALS3 the stimulation by CSPG4 of endothelial cells migration	– ITGA3 mediates – Invasiveness in HNSCC cells – ITGA3 expression is significant factor for the outcome of death in of squamous cell carcinoma of the tongue TSCC	605025	Yu et al. (2008)

(continued)

Table 5.29 (continued)

Gene	Cytogenetic location	Function	Alteration in cancer	OMIM number	References
JUP	17q21	– The membrane-associated plaques are architectural elements in an important strategic position to influence the arrangement and function of both the cytoskeleton and the cells within the tissue	– Catenins and E-cadherin are important epithelial adhesion molecules in normal epithelium – Loss of E-cadherin-catenin adhesion is an important step in the progression of many epithelial cancers – Catenins were highly under-expressed in oral tongue carcinoma, metastatic lymph node, and recurrent tumour. Gamma-catenin had predictive value for nodal metastasis – JUP over expression is a significant factor for cervical lymph node metastasis of tongue squamous cell carcinoma – High ITGB4/JUP levels are associated with a significantly high death rate in TSCC patients	173325	Kurokawa et al. (2008) Chow et al. (2001)
KRT17	17q21.2	It may play a role in the formation and maintenance of various skin appendages, specifically in determining shape and orientation of hair	– KRT17 is a molecular marker of distant metastasis in HNSCC patients – KRT 17 is upregulatedin tongue squamous cell carcinoma	148069	Ye et al. (2008)
PFDN5 (MM-1)	12q12	– This gene encodes a member of the prefoldin alpha subunit family – The encoded protein is one of six subunits of prefoldin – The encoded protein may also repress the transcriptional activity of the proto-oncogene c-Myc	– MM-1 is a candidate for a tumor suppressor in tongue cancer	604899	Fujioka et al. (2001)

(continued)

Table 5.29 (continued)

Gene	Cytogenetic location	Function	Alteration in cancer	OMIM number	References
RAB1	2p14	– This gene encodes a member of the Ras superfamily of GTPases – Members of the gene family cycle between inactive GDP-bound and active GTP-bound forms – This small GTPase controls vesicle traffic from the endoplasmic reticulum to the Golgi apparatus	– Rab1A is a potential biomarker of tongue carcinogenesis – It is overexpressed in tongue squamous cell carcinomas	179508	Shimada et al. (2005)
SCEL	13q22	– This protein localizes to the periphery of cells and may function in the assembly or regulation of proteins in the cornified envelope	– Down-regulation of SCEL gene analysis suggested a number of altered biological processes in OTSCCs, including enhancements in phosphate transport, collagen catabolism, I-kappa B kinase/NF-kappa B signaling cascade, extracellular matrix organization and biogenesis, chemotaxis, as well as suppressions of superoxide release, hydrogen peroxide metabolism, and cellular response to hydrogen peroxide, keratinization, and keratinocyte differentiation in OTSCCs	604112	Ye et al. (2008)

(continued)

Table 5.29 (continued)

Gene	Cytogenetic location	Function	Alteration in cancer	OMIM number	References
TNF	6p21.3	– It is mainly secreted by macrophages and can induce cell death of certain tumor cell lines	– Patients with SCC in the larynx present an altered TNF-alpha expression profile compared to healthy controls – TNF-alpha expression is elevated in the salivary samples of patients with SCC of tongue – Salivary levels of TNF-alpha can identify the progression of TSCC from high-risk to neoplasm, serving as potential biomarkers for cancer screening and early detection – TNF-alpha induces phosphorylation of nuclear p65 (Ser276) subunit of NF kappa B which results in NF-kB activation and promotes the cell proliferation and malignant phenotype of HNSCC	191160	Korostoff et al. (2011)
VHL	3p25.3	– The protein encoded by this gene is a component of the protein complex that includes elongin B, elongin C, and cullin-2, and possesses ubiquitin ligaseE3 activity – This protein is involved in the ubiquitination and degradation of hypoxia-inducible-factor (HIF), which is a transcription factor that plays a central role in the regulation of gene expression by oxygen	– A wide range of deletions in 3p, including at the VHL gene, may play a role in the development of tongue cancer	608537	Asakawa et al. (2008)

(continued)

Table 5.29 (continued)

Gene	Cytogenetic location	Function	Alteration in cancer	OMIM number	References
WNT5A	3p21-p14	– These proteins have been implicated in oncogenesis and in several developmental processes, including regulation of cell fate and patterning during embryogenesis	– The Wnt/beta-catenin signaling pathway plays an important role in development, tissue homeostasis, and regeneration – Inappropriate activation of the Wnt pathway is linked to a wide range of human cancers – Expression of Wnt5a is related to malignant transformation and conversion of oral mucosa – Wnt-5a was strongly expressed in OSCC cells which results in transrepression of E-cadherin and triggering epithelial-mesenchymal transition (EMT), suggesting associations of Wnt-5a with mesenchymal phenotype of SCC cells – Up-regulation of Wnt-5a is possible marker of the malignant phenotype of human OSCC – WNT5A expression is elevated in both the epithelial component of a meloblastomas, the most common	164975	Fracalossi et al. (2010), Taki et al. (2003), Sukarawan et al. (2010)

(continued)

Table 5.29 (continued)

Gene	Cytogenetic location	Function	Alteration in cancer	OMIM number	References
			epithelial odontogenic tumor, and in this tumor's likely precursor cell, the enamel epithelium located at the cervical loop of normal developing human tooth buds – WNT5A signaling is important in modulating tumorigenic behaviors of enamel epithelium cells in ameloblastomas – Overexpression of WNT5A greatly increased tumourigenic properties of enamel epithelial cell and its migration and actin reorganization		

Table 5.30 Some reported miRNAs involved in pathogenesis of TC

MiRNA	Function	Alteration in cancer	References
mir-21	– miR-21 functions as anoncogene and modulates tumorigenesis through regulation of several genes.miR-21 regulates cell growth, cytochrome C release, and apoptosis	High Expression	Li et al. (2009), Chen et al. (2017)
miR-23a	– miR-23a is a novel microRNA normalizer – miR-23a represses Runx2 in the terminally differentiated osteocyte, representing a feedback mechanism to attenuate osteoblast maturation – A regulatory network have been established with a central role for the miR cluster 23a-27a-24-2 in both progression and maintenance of the osteocyte phenotype	High Expression	Scapoli et al. (2010) Yu et al. (2010a, b, c)
miR-24	– miR-24, a ubiquitously expressed miRNA, has an anti-proliferative effect	High Expression	Lin et al. (2010a, b, c), Zhao et al. 2016)
miR-107	– This miRNAs regulate gene expression involved in cell division, metabolism, stress response, and angiogenesis in vertebrate species	Downregulatedin	Liu et al. (2009a, b)
miR-28	– miR-28 targets the 3'untranslated (3'UTR) region of MPL (myeloproliferative leukemia), inhibiting its translation, as well as other proteins potentially involved in megakaryocyte differentiation, such as E2F6 – Expression of miR-28 in CD34-derivedmegakaryocytes inhibited terminal differentiation	High Expression in	Shiiba et al. (2010)
miR-184	miR-184 plays an essential role in development andand miR-184 has been also implicated in several forms of cancer – miR-184 regulates proliferation, c-Myc expression, and apoptosis	Upregulated	Wong et al. (2008a, b)
miR-372	– An oncogenic role for miR-372 in controlling cell growth, cell cycle, and apoptosis through down-regulation of a tumor suppressor gene has been demonstrated	Upregulated	Wong et al. (2008a, b)

(continued)

Table 5.30 (continued)

MiRNA	Function	Alteration in cancer	References
miR-154	– miR-154 directly targets cyclin D2 (CCND2), which is essential for the control of cell cycle progression – miR-154 inhibited tumor cell malignancy and the G1/S transition – mir-154 enriches in embryonic tissues and is related to embryonic development	Upregulated	Shiiba et al. (2010)
miR-197	– miR-197 regulate expression of tumor suppressor gene FUS1	Upregulated	Du et al. (2009)
miR-147	– miR-147 is induced upon Toll-like receptor stimulation and regulates macrophage inflammatory responses	Upregulated	Wong et al. (2008a, b)
miR-325	– mir-325 alteration is associated with X-Chromosomal Schizophrenia	Upregulated	Lin et al. (2010a, b, c)
miR-181c	– miR-181c was proven to have the potential to regulate CD4(+) T cell activation	Upregulated	Shimada et al. (2005)
miR-198	– miR-198 inhibits HIV-1 gene expression and replication in monocytes and its mechanism of action appears to involve repression of cyclin T1	Upregulated	Shiiba et al. (2010)
miR-134	– It is a powerful inducer ofpluripotent stem cell differentiation	Upregulated	Wong et al. (2008a, b)
miR-133b	– miR-133b regulates proliferation, apoptosis, and PKM2 expression	Lowered Expression	Wong et al. (2008a, b)
miR-149	– The differentially expressed miR-149 has been identified to be involved in HCV (hepatitis C-virus) entry, replication and propagation – miR-149 is detectable in maternal plasma during pregnancy and showed reduced detection rates inpostdelivery plasma – hsa-mir-149 targets thevpr gene during HIV-1 infection	Downregulated	Liu et al. (2010a, b, c, d)
miR-195	– miR-195 may block the G(1)/S transition by repressing Rb-E2F signaling through targeting multiple molecules, including cyclin D1, CDK6, and E2F3	Downregulatedin	Wong et al. (2008a, b)

(continued)

Table 5.30 (continued)

MiRNA	Function	Alteration in cancer	References
miR-138	– miR-138 has been thought to play a role in the development of mammary gland, regulating dendritic spine morphogenesis, and modulating cardiac patterning during embryonic development – The precise regulation of these diversified biological processes is dependent on the ability of miR-138 to regulate multiple target genes in the specific physiological/pathological settings	Downregulated	Liu et al. (2009a, b), Mitomo et al. (2008)

5.13 Genetic Aspects of Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is known as the major histologic type of malignant primary liver neoplasm. It is the fifth most prevalent cancer and the third more common cause of tumor-related death around the world. Various risk factors such as hepatitis C, hepatitis B, chronic hepatitis, cirrhosis, fungal toxin aflatoxin, obesity, iron storage disease, excessive consumption of alcohol and Trisomy 18 can provide the context of this cancer. Hepatoblastomas involve 1 to 2% of every harmful neoplasm of youth, regularly occurring in infants under 3 years old. Hepatoblastomas are thought to be derived from undifferentiated hepatocytes. This cancer is more common in men than women. Abdominal mass, abdominal and back pain, nausea, anemia, jaundice, itching, weight loss and fever are the clinical symptoms of this cancer (Taniguchi et al. 2002; Nault and Zucman-Rossi 2014).

Correct function of thousands proteins encoded by our genome is a vital necessity for normal function of cells, tissues, organs, and consequently the health of body. When a gene is mutated, mutations effect on an accurate function of protein. When genetic instruction is altered, protein losses its function partially or completely, Therefore, loss of function of a key protein results in loss of normal development or causes a related medical complication. Gene mutation can be occurred through different mechanisms and could be detected as point mutation, deletion, amplification, and epigenetic modification (Zhu and Wetta 2014).

Hereditary or non-hereditary cancers are the disease of DNA and accumulation of genetic alterations in genes involved in cell cycle and proliferation control are the most genetic defect that promote cancer development. Although different types of cancers have some gene mutation in common, others are recurrently observed in a specific tumor type. In this way, the extensive information on tumor-specific genetic alterations is required. So, adequate genetic information at tumor level is an

influential approach which lead to unmask the genetic base of the cancer development. Besides, correlating these changes with clinicohistological parameters has the prognostic impact on cancer management and will improve our understanding on the multistep process of tumorigenesis (Kim 2015).

In addition to coding genes, it has been recognized that small non-coding RNAs are crucial in gene regulation and chromatin stability. miRNAs are one of these RNAs which play an important role in controlling the gene expression. Alteration of miRNAs have been extensively reported in wide spectrum of cancers. These biomolecules can act either as tumor-suppressor genes or as oncogenes. So, addressing of miRNAs in cancer can be helpful to understand the mechanism of tumorigenesis, and eventually improve the field of diagnosis and drug discovery (Braconi et al. 2011).

In Tables 5.31 and 5.32, we have summarized the most reported genes and miRNA alterations involved in liver cancer especially HCC.

5.13.1 Genetic Etiology

See Table 5.31.

5.13.2 miRNA Etiology

See Table 5.32.

5.14 Importance of Pedigree Analysis

A comprehensive pedigree is the reliable source of family history of diseases including cancer, but it is important to be updated whenever is required. Genetics information of proband and the relatives are the key data that could direct the genetic counselor to offer the most appropriate counseling for application of the essential test (s), the preventive, predictive and prognostic managements not even for the proband, but also for the relatives who may be at risk of being affected with neoplasm. Such channel is a guide for improving the individuals' present and future life style within a pedigree. Pedigree analysis is an aid to determine a pattern of a disease transmission. Additionally, pedigree help to determine if a disease is genetically or a result of lifestyle and environment factors. Basic competence in obtaining and interpreting a family medical history and pedigree are the essential gate ways for health care professionals and those who are involved in Cancer Genetics. Pedigree analysis is the most powerful and cost-effective approach to identify the individuals at risk for rare single-gene disorders as well as for common

Table 5.31 Summary of genes involved in the pathogenesis and development of liver cancer

Gene	Location	Function	OMIM	References
CASP8	2q33.1	– Caspases (cysteinyl aspartate proteases) are involved in the signaling pathways of apoptosis, necrosis and inflammation – These enzymes can be divided into initiators and effectors – The initiator isoforms are activated by, and interact with, upstream adaptor molecules – Isoform 5, isoform 6, isoform 7 and isoform 8 lack the catalytic site and may interfere with the pro-apoptotic activity of the complex	601763	Freimuth et al. (2013)
PIK3CA	3q26.32	– The protein encoded by this gene represents the catalytic subunit, which uses ATP to phosphorylate PtdIns, PtdIns4P and PtdIns (4, 5) P2 – PI 3-Kinases (phosphoinositide 3-kinases, PI 3-Ks) are a family of lipid kinases capable of phosphorylating the 3'OH of the inositol ring of phosphoinositides – They are responsible for coordinating a diverse range of cell functions including proliferation and survival	171834	Li et al. (2015a, b, c, d)
APC	5q22.2	– This gene encodes a tumor suppressor protein that acts as an antagonist of the Wnt signaling pathway – It is also involved in other processes including cell migration and adhesion, transcriptional activation, and apoptosis – Defects in this gene cause familial adenomatous polyposis (FAP), an autosomal dominant pre-malignant disease that usually progresses to malignancy	611731	Csepregi et al. (2008)
IGF2R	6q25.3	– This gene encodes a receptor for both insulin-like growth factor 2 and mannose 6-phosphate – The binding sites for each ligand are located on different segments of the protein – This receptor has various functions, including in the intracellular trafficking of lysosomal enzymes, the activation of transforming growth factor beta, and the degradation of insulin-like growth factor 2	147280	Laurent-Puig and Zucman-Rossi (2006)

(continued)

Table 5.31 (continued)

Gene	Location	Function	OMIM	References
MET	7q31.2	– This gene encodes a member of the receptor tyrosine kinase family of proteins and the product of the proto-oncogene MET – The encoded preproprotein is proteolytically processed to generate alpha and beta subunits that are linked via disulfide bonds to form the mature receptor – Further processing of the beta subunit results in the formation of the M10 peptide, which has been shown to reduce lung fibrosis – Binding of its ligand, hepatocyte growth factor, induces dimerization and activation of the receptor, which plays a role in cellular survival, embryogenesis, and cellular migration and invasion	164860	Giordano and Columbano (2014)
PDGFRL	8p22	– This gene encodes a protein with significant sequence similarity to the ligand binding domain of platelet-derived growth factor receptor beta – Mutations in this gene, or deletion of a chromosomal segment containing this gene, are associated with sporadic hepatocellular carcinomas, colorectal cancers, and non-small cell lung cancers – This suggests this gene product may function as a tumor suppressor	604584	Kahng et al. (2003)
AXIN1	16p13.3	– This gene encodes a cytoplasmic protein which contains a regulation of G-protein signaling (RGS) domain and a dishevelled and axin (DIX) domain – The encoded protein interacts with adenomatosis polyposis coli, catenin beta-1, glycogen synthase kinase 3 beta, protein phosphate 2, and itself – This protein functions as a negative regulator of the wingless-type MMTV integration site family, member 1 (WNT) signaling pathway and can induce apoptosis – Mutations in this gene have been associated with hepatocellular carcinoma, hepatoblastomas, ovarian endometriod adenocarcinomas, and medullablastomas	603816	Taniguchi et al. (2002), Feng et al. (2012a, b, c)
AXIN2	17q24.1			Mazzoni and Fearon (2014)

(continued)

Table 5.31 (continued)

Gene	Location	Function	OMIM	References
ANXA8				
TP53	17p13.1	– This gene encodes a tumor suppressor protein containing transcriptional activation, DNA binding, and oligomerization domains – The encoded protein responds to diverse cellular stresses to regulate expression of target genes, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism – p53 (TP53) is a transcription factor whose protein levels and post-translational modification state alter in response to cellular stress (e.g. hypoxia, DNA and spindle damage) – Activation of p53 occurs by several mechanisms including phosphorylation by ATM, ATR, Chk1 and MAPKs	191170	Laurent-Puig and Zucman-Rossi (2006), Friemel et al. 2016)
CTNNB1	3p22.1	– The protein encoded by this gene is part of a complex of proteins that constitute adherens junctions (AJs) – AJs are necessary for the creation and maintenance of epithelial cell layers by regulating cell growth and adhesion between cells – The encoded protein also anchors the actin cytoskeleton and may be responsible for transmitting the contact inhibition signal that causes cells to stop dividing once the epithelial sheet is complete – Finally, this protein binds to the product of the APC gene, which is mutated in adenomatous polyposis of the colon	116806	Baker et al. (2016)
IGF2	11p15.5	– This gene encodes a member of the insulin family of polypeptide growth factors, which are involved in development and growth – It is an imprinted gene, expressed only from the paternal allele, and epigenetic changes at this locus are associated with Wilms tumour, Beckwith-Wiedemann syndrome, rhabdomyosarcoma, and Silver-Russell syndrome – A read-through INS-IGF2 gene exists, whose 5' region overlaps the INS gene and the 3' region overlaps this gene	147470	Rumbajan et al. (2013)

(continued)

Table 5.31 (continued)

Gene	Location	Function	OMIM	References
HCCS	Xp22.3	– The protein encoded by this gene is an enzyme that covalently links a heme group to the apoprotein of cytochrome c – Defects in this gene are a cause of microphthalmia syndromic type 7 (MCOPS7)	300056	Nishida and Kudo (2016)
AFP	4q13.3	– This gene encodes alpha-fetoprotein, a major plasma protein produced by the yolk sac and the liver during fetal life – Alpha-fetoprotein expression in adults is often associated with hepatoma or teratoma – However, hereditary persistance of alpha-fetoprotein may also be found in individuals with no obvious pathology – The protein is thought to be the fetal counterpart of serum albumin, and the alpha-fetoprotein and albumin genes are present in tandem in the same transcriptional orientation on chromosome 4 – Alpha-fetoprotein is found in monomeric as well as dimeric and trimeric forms, and binds copper, nickel, fatty acids and bilirubin – The level of alpha-fetoprotein in amniotic fluid is used to measure renal loss of protein to screen for spina bifida and anencephaly	104150	Zuo et al. (2016)
GPC3	Xq26.1	– Cell surface proteoglycan that bears heparan sulfate Inhibits the dipeptidyl peptidase activity of DPP4 – May be involved in the suppression/modulation of growth in the predominantly mesodermal tissues and organs – May play a role in the modulation of IGF2 interactions with its receptor and thereby modulate its function – May regulate growth and tumor predisposition	300037	Jeon et al. (2016)
BAX	19q13.33	– The protein encoded by this gene belongs to the BCL2 protein family – BCL2 family members form hetero- or homodimers and act as anti- or pro-apoptotic regulators that are involved in a wide variety of cellular activities – This protein forms a heterodimer with BCL2, and functions as an apoptotic activator	600040	Mitupatum et al. (2016)

(continued)

Table 5.31 (continued)

Gene	Location	Function	OMIM	References
		– This protein is reported to interact with, and increase the opening of, the mitochondrial voltage-dependent anion channel (VDAC), which leads to the loss in membrane potential and the release of cytochrome c – The expression of this gene is regulated by the tumor suppressor P53 and has been shown to be involved in P53-mediated apoptosis		
TNF	6p21.33	– This gene encodes a multifunctional proinflammatory cytokine that belongs to the tumor necrosis factor (TNF) superfamily – This cytokine is mainly secreted by macrophages. It can bind to, and thus functions through its receptors TNFRSF1A/TNFR1 and TNFRSF1B/TNFBR – This cytokine is involved in the regulation of a wide spectrum of biological processes including cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation – This cytokine has been implicated in a variety of diseases, including autoimmune diseases, insulin resistance, and cancer – Knockout studies in mice also suggested the neuroprotective function of this cytokine	191160	Li et al. (2016a, b)
MYC	8q24.21	– The protein encoded by this gene is a multifunctional, nuclear phosphoprotein that plays a role in cell cycle progression, apoptosis and cellular transformation – It functions as a transcription factor that regulates transcription of specific target genes – Mutations, overexpression, rearrangement and translocation of this gene have been associated with a variety of hematopoietic tumors, leukemias and lymphomas, including Burkitt lymphoma	190080	Lin et al. (2010a, b, c)
STAT3	17q21.2	– The protein encoded by this gene is a member of the STAT protein family – In response to cytokines and growth factors, STAT family members are phosphorylated by the receptor associated kinases, and then form homo- or heterodimers that translocate to the cell nucleus where they act as transcription activators	102582	Fan et al. (2016)

(continued)

Table 5.31 (continued)

Gene	Location	Function	OMIM	References
		– This protein is activated through phosphorylation in response to various cytokines and growth factors including IFNs, EGF, IL5, IL6, HGF, LIF and BMP2 – This protein mediates the expression of a variety of genes in response to cell stimuli, and thus plays a key role in many cellular processes such as cell growth and apoptosis – The small GTPase Rac1 has been shown to bind and regulate the activity of this protein. PIAS3 protein is a specific inhibitor of this protein – Mutations in this gene are associated with infantile-onset multisystem autoimmune disease and hyper-immunoglobulin E syndrome		
CDKN1A	6p21.2	– May be involved in p53/TP53 mediated inhibition of cellular proliferation in response to DNA damage – Binds to and inhibits cyclin-dependent kinase activity, preventing phosphorylation of critical cyclin-dependent kinase substrates and blocking cell cycle progression – Functions in the nuclear localization and assembly of cyclin D-CDK4 complex and promotes its kinase activity towards RB1 – At higher stoichiometric ratios, inhibits the kinase activity of the cyclin D-CDK4 complex – Inhibits DNA synthesis by DNA polymerase delta by competing with POLD3 for PCNA binding	116899	Ohta et al. (2015)
PCNA	20p12.3	– The protein encoded by this gene is found in the nucleus and is a cofactor of DNA polymerase delta – The encoded protein acts as a homotrimer and helps increase the processivity of leading strand synthesis during DNA replication – In response to DNA damage, this protein is ubiquitinated and is involved in the RAD6-dependent DNA repair pathway	176740	Yuan et al. (2014)

(continued)

Table 5.31 (continued)

Gene	Location	Function	OMIM	References
HIF1A	14q23.2	– This gene encodes the alpha subunit of transcription factor hypoxia-inducible factor-1 (HIF-1), which is a heterodimer composed of an alpha and a beta subunit – HIF-1 functions as a master regulator of cellular and systemic homeostatic response to hypoxia by activating transcription of many genes, including those involved in energy metabolism, angiogenesis, apoptosis, and other genes whose protein products increase oxygen delivery or facilitate metabolic adaptation to hypoxia – HIF-1 thus plays an essential role in embryonic vascularization, tumor angiogenesis and pathophysiology of ischemic disease	603348	Guo et al. (2015)
MMP2	16q12.2	– Matrix metalloproteases (matrix metalloproteinase, MMPs), also called matrixins, are zinc-dependent endopeptidases and the major proteases in ECM degradation – MMPs are capable of degrading several extracellular molecules and a number of bioactive molecules – The protein encoded by this gene is a gelatinase A, type IV collagenase, that contains three fibronectins type II repeats in its catalytic site that allow binding of denatured type IV and V collagen and elastin – This protein is thought to be involved in multiple pathways including roles in the nervous system, endometrial menstrual breakdown, regulation of vascularization, and metastasis – Mutations in this gene have been associated with Winchester syndrome and Nodulosis-Arthropathy-Osteolysis (NAO) syndrome	120360	Dou et al. (2016)
HGF	7q21.11	– This gene encodes a protein that binds to the hepatocyte growth factor receptor to regulate cell growth, cell motility and morphogenesis in numerous cell and tissue types – Alternative splicing results in multiple transcript variants, at least one of which encodes a preproprotein that is proteolytically processed to generate alpha and beta chains, which form the mature heterodimer – This protein is secreted by mesenchymal cells and acts as a multi-functional cytokine on cells of mainly epithelial origin	142409	Liu et al. (2016)

(continued)

Table 5.31 (continued)

Gene	Location	Function	OMIM	References
		– This protein also plays a role in angiogenesis, tumorogenesis, and tissue regeneration – Although the encoded protein is a member of the peptidase S1 family of serine proteases, it lacks peptidase activity – Mutations in this gene are associated with nonsyndromic hearing loss		
FOS	14q24.3	– The Fos gene family consists of 4 members: FOS, FOSB, FOSL1, and FOSL2 – These genes encode leucine zipper proteins that can dimerize with proteins of the JUN family, thereby forming the transcription factor complex AP-1 – As such, the FOS proteins have been implicated as regulators of cell proliferation, differentiation, and transformation – In some cases, expression of the FOS gene has also been associated with apoptotic cell death	164810	Watanabe et al. (2013)
TGFB1	19q13.2	– This gene encodes a secreted ligand of the TGF-beta (transforming growth factor-beta) superfamily of proteins – Ligands of this family bind various TGF-beta receptors leading to recruitment and activation of SMAD family transcription factors that regulate gene expression – This encoded protein regulates cell proliferation, differentiation and growth, and can modulate expression and activation of other growth factors including interferon gamma and tumor necrosis factor alpha – This gene is frequently upregulated in tumor cells, and mutations in this gene result in Camurati-Engelmann disease	190180	Ibrahim et al. (2013)
VEGFA	6p12	– This gene is a member of the PDGF/VEGF growth factor family – It encodes a heparin-binding protein, which exists as a disulfide-linked homodimer – This growth factor induces proliferation and migration of vascular endothelial cells, and is essential for both physiological and pathological angiogenesis	192240	Wang et al. (2015a, b)

(continued)

Table 5.31 (continued)

Gene	Location	Function	OMIM	References
ABCC1	16p13.1	– The protein encoded by this gene is a member of the superfamily of ATP-binding cassette (ABC) transporters – ABC proteins transport various molecules across extra-and intra-cellular membranes. ABC genes are divided into seven distinct subfamilies (ABC1, MDR/TAP, MRP, ALD, OABP, GCN20, White) – This full transporter is a member of the MRP subfamily which is involved in multi-drug resistance – This protein functions as a multispecific organic anion transporter, with oxidized glutatione, cysteinyl leukotrienes, and activated aflatoxin B1 as substrates – This protein also transports glucuronides and sulfate conjugates of steroid hormones and bile salts	158343	Vander Borght et al. (2008)
TERT	5p15.33	– Telomerase is a ribonucleoprotein polymerase that maintains telomere ends by addition of the telomere repeat TTAGGG – The enzyme consists of a protein component with reverse transcriptase activity, encoded by this gene, and an RNA component which serves as a template for the telomere repeat – Telomerase expression plays a role in cellular senescence, as it is normally repressed in postnatal somatic cells resulting in progressive shortening of telomeres – Deregulation of telomerase expression in somatic cells may be involved in oncogenesis – Studies in mouse suggest that telomerase also participates in chromosomal repair, since de novo synthesis of telomere repeats may occur at double-stranded breaks	187270	Lee et al. (2017)
CDH1	16q22.1	– This gene encodes a classical cadherin of the cadherin superfamily – Alternative splicing results in multiple transcript variants, at least one of which encodes a preproprotein that is proteolytically processed to generate the mature glycoprotein This calcium-dependent cell-cell adhesion protein is comprised of five extracellular cadherin repeats, a transmembrane region and a highly conserved cytoplasmic tail	192090	Chien et al. (2011)

(continued)

Table 5.31 (continued)

Gene	Location	Function	OMIM	References
		– Mutations in this gene are correlated with gastric, breast, colorectal, thyroid and ovarian cancer – Loss of function of this gene is thought to contribute to cancer progression by increasing proliferation, invasion, and/or metastasis		
SMAD3	15q22.33	– The protein encoded by this gene belongs to the SMAD, a family of proteins similar to the gene products of the Drosophila gene 'mothers against decapentaplegic' (Mad) and the *C. elegans* gene Sma – SMAD proteins are signal transducers and transcriptional modulators that mediate multiple signaling pathways – This protein functions as a transcriptional modulator activated by transforming growth factor-beta and is thought to play a role in the regulation of carcinogenesis	603109	Li et al. (2015a, b, c, d)
RASSF1	3p21.3	– This gene encodes a protein similar to the RAS effector proteins – Loss or altered expression of this gene has been associated with the pathogenesis of a variety of cancers, which suggests the tumor suppressor function of this gene – The inactivation of this gene was found to be correlated with the hypermethylation of its CpG-island promoter region – The encoded protein was found to interact with DNA repair protein XPA. The protein was also shown to inhibit the accumulation of cyclin D1, and thus induce cell cycle arrest		Schagdarsurengin et al. (2003), Araújo et al. (2016)
E2F1	20q11.2	– The protein encoded by this gene is a member of the E2F family of transcription factors – The E2F family plays a crucial role in the control of cell cycle and action of tumor suppressor proteins and is also a target of the transforming proteins of small DNA tumor viruses – This protein binds preferentially to retinoblastoma protein pRB in a cell-cycle dependent manner – It can mediate both cell proliferation and p53-dependent/independent apoptosis	189971	Farra et al. (2015)

(continued)

Table 5.31 (continued)

Gene	Location	Function	OMIM	References
HFE	6p21.3	– The protein encoded by this gene is a membrane protein that is similar to MHC class I-type proteins and associates with beta2-microglobulin (beta2 M) – It is thought that this protein functions to regulate iron absorption by regulating the interaction of the transferrin receptor with transferrin -The iron storage disorder, hereditary haemochromatosis, is a recessive genetic disorder that results from defects in this gene	613609	
SMAD4	18q21.1	– This gene encodes a member of the Smad family of signal transduction proteins – Smad proteins are phosphorylated and activated by transmembrane serine-threonine receptor kinases in response to TGF-beta signaling – The product of this gene forms homomeric complexes and heteromeric complexes with other activated Smad proteins, which then accumulate in the nucleus and regulate the transcription of target genes – This protein binds to DNA and recognizes an 8-bp palindromic sequence (GTCTAGAC) called the Smad-binding element (SBE) – The Smad proteins are subject to complex regulation by post-translational modifications – Mutations or deletions in this gene have been shown to result in pancreatic cancer, juvenile polyposis syndrome, and hereditary hemorrhagic telangiectasia syndrome	600993	Yakicier et al. (1999), Hernanda et al. (2015)
XRCC1	19q13.2	– The protein encoded by this gene is involved in the efficient repair of DNA single-strand breaks formed by exposure to ionizing radiation and alkylating agents – This protein interacts with DNA ligase III, polymerase beta and poly (ADP-ribose) polymerase to participate in the base excision repair pathway – It may play a role in DNA processing during meiogenesis and recombination in germ cells	194360	Wang et al. (2016)

(continued)

Table 5.31 (continued)

Gene	Location	Function	OMIM	References
HLF	17q22	– This gene encodes a member of the proline and acidic-rich (PAR) protein family, a subset of the bZIP transcription factors – The encoded protein forms homodimers or heterodimers with other PAR family members and binds sequence-specific promoter elements to activate transcription – Chromosomal translocations fusing portions of this gene with the E2A gene cause a subset of childhood B-lineage acute lymphoid leukemias	142385	Miyata et al. (2015)
TCF4	18q21.1	– This gene encodes transcription factor 4, a basic helix-loop-helix transcription factor – The encoded protein recognizes an Ephrussi-box ('E-box') binding site ('CANNTG')—a motif first identified in immunoglobulin enhancers – This gene is broadly expressed, and may play an important role in nervous system development – Defects in this gene are a cause of Pitt-Hopkins syndrome – In addition, an intronic CTG repeat normally numbering 10-37 repeat units can expand to > 50 repeat units and cause Fuchs endothelial corneal dystrophy	602272	Zhao et al. (2004)
CCNB1	5q12	– The protein encoded by this gene is a regulatory protein involved in mitosis – The gene product complexes with p34 (cdc2) to form the maturation-promoting factor (MPF) – Two alternative transcripts have been found, a constitutively expressed transcript and a cell cycle-regulated transcript that is expressed predominantly during G2/M phase	123836	Jin et al. (2015)
GAPDH	12p13	– The encoded protein has been identified as a moonlighting protein based on its ability to perform mechanistically distinct functions – The product of this gene catalyzes an important energy-yielding step in carbohydrate metabolism, the reversible oxidative phosphorylation of glyceraldehyde-3-phosphate in the presence of inorganic phosphate and nicotinamide adenine dinucleotide (NAD)	138400	Liu et al. (2017)

(continued)

Table 5.31 (continued)

Gene	Location	Function	OMIM	References
		– The encoded protein has additionally been identified to have uracil DNA glycosylase activity in the nucleus – Also, this protein contains a peptide that has antimicrobial activity against *E. coli*, *P. aeruginosa*, and *C. albicans*		
BAD	11q13.1	– The protein encoded by this gene is a member of the BCL-2 family – BCL-2 family members are known to be regulators of programmed cell death – This protein positively regulates cell apoptosis by forming heterodimers with BCL-xL and BCL-2, and reversing their death repressor activity – Proapoptotic activity of this protein is regulated through its phosphorylation – Protein kinases AKT and MAP kinase, as well as protein phosphatase calcineurin were found to be involved in the regulation of this protein	603167	Kotsafti et al (2012)
DNMT1	19p132	– This gene encodes an enzyme that transfers methyl groups to cytosine nucleotides of genomic DNA – This protein is the major enzyme responsible for maintaining methylation patterns following DNA replication and shows a preference for hemi-methylated DNA – Methylation of DNA is an important component of mammalian epigenetic gene regulation – Aberrant methylation patterns are found in human tumors and associated with developmental abnormalities – Variation in this gene has been associated with cerebellar ataxia, deafness, and narcolepsy, and neuropathy, hereditary sensory, type IE	126375	Jiang and Gong (2016)
DNMT3B	20q11.2	– CpG methylation is an epigenetic modification that is important for embryonic development, imprinting, and X-chromosome inactivation – Studies in mice have demonstrated that DNA methylation is required for mammalian development – This gene encodes a DNA methyltransferase which is thought to function in de novo methylation, rather than maintenance methylation – The protein localizes primarily to the nucleus and its expression is developmentally regulated – Mutations in this gene cause the immunodeficiency-centromeric instability-facial anomalies (ICF) syndrome	602900	Chamani et al. (2015)

Table 5.32 Meta-signature miRNAs in liver cancer

miRNAs	miRNA function	miRNA effect	References
miR-216a	– Induces epithelial-mesenchymal transition – Increases stem-like cell population – Increases cell migration – Increases metastatic ability	Oncogenic	Xia et al. (2013)
miR-217	– Induces epithelial-mesenchymal transition – Increases stem-like cell population – Increases cell migration – Increases metastatic ability	Oncogenic	Xia et al. (2013)
miR-485-3p	– Promotes cell growth – Increases apoptosis – Increases cell invasion	Oncogenic	Morishita and Masaki (2015)
miR-495	– Promotes cell growth – Increases apoptosis – Increases cell invasion	Oncogenic	Marquardt and Galle (2013)
miR-664	– Promotes cell growth – Increases apoptosis – Increases cell invasion	Oncogenic	Yang et al. (2013a, b, c, d, e, f, g)
miR-138	– Inhibits cell proliferation – Inhibits cell cycle G1/S transition – Inhibits cell migration – Inhibits cell invasion	Tumor-suppressive	Wang et al. (2012a, b, c, d, e, f, g)
miR-140-5p	– Inhibits cell proliferation – Inhibits metastasis	Tumor-suppressive	Yang et al. (2013a, b, c, d, e, f, g)
miR-26a	– Inhibits cell proliferation – Inhibits cell migration – Inhibits cell invasion – Induces cell cycle G1 arrest – Induces apoptosis – Inhibits tumor growth – Inhibits metastasis	Tumor-suppressive	Yang et al. (2013a, b, c, d, e, f, g)
miR-148a	– Inhibits cell growth – Inhibits epithelial-mesenchymal transition – Inhibits cell invasion – Inhibits metastasis	Tumor-suppressive	Pan et al. (2014)

(continued)

Table 5.32 (continued)

miRNAs	miRNA function	miRNA effect	References
miR-495	– Promotes tumor growth – Promotes cell invasion – Promotes metastasis	Oncogenic	Yang et al. (2013a, b, c, d, e, f, g)
miR-485-3p	– Promotes tumor growth – Promotes cell invasion – Promotes metastasis	Oncogenic	Yang et al. (2013a, b, c, d, e, f, g)
miR-490-3p	– Increases cell proliferation – Increases cell migration – Increases cell invasion – Promotes epithelial to mesenchymal transition	Oncogenic	Zhang et al. (2013a, b, c, d)
miR-101	– Inhibits cell proliferation – Inhibits tumorigenicity	Tumor-suppressive	Lv et al. (2016)
miR-657	– Increases cell proliferation – Increases colony formation – Promotes tumor development	Oncogenic	Zhang et al. (2013a, b, c, d)
miR-550a	– Increases cell migration – Increases cell invasion	Oncogenic	Morishita and Masaki (2015)
miR-106b	– Induces cell proliferation – Induces anchorage-independent cell growth – Promotes cell cycle G1/S transition	Oncogenic	Yau et al. (2013)
miR-1	– Inhibits cell proliferation	Tumor-suppressive	Köberle et al. (2013)
miR-214	– Inhibits cell growth – Inhibits tumorigenicity – *I*nhibits EpCAM(+) stem-like cells	Tumor-suppressive	Wang et al. (2013a, b, c, d)
miR-125b	– Reduces cell proliferation – Inhibits cell cycle progression	Tumor-suppressive	Giray et al. (2014)
miR-124	– Inhibits cell proliferation – Induces cell cycle G1 arrest – Inhibits tumor growth	Tumor-suppressive	Lu et al. (2013)
miR-140	– Inhibits hepatocarcinogenesis	Tumor-suppressive	Morishita and Masaki (2015)
miR-195	– Increases apoptosis	Tumor-suppressive	Ding et al. (2013)
miR-1271	– Inhibits cell growth – Induces cell death	Tumor-suppressive	Maurel et al. (2013)
miR-130a	– Promotes cisplatin resistance – Activates Wnt/?-catenin signaling	Oncogenic	Li et al. (2014a, b, c, d)

(continued)

Table 5.32 (continued)

miRNAs	miRNA function	miRNA effect	References
miR-200c	– Reduces epithelial-mesenchymal transition	Tumor-suppressive	Karakatsanis et al. (2013)
miR-590-5p	– Promotes proliferation – Promotes invasion	Oncogenic	Yang et al. (2013a, b, c, d, e, f, g)
miR-376a	– Inhibits cell proliferation – Induces apoptosis	Tumor-suppressive	Zheng et al. (2015)
miR-182	– Increases cell invasion	Oncogenic	Wang et al. (2014a, b)
miR-499	– Inhibits cell migration – Inhibits cell invasion	Tumor-suppressive	Ma et al. (2014)
miR-10b	– Increases cell migration – Increases cell invasion	Oncogenic	Liao et al. (2014)
miR-375	– Inhibits autophagy under hypoxic conditions	Tumor-suppressive	Yin et al. (2015)
miR-148a	– Promotes cell proliferation – Promotes cell cycle progression – Promotes cell migration – Inhibits Akt signaling	Oncogenic	Pan et al. (2014)
miR-219-5p	– Inhibits cell proliferation – Blocks cell cycle G1/S transition	Tumor-suppressive	Zhou et al. (2014)
miR-21	– Increases cell migration – Increases cell invasion	Oncogenic	Karakatsanis et al. (2013)
miR-122	– Inhibits cell growth – Promotes cell apoptosis	Tumor-suppressive	Köberle et al. (2013)
miR-100	– Inhibits cell growth – Inhibits colony formation	Tumor-suppressive	Chen et al. (2013a, b)
miR-7	– Inhibits cell proliferation – Inhibits cell cycle arrest – Inhibits cell migration – Inhibits metastasis	Tumor-suppressive	Zhang et al. (2014a, b, c, d, e, f)
miR-155	– Promotes tumorigenesis	Oncogenic	Zhang et al. (2013a, b, c, d)
miR-25	– Inhibits TRAIL-induced apoptosis	Oncogenic	Su et al. (2014a, b)
miR-423	– Promotes cell growth – Promotes cell cycle G1/S transition	Oncogenic	Ma et al. (2014)
miR-99a	– Iinhibits cell growth – Induces cell cycle G1 arrest	Tumor-suppressive	Zhang et al. (2014a, b, c, d, e, f)
miR-200a	– Inhibits cell proliferation – Inhibits cell migration	Tumor-suppressive	Feng et al. (2015)

(continued)

Table 5.32 (continued)

miRNAs	miRNA function	miRNA effect	References
miR-29c	– Inhibits cell proliferation – Induces apoptosis	Tumor-suppressive	Bae et al. (2014)
miR-16	– Suppresses cell proliferation – Inhibits clonogenicity – Inhibits cell cycle G1 arrest – Inhibits apoptosis	Tumor-suppressive	Ge et al. (2013)
miR-9	– Promotes cell migration	Oncogenic	Cai and Cai (2014)
miR-199a-3p	– Suppresses tumor growth	Tumor-suppressive	Yin et al. (2015)
miR-139	– Reduces cell migration – Reduces cell invasion	Tumor-suppressive	Gu et al. (2014)
miR-183	– Inhibits TGF-beta induced apoptosis	Oncogenic	Leung et al. (2015)
let-7a	– Inhibits cell proliferation	Tumor-suppressive	Liu et al. (2014a, b, c)
let-7c	– Promotes sorafenib-induced apoptosis	Tumor-suppressive	Xie et al. (2013)
let-7g	– Inhibits cell migration – Inhibits cell growth	Tumor-suppressive	Chen et al. (2014a, b)

disorders with a genetic etiology (e.g., cancer, dementia, heart disease, stroke, diabetes, etc.).

Besides, a comprehensive family history can help the genetic counselor to find adult-onset health problems, thereby at risk individuals can act out preventive approaches and implement lifestyle changes (Mehdipour et al. 2002; Frezzo et al. 2003; Krautscheid and LaGrave 2016). Cancers are usually late-onset conditions and due to inherited mutations in some genes such as BRCA1, BRCA2, and P53 are observed in different generation of pedigrees. A family history (FH) of breast cancer is a long recognized risk factor for developing the disease. Also, there have been some reports of links between a FH and some other malignancies (mostly uterus, ovary, and prostate cancers), and an increased risk of developing BC. It is obvious that early onset of BC has direct relation with numbers of affected relatives, however lack of association between early onset of BC and numbers of affected relatives in an Iranian population has been reported (Mehdipour et al. 2002).

Approximately 5–10% of breast cancers are hereditary and can be developed in carriers of mutation (s) in particular genes, such as BRCA1 or BRCA2. In this case, if someone's family history includes more than one first-degree (mother, sister, or daughter) or second-degree relative (aunt, grandmother, or niece) with breast or ovarian cancer, therefore genetic testing can be offered to at risk pedigree members (Hashemian et al. 2009; Karami and Mehdipour 2013). Contribution of germline mutations in either BRCA1 or BRCA2 genes to inherited breast cancer in Iran has

been investigated. BRCA2 mutations c.4415_4418delAGAA and c.6033_6034 insGT have not been identified in any investigated population except the Iranian. No mutation was found in BRCA1 that could be due to lower penetrance or prevalence of BRCA1 mutations in Iran (Pietschmann et al. 2005).

Nonetheless, some genetic diseases are caused by de novo mutations, therefore, a pedigree is not adequately informative to rule out the occurrence of a genetic condition (Guttmacher et al. 2004). Additionally, the reliable information about penetrance is an important factor in genetic counseling and it is essential to be estimated in different population. The penetrance of breast cancer genes 1 and 2 of specific gene mutations in Iranian women with breast cancer has been reported to be 31.9% (<50 years) and 46.2% ($\geq$ 50 years) among BRCA1/2 carriers (Hashemian et al. 2009).

So, pedigree based analysis would direct the genetic counselor to: (1) a reliable risk estimation, (2) suggest the most appropriate test (s), and (3) inform any possible diagnostic, preventive, predictive, and prognostic managements for cancer patients and their relatives.

5.15 Conclusion

Much remains to be learned about how genetic variation contributes to cancer risk. Most of the inherited cancer genes discovered to date have a high penetrance and convey a significant predisposition to the development of cancer. Fewer low penetrance cancer genes are known, largely because such genes are more difficult to identify. Genes that may modify cancer risk in subtle ways are far more difficult to be detect but may collectively cause a significant number of cancers. Each of the roughly 100 types of human cancer is caused by the activation of proto-oncogenes and the function loss of tumor suppressor genes. Although cancer genomes are complex, but some clear mutational patterns are available. Several cancer genes are observed very frequently in some types of cancer, but rarely found in other types. Other cancer genes are much more widespread. Recent analysis of individual cancer genomes has shown that, in addition to the well-known cancer genes described in the preceding chapters, there are many mutations that arise, via clonal selection, at very low frequency during tumorigenesis. These observations imply that there are many potential combinations of cancer genes that can cooperatively allow the growth of neoplasm. In this chapter we have discussed the genetic elements of some more common cancers. At a glance, we reviewed the more reported cancer genes involved in the target cancers.

Studies of diseases such as retinoblastoma and colorectal cancer have provided fundamental insights into the behavior of cancer genes. Each of these cancers has uniquely attributes that eventually have facilitated genetic analysis. Retinoblastoma is a relatively homogenous disease that has distinguishable hereditary and sporadic forms. The two-hit hypothesis developed by Knudson provided the first model for understanding cancer predisposition. Furthermore, three-hit hypothesis by

Mehdipour and colleagues is another example which is reflective of predisposing role of ATM gene in astrocytoma. In colorectal cancer, because tumor samples can be obtained during routine colonoscopy, all stages of growth have been subject to detailed analysis. Most cancers are not understood at the same level of detail as retinoblastoma and colorectal cancer. The reasons that many cancers remain incompletely characterized include clinical heterogeneity, accessible by geneticists to insufficient numbers of clinical samples representing different stages of disease, and lack of a clearly diagnosable hereditary form of the disease that allows the mapping of a predominant gatekeeper gene (s) and pathway (s). Despite these obstacles to progress, cancer genes have been found in all of the most common types of cancer. The wider application of large-scale sequencing approaches to cancer genomes provides the promises to reveal many more cancer genes in the near future. But, it is essential to consider the functional insights, especially at protein level which may pave the ways to unmask the unknown facts in cancer. In addition, pedigree-based analysis is the key of discoveries in cancer research and cancer clinic through which the scientists and clinicians could find the real directions for selecting, (1) the most relevant target genes to be analyzed, (2) the most appropriate personalized managements for the probands and if is necessary for their relatives who may be at risk of being affected with cancer, and (3) the most logical counseling through the follow-up studies for target based diagnosis and therapy with a personalized insight.

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Chapter 6
The Relevance of Genetic Factors in Tumor Therapy and the Underlying Pharmacogenetic Principles

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Abstract In the following chapter it is shown that only by the combination of pharmacological and genetic research programs new relevant findings of tumor formation and progression can be gained. This then lead to an improved and individualized therapy of the patients in the area of application.

Keywords Drug reactions influenced by genetic and pharmacologic factors · Heterogeneity of tumor diseases · Protective effects of specific biological agents · Side effects in tumor treatment · Targeted therapy in oncology · Types of somatic mutations

6.1 Introduction

The scientific discipline of pharmacogenetics developed from the combination of the two subjects that are pharmacology and genetics.

Pharmacology comprises research with the aim to study the effects of drugs. The term "pharmakon" stands for therapeutic agents in particular but more broadly for biologically active substances. This latter definition does not necessarily postulate the requirement of a therapeutic application, it is a general definition. Consequently, a pharmakon is about a biologically active substance, that, if applied, may lead to changes in an organism. If this agent in any specific composition causes either prevention, relief or, cure of diseases in animals or humans, it is called a drug.

The topics of pharmacology coincide in many areas with subjects of similar scientific basic principles and emphasis, such as pharmacy, biochemistry, and

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physiology. Thus, research projects in pharmacology generally require close interdisciplinary teamwork.

In applied research projects, the efficiency of potential drugs first will be tested in animal experiments. Controlled studies on humans will follow before any therapeutic application can be permitted.

Pharmacokinetics and pharmacodynamics, parts of pharmacology, are being utilized in order to test application, resorption, and distribution of substances in the organism, plus biotransformation and excretion of the compounds. Besides, effects due to the molecular structure of the test substances, the relevance of bioreceptors and antagonists are analyzed. Adverse reactions of these test substances caused by genetic mutation in a patient must be taken into account. In addition, side effects and interactions of different drugs as well as cause-and-effect relations are being tested.

Within the context of drug development pre-clinical and clinical test series are being analyzed and compared to the significance of placebo effects.

The relevance of genetic differences between subjects and their importance when treating patients with drugs had already been known to our ancestors about 10,000 years ago. At that period of time, the principles and basics of genetics were familiar to humans throughout different cultural societies. Their knowledge of the inheritance of special characteristic features in plants and animals and the possibility to achieve improvements by combining those features led to the development of agriculture and thus to the evolution of science, technology, and culture (Schwanitz 1967).

The term "genetics" was defined in ancient Greece and more explicitly later by William Bateson in 1905. The laws of heredity were presented by Gregor Mendel (1866) and rediscovered by Correns, de Vries, and Tschermak in 1900. The following decades saw a rapid development of genetics mainly due to the establishment of new methods of investigation.

Human genetics segregated from the general field of genetics with relevant sub-divisions such as molecular genetics, cytogenetics, population genetics, epigenetics, research in mutation and evolution, and tumor genetics.

Similar to pharmacology, close cooperation occurred in the various areas of human genetics with fields of research like cytology, biostatistics, teratology, pathology, oncology, and clinical medicine like pediatrics, gynaecology, and neurology.

New scientific targets are the "Human Genome Project", genome mapping, genome plasticity, the development of human artificial chromosomes in order to solve diagnostic issues, and, (as expected), the correction of genetic defects in the future.

Many relevant problems have not been solved yet in a satisfactory manner, such as the shortening of the telomeres with increasing age and, in the context of cell transformation in tumors, the function of constitutive heterochromatin during the cell cycle and cell differentiation, gene-gene interactions, or the organization of the genome in a somatic cell versus a tumor cell, as well as the analysis of functional gene clusters. New investigation methods are being developed, e.g. the deactivation

of genes or even of entire chromosomes. Finally, first steps are being implemented to target selective gene therapy.

The combination of these special fields of pharmacology and genetics resulted in new perspectives in basic research as well as in areas of application in human medicine.

In many cases, it led to an improved comprehension of an existing disorder and to a personalized approach to therapy.

6.2 Historical Development in the Field of Pharmacogenetics

The special field of pharmacogenetics is a fairly recent subarea of the subjects of human genetics and pharmacology.

Even though it has been common knowledge for many centuries that numerous medications (drugs) exhibit diverse effects in different patients, a precise definition of the field did not occur until the middle of the 20th century. Motulsky (1957) formulated the theory that genetic variation must be the cause of varying success in therapy. Vogel (1959) established the term "pharmacogenetics".

In the following years, the correlation between genetic variants and diverging reactions in patients to the therapy approaches chosen was initially obtained through the analysis of studies in twins. Evidence showed that the rate of concordance in monozygotic twins was higher than in dizygotic ones (Vesell 1978).

The use of molecular-genetic methods and the enhancement of findings through the decoding of the human genome led to substantial progress in the development of the subject.

Primarily, the genetic variants of **single nucleotide polymorphisms** (SNPs) were analysed, since they were considered to be pharmacogenetically relevant. In addition, investigations of **microsatellites** and **triple repeats** were included. Not only the DNA of the nucleus but also the **mitochondrial DNA** was analysed.

In recent years, a further approach was achieved through the research of **epigenetic mutations** as well as the analysis of genetic variants leading to changes in **methylation** of DNA.

The relevance of pharmacological findings became obvious with the analysis of clinical studies concerning the efficacy of various groups of drugs. The results showed that 10–30% of the patients did not respond satisfactorily to the therapy chosen. As far as the failure rate was concerned, relevant discrepancies existed between patients with different medical conditions. Especially various studies in patients with psychiatric disorders demonstrated a dissatisfactory response to the therapy chosen in up to 70% of the cases, making it the highest percentage of therapy failure.

Currently, the research of genetic principles of relevant processes and their range of application is in a very active stage of development which will definitely benefit the importance of pharmacogenetics in medicine, pharmacology, pharmacy, and biochemistry for the years to come.

6.3 Focal Points of Research in the Subareas of Pharmacogenetics

The pharmacological as well as genetic aspects of various processes are being pursued in terms of their significance during the sequence of relevant reactions.

6.3.1 Genetic and Pharmacologic Peculiarities Influencing Drug Reactions

Non-satisfactory results in therapy prompt the qualitative and quantitative identification of the role of inter-individual aspects including gender differences regarding the effects of the substances thus differentiating between optimally reducing or failing efficacy.

Furthermore, occurring side effects in the course of the application of drugs are being characterized and their severity documented. These side effects may be slight and thus hard to register, or they may be distinct, consequently this may lead to the stop of the respective therapy and even to the necessity to take the drug off the market. In isolated cases serious side effects can actually result in the death of a patient (Lazarou et al. 1998).

Therefore, documentation of unwanted side effects is now mandatory as well as its notification to the appropriate authorities and committees.

Except for possible discrepancies in therapy results caused by differences in individual approaches, negative effects can also occur through interaction of combined substances. Except for mono-therapy, possible interactions of various substances and their prospective metabolites have to be considered. In individual cases of combination drugs though, they can prevent the identification of the particular drug-drug interaction.

In addition, the evaluation of clinical studies for effectivity of therapies has to consider that exogenous factors can impact the efficacy of drugs, besides inter-individual differences, occurring side effects, and drug-drug relations.

6.3.2 Modes of Action and Their Dependence on Specific Pharmacogenetic Features

Depending on their mode of action, substances are sub-classified in those with either pharmacokinetic or pharmacodynamic effects. Within the former, class, intake, distribution, degradation, and excretion of the substance in question are being analysed in humans and/or animals, according to pharmacological principles. Within the latter though, the interactions of the substances as well as those of their metabolites with the molecular receptors are being investigated. There is evidence

though, that not every test substance can be explicitly assigned to one of the two classes alone, instead processes occurring in both classes have to be taken into account.

6.3.3 Characterization of Relevant Entities

Prior to starting an analysis of disease patterns with inhomogeneous results in therapy, several parameters have to be closely inspected.

6.3.3.1 The Heterogeneity of the Dysfunction to Be Treated

Many clinical pictures can be divided into sub-entities with specific methods of treatment to be developed for each one of those respectively.

6.3.3.2 The Combination of Disease and Independently Occurring Mutation

A patient may present a heterozygous or homozygous variant independent of his illness.

These mutants may affect the signalling pathway, possibly leading to a modified reaction of a relevant receptor to the drug.

6.3.4 Congenital and Somatic Mutations

The majority of genetic variants are mutations that exist in all the cells of an organism. They are either inherited from the parents or occur as de novo mutations for the first time and can be passed on to the descendants.

Somatic mutations, on the other hand, always originate de novo. They are limited to the derivatives of a germ layer that is an organ or a cell system.

They are characteristic for all tumor diseases.

There is a significant difference between the types of existing mutations, among them deletions, duplications, gene disruptions, base exchanges, and the so-called position effects. These different types of mutations can either directly influence pharmaco-kinetics and pharmaco-dynamics or can lead to secondary epigenetic changes and thus to gene dysfunctions (Delwel 2015). This has been verified for normal somatic as well as for tumor tissue (Esteller 2008; Brena and Costello 2010; Burrell and Swanton 2014).

6.4 Diagnostic Procedures

6.4.1 Single Nucleotide Polymorphisms (SNPs)

SNPs are the most frequent genetic variants with about 3 million of them per genome.

They are mainly being diagnosed with the help of the following two analytical approaches.

The first one uses the candidate gene. Specific candidate genes which can be deviated from disease genes that affect the onset and/or course of the disease are being targeted.

The second approach to the analysis of pharmacogenetically relevant variants is the so-called Genome Wide Analysis (GWA). Essentially significant for this screening method is the sample size because of the differentiation between "common" and "rare" variants. The "common" variants facilitate only limited information compared to the more significant "rare" variants which can only be determined through extensive cohorts.

So far, deficits in clinic studies restricted the obtained results. Modified specifications in future studies will be able to counteract these limitations.

6.4.2 Additional Options for the Characterization of Genetic Variants

Many of the additional analytical methods available nowadays are being applied specifically for this purpose or are being selected secondarily following a pre-screening. Among these are identifications of microsatellites, RNA—and protein analyses and fluorescence in situ hybridization (Haischen et al. 2007).

6.5 The Relevance of Ethnic Differences for Pharmacogenetic Investigations

The ethnic origin of test subjects as well as patients often plays a crucial part in the success of the treatment with various drugs.

In genetic studies initially the test series differentiated only between the three main ethnic groups: Caucasian, African, and Asian.

In recent years, these three groups were further subdivided into 11 ethnic groups.

Results unfolding on this evidence showed that the frequency of variants containing a clinical risk can vary by a factor of ten.

A clear allocation of risk factors to a patient based on his affiliation to a certain ethnic group is only possible though, when his parentage cannot be deduced from two or more different ethnicities.

This special complication, caused by a multi-ethnical background in patients, increased significantly over the last decades due to the high level of migration. It is to be expected that it will grow in significance in the near future.

6.6 Prognostics and Therapy Goals in Oncology

The overall trend in oncology shows an increasing tendency to apply tumor therapy as specific and individual as possible through targeted therapies according to the collected data (Awada and Aftimos 2013).

If necessary studies as a basis for the planned treatment do not exist, a different procedure has to be selected. In this case it is essential to document the results of the therapy in each patient in detail, making it possible to change the therapy at the earliest date when no positive reaction to the applied substances can be observed or even if the adverse side reactions are not tolerable. These potentially negative results of a tumor therapy have to be taken into account in cases with an initial diagnosis of the disease as well as in patients with a tumor recidive.

For over 50 years characteristic chromosome aberrations have been diagnosed for various types of leukemia as well as for solid tumors. Their detection is of crucial importance to the classification of a cancer or precancerous condition. Due to the frequency of cancer diseases in the industrialized countries, their exact classification is of great diagnostic, prognostic, and therapeutic relevance. Among these countries, cancer represents the second most common cause of death right after cardiovascular diseases and can, in the majority of the cases, be attributed to a few types of tumors such as leukemia, breast, lung, or colon cancer.

These various tumors entail heterogeneity (Mikulasova et al. 2015), which may lead to a different prognosis and course of therapy in patients with the same type of tumor.

Already in the beginning of the 20th century, the analysis of **exogenous risk factors** could provide evidence for ionizing radiation to be one of the causes for the development of cancer. Radiologists and their assistants exposed to these mutagens suffered increasingly from leukemia, and patients with radiotherapy or isotope therapy often fell ill with a second tumor disease induced by the treatment with cancerogenic substances.

Furthermore, it could be demonstrated that multiple virus types, especially retroviruses, can account for the development of tumors.

The induced aberrations are genome, chromosome and gene mutations which consequently may lead to abnormalities in the course of the cell cycle.

Endogenous risk factors for tumorigenesis are defects of the DNA repair system such as the chromosome instability syndrome (Bellacosa 2015; Tricarico et al. 2015) as well as constitutional chromosome aberrations such as the Down-Syndrome (Harrison 2015). Evidence of specific chromosome aberrations as a causal factor for a tumor syndrome were, for the first time, achieved through the

detection of the so-called Philadelphia chromosome in CML-patients by Nowell and Hungerford in the year 1960. This observation established the subject of tumor cytogenetics which obtained a relevant and still increasing importance in oncology. Through international cooperation, the available data material was summarized, and in 1988 it was published in Mitelman's "catalogue of chromosome aberrations in cancer" for the first time.

Additional methodical developments in genetics led to the introduction of fluorescence in situ hybridization (FISH) in tumor diagnostics of metaphase and—for the first time—in interphase stages as well in the course of monitoring the disease under therapy (Schmidt-Wolf et al 2006; Busert et al 2010).

A number of additional methods has been selected in cytogenetic and molecular cytogenetic investigations to characterize the different types of leukemia and solid tumors (Cross and Burmester 2006). In the majority of cases, these include different molecular genetic techniques which nowadays are becoming the leading investigation tools in tumor diagnostics and in the prognosis of the disease (see Sects. 4.1 and 4.2; Rodriguez-Perales 2015; Park et al. 2016; Yang et al. 2011).

In contrast to the projects in applied research of tumor genetics which were directed to improve diagnostics and therapy in the patient, the investigations in experimental tumor cytogenetics led to a better understanding of the basics of genetic changes in tumors.

These investigations targeted relevant questions and problems like degeneration (apoptosis) of tumor cells, tumor progression, maintenance of cell transformation, epigenetic mutations, processes concerning the development of metastases and the karyotype evolution (Pavlova et al. 2016).

In recent years, tests on transgenic animals, particularly mice, gained in importance.

The results of cytogenetic diagnostics allowed a more precise characterization and prognosis of tumor conditions. A well-known example are patients with CML carrying a translocation 9/22 (see page 8). They have a better prognosis than those missing this mutation. The presence of additional numeric and/or structural abnormalities of the chromosomes besides this aberration though may lead to a poorer prognosis for the patient.

In the course of a karyotype evolution, the quantitative distribution of main- and sidelines of a composite tumor karyotype may change in its genetic condition causing an altered course of the disease (Mikalasova et al. 2015).

The following observation received particular attention: donor cells of a healthy person with normal karyotype administered to tumor patients in bone marrow transplantation will exhibit more and more the chromosome aberration of the recipient as revealed in the course of repeated cytogenetic testing. The cause of the aberration induction in the donor cells remains unexplained.

Frequently, FISH diagnostics serves to control the success of the tumor therapy in the patient. This, for example, is performed in therapies with bone marrow transplantation to analyse if the bone marrow of the patient (recipient) shows a quantitatively relevant increase in donor cells combined with the reduction of the tumor cells. Furthermore, in the course of the therapy, the reduction of the

percentage of aberrant cells in the patient himself in comparison to his normal somatic cells will be investigated, and it will be analysed which types of cells with pathological karyotype will disappear and in which order.

Finally, due to the results achieved through these investigations, it is possible nowadays to predict the cancer stage of a patient with a tumor condition and to choose the best individualized therapy schema by means of the aberration spectrum on hand.

In the majority of tumor therapies the surgical removal of tumor tissue is being accompanied by additional cycles of treatment. Usually these are radiation and chemotherapy either preceding or following the surgical procedure. New methods of treatment are available choosing either DNA- or RNA segments to be transferred into the tumor cells by means of adeno- or retroviruses.

Chemotherapy frequently combines the use of substances with different activity mechanisms, thus making it possible to reduce the concentration of the required substance needed. The application of a spindle inhibitor like Vincristine, for example, will lead to a synchronization of the cells in the cell cycle. That way, the subsequent application of substances with the desired effect on the DNA structure can affect the cells in the most sensitive phase of the cell cycle, and thus the concentration of the agents and the time of application can be reduced.

According to today's knowledge, the earliest therapies of a malignant tumor were performed by physicians in ancient Egypt by plant extracts, more than 2000 years ago. Regrettably, there are not many data available neither on the substances applied nor on the mode of treatment, and the medical plants chosen are unknown to us, with only general informations given and no illustrations of the plants existent.

Numerous substances that are used in tumor therapy today, e.g. Colchicine, the Vinca alkaloids, Bleomycin or Taxol, are plant extracts or isolated from fungi. In the majority of cases, the effects of substances applied in tumor therapy are not tumor-specific. But these agents can also induce lethal alterations in healthy somatic tissues, and they can result in sterility in the patient due to mutations in the germ cells and their precursors in the gonads. Primary, all meristematic cell systems are particularly affected in normal somatic tissues. The mutagenic effect of many agents can even result in the formation of new tumors.

From the very beginning, attempts have been made to reduce the occurring side effects in cancer therapy by application of so-called antimutagenic substances. This is of outstanding relevance when strong mutagens such as alkylating agents must be chosen for therapy. But even with the application of radiotherapy an additional treatment of the patient with specific biological agents showed protective effects. Experimental investigations of micro-organisms, as well of different species of animals and in vitro cultures of human cells revealed specific protective reactions in the various tested organisms. The effect of antimutagenic substances is frequently caused by its tight binding to the DNA of the nucleus. Surprisingly, a preferential combination to specific DNA structures could not be observed. The majority of projects analyzing the effect of antimutagenic substances are testing the reduction of the amount of gene mutations. Increasingly, a changed frequency of chromosome mutations is observed.

When applying antimutagenic substances in addition to the chosen tumor therapy the effects of the substance combinations and the possibility of cross reactions have to be tested in advance. Furthermore, the tolerability and any toxic reactions possible have to be analyzed before choosing agents in therapy. It could be demonstrated that the prophylactic application of antimutagenic substances is of extraordinary protective relevance. Simultaneously, analyses of patient data showed that the application of antimutagenic substances did not reduce the effect of the tumor therapy.

Due to the data available on the effect of mutagenic agents on tumor cells as well as normal somatic and germ cells of individual patients and the potential consequences of tumor therapy including mutagenic substances, each patient should be offered genetic counseling before the onset of his therapy. This is of special relevance for young patients because of the possibility to induce secondary sterility. Consequently and with relevance to the future outlook of the patient, the application of mutagenic agents in tumor therapy points to the relevance and requirement of patient information concerning negative side effects arising in addition to the effects of tumor therapy.

6.7 Outlook: Objectives for Future Projects in Research Leading Improving Therapies

In the preceding chapters a short description of main areas of research on genetic factors influencing tumor characterization, development, prognosis and therapy is given. It has been demonstrated that somatic mutations leading to cell transformation are of polygenic origin.

The causes of divergent reactions of transformed cells after application of anticancerogenic substances are presented and discussed.

It could be demonstrated that, in the field of tumor genetics, basic and applied research projects have to be combined. Longitudinal studies in patients significantly increased our knowledge of the evolution of tumors with an aberrant karyotype.

Experimental studies on different test objects, such as bacteria, mammalia, and human cells in vitro, enabled the development and establishment of simultaneous application of anticancerogenic agents together with protective substances. This led to a tumor treatment with fewer side effects for the patient.

Novel possibilities of tumor treatment are under development with special emphasis on specific gene therapy (Cross and Burmester 2006).

It can be expected, that this will lead to better results in tumor therapy and thus increase the quality of life of the patients.

6.8 Conclusion

Tumor diseases in man are defined as a group of defects and are not caused by the same specific somatic alteration. As the different types of tumors have already been described for hundreds of years their specific characterizations is nowadays

possible. This includes the knowledge about the typical age of onset, the course of the disease and the different types of therapy which were successfully selected. The characterization of a tumor includes furthermore the differentiation between benign and malign tumors and their induction and development by endogenous or by exogenous factors or by a combination of both.

Often, a change of a benign into a malign tumor in the course of its development can occur. The transformation of normal somatic cells into the immortalized tumor cells is a basic phenomenon caused by gene, chromosome, or epigenetic mutations. During further development of a tumor an increasing chromosome instability is a characteristic feature.

The so called "Cancer-families" have been diagnosed and described already for hundreds of years in the medical literature. Here, the analyses of genetic mutations presenting with an increased risk for the carrier lead to prevention and/or early detection of tumor diseases and to genetic counseling of relatives. Furthermore, it enabled a specific, individualized therapy of the patient, thus improving his life expectance significantly.

In addition to the possibilities of a standard tumor therapy new ways of an additional treatment by alternative methods have been developed. They included the analyses of specific cell functions and their relevance to the intended tumor treatment leading to specific molecular targets. They were investigated by different pharmacogenetic techniques.

The most relevant method developing new fields of tumor treatment is gene therapy which will be the most specific type of treatment and at the same time the lowest negative side effects.

6.9 Summary

In the short review presented here it could be demonstrated that cancer development is a multistep process which is caused by a number of characteristic gene, chromosome and genome mutations. This genetic complexity influencing the control mechanisms of cell function such as cell growth and division is the reason for differentiated diagnoses to characterize the tumor before treatment.

Pharmacogenetics as a combination of pharmacology and genetics thus developed as a powerful tool in tumor therapy.

In the course of the last years, tumor therapy could be significantly improved into a targeted therapy due to the analysis of tumor progression and individual risk factors of the patient.

Compared to standard therapy, the targeted therapy offers a more specific response of the tumor cells to the drug. It reacts with specific molecular targets in the tumor cells compared to standard chemotherapy which reacts with all meristematic cells-germ, normal somatic and cancerous.

While targeted therapy blocks the proliferation of the tumor cells, standard therapies are cytotoxic, meaning that they do not only block but kill the tumor plus somatic cells.

In addition to a growing individualized tumor therapy, the additional application of antimutagenic substances has reduced negative side effects of the chosen drugs in standard chemotherapy.

The future of cancer treatment will be further dominated by the development of gene therapy. Investigations on cells in vitro and animal models testing a wide variety of agents showed remarkable efficacy.

In different types of cancer for example, the transfer of viruses or genes led to cell death, decrease of blood supply to the tumor, or restored the normal cell function.

These new fields of therapy will—in future—be used alone or in combination with different types of chemotherapy and thus lead to an improved management of a cancer disease.

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Chapter 7
Genetic Counselling for Cancer Susceptibility

Shirley V. Hodgson

Abstract This chapter describes the basic principles of genetic counselling for inherited cancer susceptibility, the types of genes involved in causing cancer susceptibility and their inheritance. The difference between diagnostic tests to determine the molecular defect underlying a genetic condition in an affected individual, and predictive testing of a relative for a germline mutation in a cancer predisposing gene already identified in a close relative is explained. The process of pedigree analysis and risk assessment, and the appropriate level of risk for surveillance and prophylactic interventions, is discussed with a detailed discussion of how this is done. Examples are given of genetic counselling in situations where a genetic mutation causing a high risk of a specific cancer or cancers has been identified, with some challenging ethical and social aspects of the process of informing relatives about a germline mutation detected in an affected individual.

Keywords Cancer susceptibility · Genetic counselling · Pedigree

Abbreviations

AJ	Ashkenazi Jewish
CRC	Colorectal Cancer
DNA	Deoxyribose nucleic acid
FAP	Familial Adenomatous Polyposis
HNPCC	Hereditary Non-Polyposis Colon Cancer
MMR	Mismatch repair
OR	Odds ratio
PARP	Poly (ADP-ribose) polymerase
SNP	Single Nucleotide Polymorphism

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7.1 Introduction

In the UK, clinical cancer genetics clinics were only just being set up in a few centres in the 1980s, with the recognition of the contribution of mutations in some strongly predisposing genes to cancer susceptibility. Initially, studies of Familial Adenomatous Polyposis (FAP) led to the setting up of registries for families with the condition in the 1970s, with the aim of facilitating the contacting of at-risk relatives of index cases so that they could be offered screening for the condition and early intervention (colectomy once polyposis was established) in affected individuals. In the 1980s, Henry and Patrick Lynch were developing services for colonoscopy screening of individuals from families with hereditary non-polyposis colorectal cancer (HNPCC), now known as Lynch syndrome, because they clearly demonstrated the clinical phenotype and autosomal dominant inheritance pattern of the condition. They and a Finnish group were able to demonstrate the reduction in morbidity and mortality from colorectal cancer (CRC) by colonoscopy screening in at-risk individuals from such families. The FAP gene, the genes for Lynch syndrome and the BRCA1 and BRCA2 genes for breast and ovarian cancer susceptibility were identified in the 1980–90s. Cancer genetics clinics began to be set up, perhaps the first being in Denmark, Holland, and in London and Manchester in the UK, where individuals with a personal or family history of certain cancers could be seen and their family histories assessed for the likelihood that there was a strong cancer susceptibility in the family. Increasing numbers of patients were referred over the subsequent years, so that nowadays cancer genetics referrals comprise more than half the workload of clinical genetics services in the UK at the present time. My interest in cancer genetics was first awoken by a strange and tragic co-incidence. My husband, a gastroenterologist, was looking after a very nice lady who had developed colorectal cancer in her late 50s, and she had previously had endometrial cancer. He noticed that she resembled a close friend of ours, and soon found out that she was our friend's mother! I contacted our friend to arrange for her to be seen in the new cancer family clinic in London, and screening was arranged, but deferred because she found she was pregnant. Subsequently she was diagnosed with ovarian cancer and tragically died. The family was seen in a genetics centre in London when the genes for Lynch syndrome had been identified, and a strong but previously unrecognised family history of colorectal and endometrial cancer was ascertained. A mutation in one of the Lynch syndrome genes was identified. This enabled the younger generations of this family to be offered testing for the condition and those who tested positive for the mutation offered screening. This example clearly shows how important it is to ascertain families with inherited cancer susceptibility so that they can be offered genetic testing and screening (although screening for ovarian cancer remains one of the least useful screening tools that we have). Young age at diagnosis and individuals who develop more than one cancer are important indicators of familial cancer susceptibility, in addition to a strong family history of the same or related cancer on the same side of the family.

Demand for cancer risk assessment based upon the estimation of the genetic component of cancer risk to a given individual is increasing rapidly. This is both because of increased public awareness of the genetic aspects of cancer susceptibility, and as a result of requests from clinicians for evaluation of their patients' cancer risk so that appropriate surveillance protocols can be developed. Risk prediction for common cancers is based upon careful assessment of family history of cancer and cancer-related syndromes, and a personal history and examination (where appropriate). The genetic risk assessment requires confirmation of the diagnosis in affected relatives whenever possible. Evaluation of the pedigree will allow the clinician to determine whether there is likely to be a strong inherited cancer susceptibility condition present in an affected individual in the family, allowing genetic testing to be done to try and identify the mutation causing the susceptibility in an affected individual in the family, with downstream cascade testing offered if a mutation is detected. Alternatively, the family history may be due to a combination of less strong genetic factors and environmental influences, where genetic tests are not appropriate but risk assessment is available for individuals in the family based on pedigree analysis, leading to appropriate surveillance and preventative measures being offered to those individuals at increased cancer risk. Risk assessment allows the person counselled (the consultand) to be assigned to a risk category (high, moderate or low) which then can allocate them for appropriate management. Close links with oncologists and clinicians such as in joint or multi-disciplinary meetings are helpful for arranging surveillance and prophylactic measures. Education should be provided for primary care and other referral clinicians with guidelines for appropriate referrals for screening or genetic testing.

7.2 Genes Involved in Cancer Susceptibility

Cancer generally occurs as a result of interacting genetic and environmental factors, and disease susceptibility is multifactorial. In inherited cancer susceptibility, the inherited component of risk may be subdivided by the degree of risk conferred by the germline mutation in question: (1) Rare, high penetrance genes conferring a strong susceptibility to certain cancers, such as *BRCA1, BRCA2* mutations which confer a strongly increased risk of breast and ovarian cancer, and the genes causing Lynch syndrome (Hereditary non-polyposis colon cancer, HNPCC, which causes an increased risk of colorectal and uterine cancer), which may account for about 5% of cases of the relevant cancers, (2) Uncommon, moderately penetrant genes conferring a moderate increase in risk (e.g. conferring an odds ratio (OR) for the specific cancer(s) of 2–2.5, such as *CHEK2* for breast cancer susceptibility, and (3) Common polymorphisms which alter disease risk by a very small amount (e.g. OR = <1.2). These low risk variants are increasingly being identified in large

case-control studies (Pharoah et al. 2008; Stratton and Rahman 2008). On their own these polymorphisms cannot usefully predict the susceptibility of an individual to a certain cancer, since many other genetic and environmental factors will also have a bearing on disease susceptibility, but it is possible that in the future, individuals carrying several high risk variants will be identifiable, in whom the relative risk of a disease could be elevated sufficiently to warrant surveillance and preventative strategies to be offered. Thus a panel of several such polymorphisms could in the future be used for identifying patients at a significantly increased cancer risk, so that screening and appropriate prophylactic measures could be offered based on such a risk assessment for individuals in the population. Currently, however, such low risk polymorphisms are not in general use for genetic testing. Moderate risk genes such as CHEK2 are tested in some countries (e.g. Poland) but not in others, since the relative risk of cancer (in this case breast cancer) in mutation carriers is not as great as with the high-risk gene mutations such as in BRCA1 and BRCA2, so the benefits of risk reducing options available are not so clear cut. BRCA1 and BRCA2 mutations confer a high risk of breast cancer (up to 70–80% over a woman's lifetime) which clearly indicates that offering screening and preventative surgery to female mutation carriers would be appropriate.

Genes which when mutated cause a high risk of developing specific cancers are usually tumour suppressor genes, DNA repair genes, or rarely, oncogenes. The usual mode of inheritance of conditions causing cancer susceptibility (most due to mutations in tumour suppressor genes) is autosomal dominant, such that the offspring of an affected individual will have a 50:50 chance of inheriting the defective gene. This is characteristic of Familial adenomatous Polyposis (FAP), BRCA1/2 breast cancer susceptibility gene mutations, Lynch syndrome and many syndromic cancer predisposing conditions such as Gorlin syndrome, Peutz-Jegher syndrome, Cowden syndrome and Multiple Endocrine Neoplasia type 1. Multiple Endocrine Neoplasia type 2 is also an autosomal dominant condition but caused by a germline mutation in an oncogene. Some other cancer predisposing conditions are inherited as autosomal recessive conditions, usually those caused by DNA repair defects, such as Fanconi Anaemia and Ataxia Telangiectasia, and also some forms of adenomatous polyposis.

When a high risk mutation has been identified in an individual it is important to consider their own clinical risk reducing management, and also who else in the family may be at risk and could benefit from genetic testing. This requires arranging for such relatives to be informed of this in an appropriate manner. In some cases, the treatment of cancer may also be altered by knowing that the individual had a germline mutation in a cancer predisposing gene.

There are a number of inherited conditions which have a clear clinical phenotype which carry an increased risk of certain specific cancers, such as Familial Adenomatous Polyposis (FAP), (characterised by multiple colonic adenomas from the teenage years), Von-Hippel Lindau disease, (characterised by cerebellar tumours, renal cell cancers and phaeochromocytomas), Cowden syndrome (characterised by mucucutaneous lesions, macrocephaly and nodular breast and thyroid lesions), Gorlin syndrome (with multiple basal cell cancers on the skin), Neurofibromatosis type 1 (skin neurofibromas and café au lait patches), and

Peutz-Jegher Syndrome (with mucocutaneous skin pigmentation). Many of these are rare, but when diagnosed clinically in an individual there is a clear inheritance pattern (usually autosomal dominant) and known cancer risks, with well-established screening and prophylactic protocols to reduce the associated cancer risks. Genetic counselling for such conditions involves making the correct diagnosis, identifying the causative mutation if possible, discussing risk-reducing options and arranging predictive testing for at-risk relatives of the index case. However in many situations there is no clear clinical phenotype to help make the diagnosis, such as in breast cancer susceptibility and Lynch syndrome, so often pedigree analysis is necessary to help make the diagnosis (van Lier et al. 2010).

7.3 Genetic Counselling

The mainstay of genetic risk evaluation and counselling is taking a family history. The history should be ascertained carefully, obtaining details about first, second and third degree relatives on both sides of the consultand's family, determining the age at diagnosis of cancer, histology if known, and number and types of cancer and pre-malignant lesions present in each individual. If a genetic test result for a mutation in a cancer predisposing gene is available in a relative, consent should be sought from the affected individual to obtain details of this. Pathology details of the cancers in affected relatives should be confirmed if possible from the treating hospital or cancer registries, as it should be borne in mind that sometimes the consultand may have erroneous knowledge of their relatives' cancers. It is recommended that where possible a family history questionnaire be sent to the consultand before their appointment so that these details can be obtained at leisure. It is also important to obtain a complete past and current history of any illnesses in the consultand that could indicate a cancer susceptibility, e.g. freckly pigmentation of the lips in Peutz-Jegher syndrome, or multiple colorectal polyps in Familial Adenomatous Polyposis. Features in the family history that indicate an increased risk for cancer in the consultand are: several close relatives affected with the same cancer or a related cancer (e.g. breast and ovarian cancer) on the same side of the family, cancers diagnosed at a young age, individuals affected with more than one related cancer, or individuals with features of cancer predisposing conditions such as Familial Adenomatous Polyposis.

Genetic counselling is the process by which the personal and family history is taken and assessed, and information derived from pedigree analysis and genetic testing is explained to the individual attending the genetic clinic (the consultand) to indicate their risk of developing a disease and their risk of handing on a genetic susceptibility to their offspring.

Genetic testing can be divided as follows: (1) The diagnosis of a genetic disorder or disease susceptibility in the consultand by pedigree analysis, clinical examination and genetic testing, and (2) predictive testing in an unaffected relative of an index case, by a genetic test for a known genetic alteration identified in the affected relative (the proband).

Genetic testing looks for inherited mutations or chromosomal abnormalities. Such tests may be divided into two main groups: the first includes tests for disorders which will inevitably develop, with known age-related risks of developing cancer, and where there are clearly established prophylactic and surveillance measures that can be put in place to lower the risk of cancer, such as Familial Adenomatous polyposis or Peutz-Jegher syndrome, and the second includes tests for genes conferring an inherited susceptibility to certain cancers without a clear clinical phenotype. These include Lynch syndrome (Hereditary non-Polyposis Colorectal Cancer, HNPCC), and hereditary breast/ovarian cancer susceptibility due to inherited mutations in *BRCA1* or *BRCA2*. In these conditions it is not inevitable that the affected individual will develop cancer, but the probability that they will do so is significantly increased. There is good evidence that environmental factors influence whether cancer will develop in such individuals, and there are many lifestyle, surveillance and prophylactic options that can be offered to them to reduce their cancer risks, such as taking regular aspirin to reduce the risk of colorectal cancer in Lynch syndrome.

7.4 Risk Assessment in Familial Cancer

In many situations the family history may indicate that it is unlikely that a strong genetic susceptibility condition is present in the family, so the counselling session would be based on giving the consultand an estimate of their own empirical risk of developing the cancers seen in relatives, which may be increased due to shared genetic and environmental factors in the family. The risk estimate can be divided into low, moderate and high risk, and surveillance protocols tailored to the risk estimate. The consultand should be advised to inform the genetics centre if there are any changes to the family history going forward.

The estimate of risk is made based on the number of affected individuals with the same or related cancer (e.g. breast and ovarian cancer), the ages at diagnosis and the closeness of the relationship to the consultand (on the same side of the family). Early age at diagnosis and a strong family history of the same or related cancers indicates an increased risk of a genetic predisposition, and certain characteristics of the cancers present are important. Thus, breast cancers with a "triple negative" phenotype (oestrogen and progestogen receptor negative and Her2-neu negative) are more likely to occur in women with germline *BRCA1* mutations, so a woman with this type of cancer is more likely to carry a BRCA1 mutation, and colorectal cancers which are microsatellite unstable and proximally sited are commoner in Lynch syndrome than in sporadic cancers. Information may be available regarding the staining pattern in tumours using antibodies to the proteins produced by Lynch syndrome genes in affected relatives' cancers which can also help determine whether the affected individual has Lynch syndrome. This can allow an estimate to be made firstly of the likelihood that there is a germline cancer susceptibility mutation present in a person in the family, and secondly what the estimated risk of

that cancer of developing in the consultand is. Surveillance protocols are available in many countries which vary depending on the estimated degree of risk, and such interventions should be discussed and put in place if appropriate (e.g. mammograms annually from 50 year age, or a single colonoscopy at 55 year, depending on local guidelines). These protocols are developed based on the age-related risk of cancer and the economic cost-benefit considerations for the health service. Details of surveillance protocols of this type can be found at NICE (UK) for breast cancer, and Dunlop et al. on the GUT website for colorectal cancer.

In many healthcare systems there are thresholds for the risk estimates above which genetic testing for a cancer susceptibility is available in the health service (www.nice.org.uk/pdf/CG014Fullguideline.pdf; http://www.cancer.gov/cancerinfo/pdq/genetics/risk-assessment-and-counseling). There are several computer programmes that can analyse pedigrees and give estimates of the likelihood that there is a mutation in the family (Kelly and Sweet 2007; Antoniou et al. 2008; Hampel et al. 2004), and these, and the "Manchester scoring" system can be used to help select families for mutation testing (Evans and Lalloo 2002). The Manchester scoring system is shown below; a score of 17 and above indicates a sufficiently high chance of detecting a mutation to recommend such testing in an affected individual with this family history. Other methods of assessing the risk of an inherited *BRCA1/2* mutation being present in a family have been developed, one of the most well-known being BRCAPRO, that uses a Bayesian probabilistic model, and BOADICEA which can also predict the risk of breast cancer accurately, taking into account unmeasured polygenic factors (Antoniou et al. 2008).

Cancer. age	BRCA1	BRCA2
FBC. <30	6	5
FBC. 30–39	4	4
FBC. 40–49	3	3
FBC. 50–59	2	2
FBC. >59	1	1
MBC. <60	5	8
MBC. >59	5	5
Ovarian. <60	8	5
Ovarian. >59	5	5
Pancreatic	0	1
Prostate. <60	0	2
Prostate. >59	0	1

Genetic counsellors and trained genetic nurses may be increasingly employed in specialised familial cancer clinics in cancer units and primary care, with the remit of assessing empiric cancer risks on the basis of personal and family histories, and to arrange surveillance protocols (audited centrally, if possible) for individuals at moderately increased risk, reassure those at low risk, and refer those at high risk of

a genetic cancer susceptibility to the Regional Genetics Centre for further evaluation, advice and management.

There are a number of questions that can help when trying to assess an individual's risk. Ask about
(a) Age of onset in the family member
(b) Site of primary tumour
(c) Number of affected members in the family
(d) Multiple primary tumours
Possible indications for referrals
Personal history
• Early onset of cancer (e.g. breast cancer diagnosed <40 years, colorectal cancer diagnosed <45 years, etc.)
• Multiple primary cancers
Family history
• 3 close relatives (same side of family) with cancer of the same or syndromicaly-related type (e.g. breast and ovarian or colorectal and uterine)
• 2 close relatives (same side of family) with cancer, or the same or related type, with at least one affected under 50 years
• 1 first degree relative (mother or sister) with early onset cancer (e.g. breast cancer) diagnosed <40 years, or <45 years if colorectal cancer
• 1 first degree relative with multiple primary cancers
• Two or more relatives with uncommon cancers e.g.: sarcomas, gliomas, pancreatic cancer, glioma haemangioblastomas, etc

Most risk estimates for cancer risk are empiric, based on the likelihood of a genetic contribution in the individual, and this risk estimate is increased if the individual has several affected relations on the same side to the family with the same or related cancers, multiple or early-onset cancers, and if the proband has clinical features of a cancer-predisposing condition or has previously had cancer or a cancer precursor lesion (Hampel et al. 2004).

Risk estimates can be given as a risk of developing cancer per year, or before a certain age, or as an overall life-time risk relative to the population risk. It is appropriate to compare this risk with the background population risk (relative risk). Screening and preventative options should be discussed, with consideration of the possibility of false-positive and false-negative results of tests and the anxiety these could cause. It should be made clear that no surveillance programme is totally

reliable and it should be emphasized that the individual being screened should never ignore abnormal symptoms between screening procedures. The current state of knowledge about the efficacy of screening should be fully explained. The individual's perception of his or her cancer risk should be assessed, as should its possible effects on the individual's health- behavior (Printz 2016; Ramirez et al. 2015; Evans and Lalloo 2002).

NICE guidelines for management of women at increased risk of breast cancer:

- Population risk—3% 40–50 years, lifetime risk <17% (Routine care)
- Moderate—3–8% 40–50 years, lifetime risk 17–30% (Care in Secondary centre)
- High risk— >8% 40–50 years, lifetime risk
  >30% or >20% chance BRCA1,
  BRCA2, TP53 gene in family (Care in Tertiary centre)
- Gene Test Guidelines >20% chance of finding a gene mutation, or Jewish ancestry.

7.5 Genetic Testing

The genetic counselling process for mendelian high-risk susceptibility gene mutations can be divided into two types of genetic testing situations: "mutation searching" diagnostic tests in an affected individual with clinical features or a strong family history of the cancer indicating that they have an increased chance of being a mutation carrier, and predictive testing, where an at-risk individual, who has an affected close relative with a known mutation, is tested to determine whether they have inherited the mutation or not.

To identify a pathogenic germline mutation in a family, it is usual to start by testing a blood (or tissue) sample from an affected relative, following informed consent for testing for a genetic cancer susceptibility. This requires an initial approach from the individual being counselled, and some family and confidentiality problems can arise over this. It is essential that the affected relative understands the nature of the tests being performed and the possible emotional impact of a positive (or a negative) result, and its relevance in terms of insurance and employment. When arranging diagnostic genetic tests it is important to have a rapport with the tested individual, and to explain possible test outcomes before the test is undertaken, with a clear plan for the communication of results.

7.6 Counselling for Diagnostic Genetic Tests in an Affected Individual

An affected individual (proband) may present wishing to be tested because they want to know what the risks are to their children of inheriting a cancer susceptibility from them, or because knowing that an individual has a germline susceptibility

mutation may alter their management. Thus an affected woman found to carry a pathogenic *BRCA1* or *BRCA2* mutation might opt for prophylactic mastectomy, more radical breast surgery at initial treatment for cancer, or prophylactic oophorectomy (Weitzel et al. 2003; Evans and Lalloo 2002), and subtotal colectomy, and hysterectomy in postmenopausal women might be recommended for treatment of early colorectal cancer in individuals with Lynch syndrome. Cancers in patients who possess germline *BRCA1/2* mutations may be more susceptible to poly (ADP-ribose) polymerase (PARP) inhibitors, as they are unable to repair double strand breaks in DNA in the tumour due to the bi-allelic *BRCA1/2* mutations present in the tumour. (Ratnam and Low 2007). Tumours with microsatellite instability, as in individuals with Lynch syndrome (caused by germline mutations in genes encoding DNA mismatch repair (MMR) enzymes) have greater resistance to cisplatin and 5-Fluoro-Uracil than MMR proficient patients (Papouli et al. 2004). For this reason, testing affected individuals is sometimes performed for management indications, with little pre-test counselling. Early indications are that the emotional impact of being informed that they carry a susceptibility mutation with only minimal genetic counselling has little adverse effect on individuals already affected with cancer. However some mutation carriers may experience severe guilt feelings because they may have handed on the mutation to their children who may have developed cancer, so pre-test counselling should include a careful exploration of the reasons for having the test, the cancer risks in individuals carrying mutations in the gene tested, and the anticipated impact of a positive, negative and uncertain result. The surveillance, prophylactic and treatment options available to mutation carriers should be clearly explained, and a strategy for management in the event of a positive result clearly outlined prior to testing. It is also helpful to discuss the way in which the results are to be conveyed, to whom, and any confidentiality constraints (Schwartz et al. 2004). Where a positive result was not anticipated, such a result can be unexpected and upsetting in an affected individual, for instance, in a young woman affected with breast cancer but with no family history of cancer, or in a woman of Askenazi Jewish (AJ) descent with little family history of breast/ovarian cancer, tested for a founder mutation in the *BRCA* genes (common in the AJ population). Increasingly, as tests become cheaper and more widely available, there is a case to be made for testing all individuals newly diagnosed with cancer in certain categories, such as ovarian cancer, for instance, or individuals diagnosed at a young age especially if they have features of hereditary cancers such as triple negative breast cancer. This could influence the type of surgery or treatment offered. Here it is important to have a rapid turnaround for the test result; the time-scale for testing should always be explained prior to testing. It should not be forgotten that individuals who have had cancer may be psychologically affected by the news that they have an inherited cancer susceptibility, particularly as it may indicate that they have an increased risk for metachronous cancers, and that they could be ‘responsible’ for handing on the susceptibility to their children—a potential cause of profound guilt feelings (Jagsi et al. 2015; Mcallister and Dearing 2014; McAllister et al. 2007).

There may be one of three outcomes of a diagnostic genetic test:

(1) The test may reveal a pathogenic mutation, which explains the disease in the proband and allows predictive genetic tests to be offered to their close relatives.
(2) The test may not reveal a pathogenic mutation. Where no mutation is detected, no genetic test will be available for close relatives, and no explanation will have been found for the cancer in that individual. However, it does not rule out the possibility that other (probably lower penetrance) cancer predisposing genes may have contributed to the aetiology of the cancer.
(3) A sequence change (or variant) may be detected in the gene tested whose significance may not be clear, necessitating further tests (e.g. segregation of the mutation with disease in the family, loss of the normal allele in tumour tissue, in silico and functional analysis of the variant, consultation with databases of mutations detected in other patients) to clarify this (Goldgar et al. 2004; www.genetests.org). Such variants are not uncommon. There is now a good deal of information available about such polymorphisms on websites, gleaned from the experience of other centres world-wide, and databases are being developed utilizing information accrued worldwide of the pathogenicity of such gene variants which are helpful. In such cases it is important that the tested individual understands their results and their implications. The development of improved methods of mutation testing using sequencing has resulted in the detection of increasing numbers of such polymorphisms, and the uncertain nature of their implications is sometimes very difficult to explain to the tested individual. For this reason it is helpful to mention the possibility of detecting such a variant before the test is initiated. Clearly, when the pathogenicity of a variant is unknown, it cannot be used for predictive testing in the unaffected relatives in that family.

7.7 Predictive Tests

Where an unaffected individual presents for genetic risk assessment with a family history of cancer which suggests that there may be a highly penetrant cancer susceptibility gene mutation in the family, it is generally considered inappropriate to test the unaffected individual without knowing whether there is a detectable pathogenic germline mutation in the family. In order to identify the mutation in a family, it is necessary to obtain blood (or tissue) from an affected relative, with informed consent for testing for an inherited cancer susceptibility. The initial approach to this individual should ideally be made by the individual being counselled. It is important that the affected relative understands the nature of the tests being performed, its relevance to their own cancer risks and management, and the possible emotional impact of a positive or negative result. This should usually be achieved by arranging for the individual to be seen in a local genetics centre. When a pathogenic mutation is detected, the affected individual needs to agree to the

release of their results to the family to enable predictive testing to be offered to at-risk individuals in that family. Occasionally, difficulties are encountered with this, and the ethical dilemmas involved in deciding whether to release genetic test information to at-risk relatives without the consent of the individual tested (thus breaking confidentiality) are complex. This may be resolved by further discussions with the family, and considering whether the interests of the individual or the family takes priority (McGivern et al. 2004; Costalas et al. 2003; Claes et al. 2003; Hughes et al. 2002; Smith et al. 2002; Sermijn et al. 2004; Daly et al. 2001; Parker and Lucassen 2003). Classically, genetic counselling for a predictive test in an unaffected individual at risk of inheriting a known mutation involves a pre-test session, during which the disorder and its inheritance is fully discussed, with a detailed explanation of the likelihood that the consultand will have inherited the mutation, the cancer risks associated with the disease—causing mutation, and the screening and prophylactic options available to mutation carriers to reduce their risks. Issues such as implications for insurance cover, employment, and childbearing should be discussed. The counsellor should also explore how the consultand may react psychologically to either test result, their reasons for wishing to be tested, what they would do if found to have inherited the mutation, and who they would inform of their test results. In some families there are issues of confidentiality and release of information to third parties that should be discussed before undertaking the tests. The pre-test counselling session may be followed after an interval by a further counselling visit to the clinic, at which time blood is drawn for the test if the individual still wishes to go ahead. The idea is that they will have had some time in the intervening period to reflect on the implications of the test, and will be prepared emotionally for the test results. However, often the individual wishing to undergo testing may feel that they have already deliberated sufficiently about the test to feel comfortable with going ahead at the first counselling visit. At the time the blood is drawn, the consultand will be told when the results would be expected, and how they will be given. Often an appointment is made for the results appointment at that time. Usually the results are given at a face-to-face meeting, although in some cases they may be given by telephone or letter. In all sessions it is recommended that the consultand brings a confidant with them with whom they can discuss the implications of the test after the counselling session.

Problems may arise in the context of genetic testing. Sometimes certain individuals prefer not to release the results of their genetic test to their relatives, which leaves the counsellor with the dilemma about whether it is right to breach their confidentiality as a "duty to warn" the at-risk relatives. Genetic tests in at-risk relatives may test an intervening relative inadvertently (since they would be an "obligate carrier") and this requires careful handling. Thus, in some cases, where the consultand is not the first-degree relative of the affected individual in the family, a positive test result in the consultand may indicate that the intervening relative (e.g. the mother of the consultand, whose own mother is a mutation carrier) is also a mutation carrier, and it is very important that this possibility is discussed prior to testing. There should be a clear decision about how the intervening relative is to be informed about the result, preferably to include genetic counselling of that individual

at the same time as the consultand. In some cases the consultand may wish to undergo testing and not inform the intervening relative, possibly because they are elderly or infirm, but sometimes because of poor family relationships. This is often a difficult counselling situation. Another counselling conundrum may arise when only one member of an identical twin pair wishes to undergo predictive testing. Again, it is preferable that they both receive pre-test counselling at the same time, because the results for both would be expected to be the same. An example is to ask both twins to come together for the test counselling; problems can arise if one twin wishes to be tested and the other twin does not. Careful counselling is needed to resolve this, since if the test identifies a germline mutation in a cancer predisposing gene in one twin, this is likely to be present in the other twin. In my experience this can be difficult, particularly if the twins live in different areas and have different agendas. I once tried to test one twin in my clinic whilst her twin was seen in a different genetics centre at the same time. My twin came to the clinic because she wanted the test for the benefit of her children. However her twin failed to attend the other genetic centre because she had no children and felt ambivalent about being tested. Eventually, at a different appointment, they did agree both to be tested!

Individuals with a low-risk result (i.e. they have not inherited the susceptibility mutation) may require post-test support because they can suffer from 'survivor guilt', particularly if their close relatives have suffered or died from cancer. Individuals who have inherited the mutation should be offered psychological support and a clear protocol for surveillance and prophylactic options. Patient support groups are established for familial cancer conditions such as retinoblastoma, and for many other cancer predisposition conditions. "Carrier clinics" specifically for carriers of mutations in *BRCA1* and *BRCA2* have been set up in some genetic centres, where such individuals may be seen regularly and management issues can be addressed. The Hereditary Breast and Ovarian Cancer Foundation (http://www.hboc.ca/) is devoted to women at increased genetic risk of breast and ovarian cancer.

The general rule that predictive testing is usually only performed when the pathogenic mutation in the family is known may sometimes be broken. For instance, where there is a strong family history of disease, e.g. breast cancer, with a high probability of a mutation being present in affected individuals in the family, or in families from certain ethnic groups such as Ashkenazi Jewish (AJ), where there is an increased chance of a founder mutation being present, an unaffected individual in the family may be tested in the absence of a known familial mutation. Such testing may be offered particularly in cases where the outcome would impact on management (e.g. where the consultand is requesting prophylactic surgery). Counselling for such tests needs to include the implications of a possibly unexpected detection of a mutation, which can be a shock to someone unprepared for such a result. They should also understand that the receipt of a negative mutation result cannot confidently indicate the absence of the familial genetic susceptibility in the individual tested, since it is not known whether the disease in the family was due to mutations in the gene tested or not. The test could have been, in effect, for the wrong susceptibility gene, and give an unrealistic perception of low risk.

In AJ families, the at-risk individual may be tested only for the three founder *BRCA1/BRCA2* mutations which are common in individuals of AJ descent, and the absence of a mutation is reassuring since these mutations are the most likely cause of familial breast/ovarian cancer in individuals from this ethnic group. However the absence of a founder mutation does not mean that the consultand does not have a different mutation in the BRCA1 or BRCA2 gene that has not been tested. One situation encountered is that the unaffected daughter of a woman diagnosed with triple negative breast cancer, and who had multiple affected relatives, insisted on being tested for a BRCA1/2 mutation without informing her mother, who she said declined testing for emotional reasons. The daughter tested positive for a mutation, but was reluctant to inform her mother, despite the possibility that the mother's cancer treatment could be modified if it was known that she almost certainly carried the same mutation. It can be helpful to involve the psychiatric team in such situations.

Systematic reviews of studies assessing the psychological impact of testing have so far concluded that there is little evidence of adverse psychological outcomes in individuals who undergo predictive genetic testing. Most studies found that the level of distress, anxiety or depression in carriers was not significantly increased after disclosure of their positive result, and that non-carriers experience psychological benefits, such as significant relief, after disclosure of results. (Broadstock et al. 2000; Butow et al. 2003; Trepanier et al. 2004). In my personal experience I find that several discussions with the family can overcome most misunderstandings and difficulties with the acceptance of genetic counselling and the dissemination of results to at-risk relatives, without too many concerns about adverse psychological consequences, and as genetic tests become more widely accepted as part of routine medical management this will increasingly become normal practice. The main concern with new genetic tests which may identify low-penetrance variants of small effect is the potential for misinterpretation and over-interpretation of the results.

7.8 Testing of Minors

The risk of cancer in many cancer predisposing conditions is often not significant until adult life, although this is not always the case (viz. early onset of colorectal adenomas and cancer in Familial Adenomatous Polyposis), and cancer can sometimes develop in very young individuals with certain predisposing conditions (e.g. retinoblastoma). In these conditions, where surveillance would be initiated in childhood, predictive testing is advisable in childhood before the cancer risk becomes actionable. However, in cases where the cancer risk is not significant until adulthood, and genetic testing in childhood for such a susceptibility would not influence management, it is generally considered preferable to avoid testing in children. This is because a positive outcome could generate unnecessary anxiety at a time when no intervention would be advised, and it is argued that it would take away the autonomy of the child to decide whether to be tested or not. It is sometimes difficult to argue this point of view to anxious parents who find the

uncertainty without testing very difficult to bear (Harris et al. 2005; Working Party of the Clinical Genetics Society, UK 1994). There are occasions where the parents of a child at risk of inheriting an adult onset cancer predisposing gene from one of their parents are adamant that the child be tested in childhood. In such cases it is advised that the counsellor explore the reasons for wanting the test at that age, and discuss all aspects of the test with the implication that the test at this age is inadvisable. This is because it might cause undue anxiety to the parents if a child who had inherited the condition developed an unrelated illness, and might cause the parents to be over-protective. The benefits of waiting until the child is able to make an informed decision in adulthood to be tested include maintaining the autonomy of the child, including the child's right not to know their result, and avoid the child being "labelled" as ill and/or feeling a burden to the family. An exceptional indication for testing a newborn baby is to ascertain whether he or she might be a donor for a transplant to treat or cure an affected sibling with an autosomal recessive condition such as Fanconi Anaemia.

7.9 Testing in Pregnancy

Another specific issue that can arise is genetic testing in pregnancy. Clearly only certain conditions would be considered "severe" enough to warrant this, done with the intention of terminating an affected pregnancy, and these conditions should be carefully chosen by an expert panel. A possible problem with such testing is that if a pregnancy is tested and the unborn child does carry the high risk mutation, if the couple then decides to continue the pregnancy they will in effect have tested the child for the mutation without its consent. Decisions about testing in pregnancy are taken at a very emotional time with time limitations, and there can sometimes be disagreements between the parental couple about testing. One of my patients with Familial Adenomatous polyposis said she wanted a genetic test for FAP in her pregnancy, and the molecular team did all the preliminary work for the test, but the lady then changed her mind. If she had gone ahead and been tested, and the baby had tested positive for the mutation, she said she would have been reluctant to terminate the pregnancy, so preferred not to know whether the child would be affected until such time as screening would have been initiated (at about 12–13 year age).

7.10 Disclosure of Results

The situation sometimes arises where an individual who has tested positive for a cancer predisposing condition refuses to tell their at-risk relatives. In such a situation is there a duty of the doctor to inform such relatives? There is a discussion as to whether such genetic test information is the confidential information for one person only, or whether it is the family's information. There is much debate about

whether genetic information is the property of the individual or the whole family, since genetic information about one person has implications for relatives (BMJ 7 July 2007, V335, 22–23). There is therefore a conflict between patient confidentiality and autonomy, and a tension between maintaining the duty of confidentiality and disseminating genetic information to others, if intervention could decrease morbidity and mortality. In general if the family is counselled carefully, and the reasons for wishing to withhold information explored, (e.g. Limited understanding of the genetic diagnosis, denial, not wishing to be the messenger of bad news to the relative or a desire to protect them, uncertainty about the relative's ability to cope, conflict within the family or difficult family relationships, and the proband being unable to find the right time or way to give the information) people may eventually decide to release their genetic test results. However, in some countries it is felt that if this fails the doctor does have a duty to over-ride the confidentiality for the benefit of their relatives. Counsellors can help in these situations by identifying barriers to disclosure and strategies to overcome these, discussing when to communicate with their child or other relative, what information to give, and provide written information with contact details of the genetic counsellor and other support, and help recognise the implications of non-disclosure.

7.11 Testing for Low Penetrance Genetic Variants

Genetic testing for moderate risk susceptibility gene mutations are not in common use in the health service because the relative risk conferred is insufficient for alterations in clinical management, although in some countries testing for CHEK2 mutations (e.g. Poland) is offered and testing for some other similar gene mutations is being considered. Genome-wide scans for single nucleotide polymorphisms (SNPs) in large populations of cancer cases and controls have ascertained SNPs which confer a very small increased risk of specific cancers. This may allow individuals to be typed for a panel of SNPs, which individually may only confer a relative risk (OR) of 1.2–1.3 but collectively could identify a small proportion of individuals who have a more substantial increase in risk. In the case of colorectal cancer for instance, individuals with several such SNPs could have an overall relative risk of colorectal cancer >3, where regular colonoscopy screening might be offered. A similar situation may be encountered in breast cancer susceptibility, where about six common polymorphic loci identified in case-control studies could be tested, where the high risk allele of each confers an odds ratio of <1.2 on average. Women with six high risk alleles would have a significantly increased risk of breast cancer, (OR about 2) and could be offered breast cancer screening earlier than that offered in the general population, and women with several low-risk alleles could be screened from a later age (Pharoah et al. 2008). Such testing has not been clinically validated and is not currently offered in the UK health service. It is also very difficult to interpret, particularly as more and more low penetrance SNPs are identified.

Companies offering SNP risk profile services do not routinely offer pre- and post-test counselling and explanation of the results. Thus individuals informed that they possess a single SNP conferring increased disease risk may wrongly perceive this risk to be substantial, and may be unaware that the information does not take account of all other predictive factors (including other polymorphic loci, family history, mutations in high-penetrance genes and lifestyle factors). Alternatively, one low risk SNP result could lead to false reassurance and increased risk-taking behaviour. Unregulated testing could lead to an increased workload for primary care practitioners, as patients may present requesting explanation of the results of the test, and access to further diagnostic testing. The implications of such multi-SNP tests are complex and difficult to interpret by most clinicians not working in the field of genetics. I have seen individuals in the clinic who were perplexed and frightened by the results of a commercial test for such variants, wrongly perceiving the risk conferred by a single SNP as being substantial. My task was to explain to them that a single SNP confers only a very small alteration in risk, and their total risk is dependent on the combination of the effects of many variants and environmental factors. Such tests require complex algorithms for interpretation, and are currently too complex for routine use in the health service.

7.12 Conclusions

Genetic testing for cancer susceptibility is a paradigm for testing for susceptibility to common diseases. Such testing will become increasingly important as we understand more about the complex interplay of rare high penetrance gene mutations and less common variants of low penetrance, and environmental and lifestyle factors in the causation of cancer. The development of strategies for genetic counselling for such diseases should take account of the importance of environmental risk factors and avoid the pitfall of genetic determinism. The provision of balanced information about the predictive value of different tests is extremely important, both for the wide variety of healthcare professionals who will be required to interpret test results, and for the individuals taking the tests.

7.13 Summary

We now know a considerable amount about the genes which, if inherited as faulty copies, contribute to an increased risk of developing certain cancers. We are able to utilise genetic testing for such faulty genes to determine whether an individual has a susceptibility to particular cancers, and genetic counselling is available to inform individuals of the implications of such genetic tests. Genetic counselling involves the assessment of a family history to determine how likely it is that a strong genetic susceptibility may be present in some members of that family, and also to assess the

cancer risks for the person seeking advice. Diagnostic tests to determine the molecular defect underlying a genetic susceptibility in an affected individual are available, and predictive testing of a relative for a germline mutation in a cancer predisposing gene already identified in a close relative can then be offered. Genetic counselling procedures are developed to optimise the presentation of such information and to help individuals tested to choose appropriate screening and risk-reducing strategies based on the results of such genetic tests. The appropriate level of risk for surveillance and prophylactic interventions needs to be established. Counselling for individuals in such families may present ethical and social challenges. Now that low-penetrance genes are increasingly being identified and used for genetic testing, the interpretation of the results of these tests presents particular difficulties.

Glossary

Consultand person attending the clinic wishing to have genetic advice

Proband person with a genetic condition attending the clinic

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Chapter 8
Cancer, Psychotherapy and the Airway

Zahid Hussain Khan

Abstract Cancer is the leading cause of death in the developed countries only surpassed by cardiovascular diseases, and one of every four deaths is from cancer. This chapter dwells on cancer in general and airway in particular, the role of genes in its causation, the different modalities of treatment currently in vogue and finally tackles the much talked about role of psychotherapy in cancer patients. The prospects of psychotherapy in cancer patients are being brought to the limelight and an effort being made to make some subtle conclusions about the potential role of psychotherapy in offering a ray of hope to the cancer patients whose prospects are dismal and dreadful. Any claims about survival improvement following psychotherapy are open to criticism because of the heterogeneity across the studies and the short comings of the studies.

Keywords Cancer · Psychotherapy · Airway

Abbreviations

AJCC	American Joint Committee on Cancer
ANV	Anticipatory Nausea and Vomiting
Tis	Carcinoma in situ
CBI	Cognitive Behavioral Interventions
FDR	False Discovery Rate
FDA	Food and Drug Administration
HNSCC	Head and Neck Squamous Cell Carcinoma
IHC	Immunohistochemistry
NGS	Next Generation Sequencing
QOL	Quality of Life
RCTS	Randomized Clinical Trials
SCC	Squamous Cell Carcinoma

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UICC Union for International Cancer Control
WHO World Health Organization

8.1 Introduction

In the not very distant past, physicians' role was to provide a total cure for the illness and heal their patients. The length of life or in other words the life expectancy was being pursued. Thus survival was the only gadget to qualify the success of treatment strategy. It is beyond our knowledge, comprehension and expertise to provide a total cure and full recovery to a cancer patient, although there might be instances in literature where cancer patients have attained full recovery. It must be understood without mollycoddling that cancer limits life expectancy, thus the quality of life (QOL) becomes of paramount importance to us as physicians. Patients are unaware of the ephemeral nature of their life-fate and the ultimate doom that awaits them. Under such circumstances when we as physicians cannot provide disease free life or total recovery to our patients, we should endeavor at least to improve the QOL in terms of curtailing their depression, anxiety, pain, insomnia, and at the same time inculcating in the patients the spirit and feeling of optimism and a sense of well-being through counselling and psychosocial interventions. Cancer is a diabolical term as it frightens a patient and its diagnosis once clinched evokes images of suffering and death. The life of such a patient undoubtedly receives a lethal blow and runs a downward course as far as mood and happiness are concerned.

Cancer is a difficult region where we balance probabilities and choose the most likely. However, it should be understood that the QOL and its improvement is difficult to achieve in all patients because it is affected by a host of factors such as age, sex, demographic factor, socioeconomic status, stage and state of the disease, effect of treatment, physical complaints, mood disorders, identity change, forced premature retirement or job loss, marital life and sexuality (Babin et al. 2008). It is still being debated whether female sex and advanced age could be predictive factors in altering the health related QOL. However, seemingly destructive or mutilating surgical procedures reduce it (Woodward et al. 2007). Smoking and depression are said to be affecting QOL (Duffs et al. 2002). Smoking is addictive and if quitted could adversely affect the QOL because smoking elates the mood and gives a psychological boost to the psyche of a person (Khan 1994). I remember a patient who had a colonic cancer for which he had been treated but like all cancers it had become dormant like a volcano. This patient had been an inveterate smoker for more than 30 years. I had weaned him from his tobacco mania, but I was well aware that the fiend was not dead but sleeping (Khan). Alcohol intake may improve QOL (Allison 2002). Weaning from tobacco and alcohol is at times challenging and

can both induce and exacerbate depressive states (Bjordal and Kaasa 1995). Those with higher economic status and without comorbidity tend to enjoy better QOL. A letter of unemployment from the employer would drastically affect the QOL as the household income is reduced affecting the entire infra-structure of the house (Vartanian et al. 2006). Cancer if it affects a person in the deprived stratum of the society literally shatters the determination and hopes of all concerned especially the patient if by a stroke of luck he happens to be the sole person catering for the needs of the family.

Advanced cancers certainly affect the QOL because of the severity of associated symptoms. The treatment protocols in itself affect the QOL. Multimodality treatment is believed to decrease the QOL (Lee-Preston et al. 2004). Some treatment regimens for cancer patients leave behind indelible scars and other stigma that adversely affect the self-esteem thus influencing their health related QOL (Babin 2002). QOL is often measured by emotional, physical, functional, financial, and spiritual components. It can be well imagined how dysphagia would be affecting the QOL. Again if a patient is robbed off the senses of taste and smell, the food cannot be relished and this feeling or situation affects the QOL. Moreover, if a patient takes too long to swallow his/her food, a natural course left open for such a patient would be to avoid eating in public places to avoid embarrassment and the agonizing feeling that eyes are focused on him/her. Thus such patients become socially isolated, withdrawn and secluded. These patients suffer from extreme depression, anxiety and stress and suffer also from a constant fear of choking while eating which is specially seen in patients having undergone laryngectomies for carcinoma of the larynx (Maclean et al. 2009).

When sorrows come, they don't come single but they come in batallions. Cancer is no exception. It engulfs you in a labyrinth of troubles too great to get out at all.

8.2 Cancer Genetics

There is a common belief that cancer is a single disease. However this notion has been negated, and cancer in fact is regarded as a generic term encompassing a group of more than 100 diseases in which the damaged DNA of a cell causes it to grow out of control. This uncontrollable growth and accumulation of abnormal cells is a feature common to all types of cancers. Normal cells behave according to a genetically pre-determined set of rules unique to the particular cell type. These normal cells divide, mature, die and are replaced systematically. Cancer cells on the contrary markedly differ from normal cells as far as their growth is concerned. Because of damaged DNA, instead of dying, cancer cells exhibit a perpetual growth and continue to form abnormal cells that grow all the more rapidly, erratically, in an altogether disorderly fashion and as they fail to abide by set rules, they do not mature correctly. The cells can grow into malignant tumors replacing normal tissue and spreading throughout the body. In cancer cells unlike normal cells, the damaged DNA is neither repaired nor does the cell die. Instead the cells go on making

new cells that the body does not need. These new cells will all have the same damaged DNA like the very first cell. It is believed that the damage to the DNA is caused due to some error while the normal cell is reproducing.

The nuclear genome comprises approximately 3.2 billion nucleotides of DNA, divided into 24 linear molecules, the shortest 50,000,000 nucleotides in length and the longest 260,000,000 nucleotides, each contained in a different chromosome (Gregory et al. 2007). There are estimated 20,000–25,000 human protein-coding genes (Gregory et al. 2007). The content of the human genome is commonly divided into coding and noncoding DNA sequences. Coding DNA occupy only a small fraction of the genome (<2%). The genomic loci and length of certain types of small repetitive sequences are highly variable from person to person, which is the basis of DNA fingerprinting and DNA paternity testing technologies (Gregory et al. 2007).

A hereditary component does exist that can cause malfunction of the DNA. Some people inherit DNA mutation from their parents that greatly increase their risk for developing certain cancers. However, it is believed that such inherited gene mutations do not cause most of the laryngeal cancers or those cancers originating from the hypopharynx. These cancers develop as a result of gene mutations later in life and are thus called acquired mutations probably related to environmental factors. Acquired changes in genes such as TP 53 and p16 tumor suppressor genes seem to be of importance in laryngeal and hypolaryngeal cancers.

8.3 Environmental Impact on Gene Expression

It has been upheld that the environment does have a powerful impact in the development or tumor of cancer. For instance, inherited mutations of oncogenes or suppressor genes rarely cause laryngeal and hypolaryngeal cancers, but some people probably inherit a reduced ability to detoxify certain types of cancer causing chemicals and thus show an exaggerated vulnerability to the carcinogenic effect of cigarette, alcohol, and industrial chemicals or byproducts. Yule Bryner and Elizabeth Taylor, the two legendary figures from the film industry developed cancer of the voice box and succumbed to it. They both consumed tobacco and alcohol, and these could be the causes of their cancers or else could have triggered the process. This was just to quote a few examples. On the contrary history is replete with instances where people have lived beyond their 9th decade of life despite the fact that they had been chain smokers throughout their life. Through these examples, I am certainly not underestimating the known and established detrimental effect of tobacco smoke for our health in general and as an incriminating factor of cancer in particular, but would like to emphasize that there might perhaps be other factors at work that enhance the vulnerability of certain persons in developing cancers and which are probably beyond our comprehension and beyond our present day knowledge to understand.

Kandel has elegantly demonstrated how synaptic connections can be permanently altered and strengthened through the regulation of gene expression connected with learning from the environment. The number of synapses doubles or triples as a result of learning, in the marine snail. Kandel postulated that psychotherapy might bring about similar changes in brain synapses. The brain possesses plasticity. If psychotherapy is conceptualized as a form of learning, then the learning process that emanates in psychotherapy may produce alterations of gene expression and hence culminate in altering the strength of synaptic connections. There is enough evidence to support that environmental factors do have an impact on the function of the genes. Learning induces greater number of synapses per neuron and this could be attributed to the greater power of plasticity in the brain (Kandel 1998).

8.4 Gene Amplification and Genomic Alterations

Mammalian cells use the mechanism of gene-amplification to overexpress particular genes for survival under stress, such as when exposed to cytotoxic drugs. Gene amplification is a typical genetic alteration in cancer, and oncogenes have been identified in the amplified regions. Thus cancer associated genes may remain to be identified in the amplified regions. It has also been revealed that co-amplified genes do contribute to tumorigenesis in concert with known oncogenes in the same amplicons (Matsu et al. 2013). The fact that cancer genes play causal roles in carcinogenesis is being considered as an indispensable task in cancer research. It can provide an understanding on the functional cooperation of gene mutations in cancer.

In a survey, it was concluded that False Discovery Rate (FDR) strategy is efficient in identifying co-mutated gene pairs and the genes in the identified co-mutate gene pairs can be considered as candidate cancer genes (Wang et al. 2011).

Genomic alterations that cause or else promote cancer are referred to as drivers in lay man terms whereas passengers are referred to as alterations existing in the cancer genomes but without any apparent advantage to the cancerous cells when they do occur. During the past decade and a half, improvements in genomic technology have led to our understanding of cancer genetics, and apart from that the advent of second generation sequencing technologies and their applications to cancer have given a rapid boost to the pace of genome discovery. Whereas, the earlier technologies could focus on only one modality of cancer genome alteration such as mutation or expression, the second generation analyses enable us in identifying all such alterations simultaneously (Chin et al. 2011).

There is enormous evidence supporting the premise that changes in certain genes cause cells in the larynx and hypopharynx to become cancerous.

An alteration in the TP53 tumors suppressor gene is observed in these cancers. Changes in other genes such as P16, NOTCHI and Cyclin D1 genes have also been

found leading to cancer of the voice box and hypopharynx. The larynx or voice box contains the vocal cords and is located above the opening of the trachea-colloquially termed the windpipe.

Although there are no studies to support it but there had been singers with angelic voice and renowned fame who developed carcinoma of the larynx either during their career or towards the end of their career and died because of this harrowing disease. It is yet to be proved whether persistent movement of the vocal cords as is seen in professional singers could be an incriminating factor in perpetuating alteration in some genes and eventually leading to cancer of voice—box!

8.5 An Overview of the Carcinoma of the Larynx or the Airway

A: Classification and epidemiology

Cancer of the larynx, colloquially called the voice box is commonly a Squamous Cell Carcinoma (SCC) reflecting the origin of the squamous cells from the laryngeal epithelium. Thus cancer can develop in any part of the larynx. Anatomically, the pharynx is a musculo-facial tube that connects the nasal and oral cavities with the larynx and the esophagus. The pharynx is divided into three sections: the nasopharynx, situated directly posterior behind the nasal cavity; the oropharynx, lying directly posterior to the oral cavity and extending from the soft palate superiorly to the tip of the epiglottis inferiorly; and the oropharynx, that extends inferiorly from the upper edge of the epiglottis to the inferior edge of the cricoid cartilage and communicates with the oropharynx, the laryngeal inlet, and the esophagus. The larynx is continuous with the trachea and has a specialized constrictor-dilator mechanism in the airway. Although an exact classification does not appear in the literature, but an airway obstruction from an invading tumor can be demarcated or classified into: (a) a proximal or large airway obstruction and (b) distal airway obstruction. The proximal large airways which include the hypopharynx, larynx and the trachea lower down up to the carina. It has been further subdivided into the upper airway, which includes the part above the mid-trachea, and the lower airway, which is the part distal to the mid-trachea. This classification has definite implications as far as management of these malignancies is concerned, as proximal airway obstruction caused by an invading cancer can be safely bypassed by a surgical tracheostomy, whereas the lower airway obstruction may not be amenable to such surgical intervention.

Squamous cell carcinoma of the head and neck region, affecting the oral cavity, the larynx and the hypopharynx, is the sixth most common cancer among the male population in the developed world (Regin et al. 2007) and has a high morbidity and mortality. The primary etiologic agents in SCC are tobacco and alcohol (Davies and Welch 2006).

B: **Airway pathology**

The sphincteric, protective function is the oldest phylogenetic function of the larynx, which has evolved and developed from equatic amphibian predecessors. The constrictor mechanism of the larynx, imparts it an effective and rapid closure that prevents food, liquid and other foreign material from entering the lower airway. Apart from that the vocal cords have a vibratory effect on the expiratory air column and produce the sound used in voice production. Any tumor within the vicinity of this region will impair swallowing, cause aspiration and inflict a lethal blow on the voice box thus depriving the patient of speech.

An endotracheal tube passed through the larynx interferes with these laryngeal functions. The intubated patient experiences loss of voice and possible aspiration of foreign material into the airway. Aspiration of foreign material such as secretions or solid material may trickle down into the lungs causing atelectasis, infection and even death. The presence of an endotracheal tube also prevents the production of an adequate protective cough thus potentiating the aforementioned complications.

The clinical appearance of premalignant or incipient lesions of the mucosal surface of the aerodigestive tract include leukoplakia, erythroplakia, or speckled leukoplakia reflecting the presence of a white, red or mixed white/red lesion, respectively.

Laryngeal cancers are considered by some authors as microinvasive cancer that includes the presence of scattered malignant cells within the mucosa just below the basement membrane (Miller 1976) or within 1–2 mm below the basement membrane (Ferlito et al. 1996). Microinvasive carcinoma excludes those lesions that are restricted to the surface epithelium or carcinoma in situ (Tis) and those carcinomas that are deeply invasive into the muscle and cartilage and extralaryngeal structures (T2 or greater tumors). In the larynx, full cord mobility is present, which is a prerequisite for voice production and the prevention of aspiration of foreign particles. Any dysfunction in vocal cord mobility (fixation) means muscle invasion, which excludes the diagnosis of microinvasive cancer (Wenig 2002).

C: **Tumor staging**

In order to understand tumor staging, the larynx can be divided into three distinct anatomical regions: the glottis (true vocal cords, anterior and posterior commissures); the supraglottis (epiglottis, arytenoids, aryepiglottic folds, and false cords), and the subglottis. Almost all laryngeal cancers originate in the glottis. Cancers originating in the supraglottic region are less common, and subglottic or infraglottic tumors are less frequent. Laryngeal cancer may spread to adjacent structures by direct extension to the regional cervical lymph nodes by metastasis, and to far away distant organs through the blood stream (Latifi et al. 2012).

The staging can be further enumerated for ease of understanding and in order to better grasp the treatment protocols as under:

Tis: CA in situ
T1: Limited to subglottis
T2: Extends to vocal cord with normal or impaired mobility
T3: Limited to larynx with vocal cord fixation
T4a: Invades cricoid or thyroid cartilage, and/or invades tissue beyond the larynx
T4b: Invades prevertebral space, encases carotid artery, or invades mediastinal structures

Stages Tis, 1 and 2 are considered to be early stage carcinoma, whereas 3 and 4 are included in the advanced stage of laryngeal cancer. The term advanced laryngeal cancer generally denotes 3 or 4 laryngeal cancers according to the Union for International Cancer Control (UICC)/American Joint Committee on Cancer (AJCC) staging (Edge et al. 2010).

D: **Surgical and other treatment modalities**

The larynx plays a key role for many essential functions, including breathing, voice production, airway protection, and swallowing. Keeping these highly vital functions in mind, the treatment modalities should be tailored to the age, sex, occupation and staging of the tumor. The most important adverse prognostic factors for laryngeal cancer include increasing tumor (T) stage and nodal (N) stage. Other prognostic factors may include sex, age, performance status and a variety of pathologic features of the tumor, including grade and depth of invasion (Yilmaz et al. 1998).

Surgical procedures frequently employed to treat cancer of the larynx include cordectomy (removal of the vocal cords), microlaryngeal surgery, supraglottic laryngectomy (supraglottis removal), hemilaryngectomy (removal of half of the voice box, saving the voice), partial laryngectomy (partial voice box removal, saving the ability to talk), total laryngectomy (removal of the entire larynx), thyroidectomy (removal of the thyroid gland), and laser surgery to remove a surface tumor through a bloodless cut in the tissue (Zhang et al. 1994).

Besides that, neck dissection is also performed to remove any malignant tissue and to remove lymph nodes that harbor malignant tissue or cells.

E: **Early stage carcinoma (stage I and II)**

Current therapeutic options include transoral laser microsurgery, open partial laryngectomy and radiation therapy. Rate of control has been found to be similar after surgery and radiation. However, no randomized clinical trials have been performed to compare surgery with radiation therapy. While comparing radiotherapy and surgery for T1—T2 glottic cancers, vocal cord function assessed by videostroboscopy was found to be superior in the radiotherapy group of patients (Mylnarek et al. 2006). Radiotherapy along with surgery is reserved for salvage therapy with local recurrences. Laser resection has also shown considerable success

in early stage SCC and minimizes functional changes after surgery. Treatment of hypopharyngeal carcinomas is similar to that of laryngeal cancer. Early stage lesions are usually treated with radiation alone, although endoscopic resection is an option. However, the majority of patients with hypopharyngeal cancer have advanced stage disease because of the silent nature of the disease and frequent regional lymphatic spread, thus such patients are treated with chemotherapy and radiation therapy primarily, with surgical salvage.

F: **Advanced laryngeal cancer**

In recent decades, the treatment paradigm for advanced laryngeal cancer has shifted from one of primary surgery (total laryngectomy) as gold standard toward non-surgical organ preserving treatment using radiotherapy or chemotherapy. However, it is to be emphasized that the mainstay of treatment for advanced glottic lesions in most centers is total laryngectomy with or without postoperative irradiation. However, in some centers, radiation therapy is the initial treatment modality for T3 lesions (Mendenhall et al. 2005). For patients with locally advanced laryngeal cancer, radiotherapy with concurrent cisplatin is the standard alternative to total laryngectomy. The value of induction chemotherapy in larynx preservation therapies remains unknown (Majem et al. 2006). Major advantages of radiotherapy or chemotherapy for treatment of advanced laryngeal cancer are avoidance of surgery and anatomic preservation of the larynx, with no definite compromise in overall survival (Ganly et al. 2009; Forastiere et al. 2003). The disadvantages include a high incidence of severe acute toxicity, and a high incidence of long term laryngeal problems, particularly in patients treated with concurrent chemoradiotherapy (Matchtay et al. 2008; Ghadjar et al. 2012).

As with glottis carcinoma, most advanced stage supraglottic cancers initially are treated with chemotherapy and radiation therapy. The supraglottis has a rich lymphatic network so the neck must be explored and diligently addressed in all patients with supraglottic cancer.

G: **Securing the airway**

To safeguard the airway, an organ of paramount importance, a multidisciplinary team approach including radiologists, anesthesiologists, medical oncologists, head and neck and thoracic surgeons and the intensivist is warranted. In proximal airway obstruction, patency of the airway should be the goal. Malignancies in the upper airway that include the base of the tongue, nasopharynx, pyriform fossa, epiglottis or vocal cords will usually require a surgical airway such as tracheotomy or cricothyrotomy. In these situations, intubation should only be attempted in the presence of a surgeon, because these cases are not only difficult, but can lead to profuse bleeding as the tumors are highly fragile. Sedatives, respiratory depressants and muscle relaxants should be altogether avoided. Fibreoptic intubation is also potentially fraught with perils owing to the fragility of the tumor and especially so when it is already bleeding. At the same time, the anatomy of the airway may be distorted and it would be exceedingly difficult to visualize the glottic aperture. In

patients who are not cooperative or are in respiratory distress, fibreoptic intubation in securing the airway should be avoided. Studies have shown that as there is no survival benefit, there is no role for emergency laryngectomy for patients with carcinoma of the larynx. Such patients should preferably undergo tracheostomy and their elective surgery postponed for a later date (Yotakis et al. 1996; Narula et al. 1993). For patients in distress and who cannot lie supine for tracheostomy, stab cricothyrotomy can be lifesaving.

For lesions in the distal airway, intubation and tracheostomy are essentially of no use to alleviate the distressing symptoms. On the contrary, rigid bronchoscope has been found to be of help in patients with central airway obstruction. The rigid bronchoscope can relieve the obstruction due to blood clot or a fragment of the tumor. The bronchoscope can keep the airway patent if the lower airway obstruction is from an extrinsic cause (Pelton and Ratner 1989). The rigid bronchoscope provides a secure airway permitting excellent control of oxygenation and at the same time creates a channel through which a number of instruments can be passed. Apart from that, the commonly used surgical techniques in the trachea are primary end-to-end anastomosis and tracheal sleeve resection. In patients in whom airway obstruction is unresectable, stent insertion is preferred.

It is to be emphasized that as far as the children are concerned when they present with acute airway obstruction, endotracheal intubation is the preferred option even in an emergency situation. They should be preferably intubated while being awake and breathing spontaneously.

H: **Clinical outcome and survival in carcinoma of the larynx**

Although most early lesions can be cured by either radiation or surgery, radiation therapy may be a reasonable choice to preserve voice, leaving surgery for salvage.

Total laryngectomy in combination with postoperative radiotherapy affords a longer period of survival for advanced laryngeal cancers as compared to radiotherapy alone or total laryngectomy alone or no treatment (Iseh 2011).

Surgery and postoperative radiotherapy can produce substantial long term cancer control and survival for patients with T4 larynx cancer. Caution should be exercised in selecting patients for initial non-surgical treatment because of significant rates of functional impairment despite survival equivalence (Rosenthal et al. 2015).

Rehabilitation may be required after either surgical or non-surgical treatment. Significant swallowing problems are common after chemotherapy and radiotherapy, and some patients may require esophageal dilatation, swallowing therapy, or in severe cases, surgical replacement of the pharynx or gastrostomy tube feedings.

Body mass index has been found to be a prognostic factor for survival, independent of primary site, and tumor stage (Takenaka et al. 2015).

Similar outcomes for head and neck cancer patients treated with surgical and non-surgical approaches have been reported (Soo et al. 2015). Again in another study, no difference in outcome, nor a slight advantage favoring surgery plus radiotherapy compared with concurrent chemoradiotherapy for advanced, non-metastatic SCC of the head and neck could be found (Iyer et al. 2015).

8.6 A Philosophical Overview of Psychotherapy

If we take a brief glance at the prevailing literature, it dawns on us that the words 'psychotherapist' and 'counsellor' are often used interchangeably, with uncertain clarity of definition thus leaving the reader to draw his own conclusions as far as these and similar terms or terminologies are concerned. Customarily counsellors/therapists train with in specific schools, adopting preferred models, according to favorite theories and theoreticians. But the main question that arises in the deepest recesses of one's brain is that what exactly are the intellectual foundations of those theories. How are those theories that are being put forward by therapists intellectually grounded? These are the sort of questions applied philosophy attempts to answer within the context of psychotherapy.

Before going further, it would be better to look at the Oxford Dictionary and dig out the meanings of these words to enable the reader to easily grasp the rest of the chapter. Philosophy is the 'study of nature and meaning of the universe and of human life'. Psychology is the scientific study of the mind and the way it influences behavior. Psychotherapy on the other hand is a branch of medicine related to the treatment of mental illness by discussing the problems with the subject or patients other than by giving them drugs (Oxford Advanced Learners' Dictionary 2000).

The relationship between the counsellor and the client is dialogical and dialectic, and not authoritative and hierarchical. The counsellor thus gets into the depth of the problem through discussion and logical argument. The counsellor thus aims to assist the client to think through their situation and replace the problem with a thorough understanding and philosophical insight. Thus philosophical counselling is essentially a dialogue and attempts to divert our attention away from medicine and so we fail to be convinced that this 'beyond method' eventually constitutes a form of therapy. It could be interpreted as an insight, self-understanding, intellectual balance but not therapy because we as physicians have a total different concept of therapy. Philosophy and to be more exact philosophical counselling is an altogether different discipline that requires a healthy, radically active intellect. How a dejected, traumatized and a mentally ill patient will meet the requirement is far from clear and remains the crux of the conundrum. Thus philosophical counselling negates the fundamental fact that some of the patients need medicine. Moreover, it is very difficult to conduct philosophical counselling in a frenzied, dionistic state of mind. It could be interpreted differently that an interdisciplinary does exist between philosophical counselling and psychological counselling and demarcating the two would be as difficult as removing the wheat from the chaff. A number of methods of psychological counselling, such as cognitive therapy and rational motive behavior therapy, deal extensively with philosophic ideas, albeit not necessarily in an explicit fashion.

To clarify it further, there are some problems encountered in life that are clearly medical problems where as others are labelled educational problems. Thus on one side of the boundary is the terrain of the therapist, and on the other side of that boundary is the domain of the philosopher. Despite these succinct and definite

demarcations and the fact that there does exist an interplay between philosophical counselling and psychotherapy, it has been upheld by some that philosophy alone, without any kind of interdisciplinary, can claim a therapeutic status. If we go through history, we find that philosophers from Pythagoras to the contemporary deconstructionists, have a lot to teach therapists about the meaning, healing and wisdom in life. If therapists try to learn from philosophers, they can develop a broader deeper vision of therapeutics and its actions. The commonly held notion is that a therapist is just listening. It alone would not fetch us more because listening cannot take place without utilizing the ideas, experiences and values that matter to us (Howard 2000).

Philosophers are primarily interested in critique and not in health. Psychotherapists have an altogether different objective i.e., to guide and heal by means of anthropologies which are not necessarily drawn on the time—honored philosophical goal of securing epistemological and metaphysical foundations.

Psychotherapy is being regarded as a treatment of psychologically based disorders while 'biologically based disorders' should principally be treated with medicine. This view is related to a Cartesian dualism that splits people into a mind and a brain. What we call mind can be misunderstood as the activity of the brain (Andreasen 1997). Mental phenomena arise from the brain, but subjective experience also affects the brain.

8.7 Conventional Treatment Modalities for Cancer

Treatment modalities differ from patient to patient and from cancer to cancer. The commonly employed definitive treatments include chemotherapy, radiotherapy and surgery either singly or in combination and vary depending upon the stage and size of the tumor. Definitive treatment uses medical procedures (i.e., radiation) only as an attempt to cure the condition. Concomitant treatment combines chemotherapy and radiation with another treatment, usually surgery. Adjuvant treatments on the other hand are those therapies that follow surgery in an attempt to improve the outcome and constrain or prevent the cancer in spreading locally or else spreading its tentacles to other parts of the body. However each treatment has its own advantages and side effects. Radiation therapy for instance ushers in adverse effects such as mucositis, xerostomia, sensory changes in taste and smell, fibrosis, neuropathy, changed anatomy, odynophagia, loss of appetite, edema, infection, dental changes, all of which can eventually lead to yet another and perhaps a devastating complication i.e., dysphagia.

Chemotherapy also has its own side effects such as nausea and vomiting, loss of appetite, mouth sores, diarrhea, hair loss, increased vulnerability to infections, bleeding problems and a perpetual feeling of fatigue and tiredness.

The palliative treatment as the term signifies brings palliation or relieves symptoms that are bothersome. For instance, patients with cancer often complain of excruciating bone pain, and radiation might be of value to relieve this type of pain.

Similarly, patients whose airway has been encroached by a tumor resulting in blocking of the airway might benefit from a course of chemotherapy causing shrinkage of the tumor and thus providing a free passage of air during inhalation and exhalation. Although chemotherapy is commonly employed as a singular treatment, it is often an adjuvant treatment to surgery or radiation therapy. Radiation likewise may be given before surgery to shrink the size of the tumor or can be employed after surgery to diminish or else eradicate the remaining cancer cells.

Biological therapy also called immunotherapy uses the body's immune system to stop or suppress the cancer growth. It involves interferons, interleukins, colony stimulating factors, monoclonal antibodies, and vaccine gene therapy to defend the body against attacks from cancer cells.

The problem of fatigue and loss of energy affects up to 70% of cancer patients during chemo and radiotherapy or after surgery (Blesck et al. 1991). The detrimental effects of fatigue on patients' QOL are clearly discernable. Patients are thus inclined to take rest and since inactivity induces muscular catabolism, prolonged rest in fact perpetuates fatigue. Aerobic exercise has been shown to reduce fatigue and increase effectors cells which play a role in natural immunity against cancer cells (Johnsdottir et al. 1997; Nieman et al. 1995). Patients with terminal cancer experience agonizing pain and it has been confirmed that half of these patients receive inadequate analgesia (Cleeland et al. 1994).

8.8 Surgeon's Appreciation of Expression Profiling or Next Generation Sequencing!

When Robert Hooke published micrographia for the first time in 1665, he explained his observations through different lenses and coined the biological term cell (West 2014). He revolutionized the field of biology and our understanding of the Universe, as Galileo Galilei did through telescope (Burns 2010). The data provided by the new instruments were incomparable to traditional scientific information gathered through the history of human (West 2014; Burns 2010). For decades light and electron microscopy, has been the basis for cell study and morphological classification of cancers (Pollo 2012; Malzkorn and Reifenberger 2016).

Considering glioma as the most prevalent primary brain lesion, more than 16 morphological subtypes exist for high grade glioma (Figarella-Branger et al. 2010). The biologic behavior, natural history and prognosis of many cases could not be explained by routine classifications (Malzkorn and Reifenberger 2016; Figarella-Branger et al. 2010; Karsy et al. 2012). Several cases of high grade glioma are reported with favorable outcome; however, some of the low grade tumors even in young patients would lead in short overall survivals (Figarella-Branger et al. 2010; Karsy et al. 2012). Furthermore, the response to uniform adjuvant therapies varies widely among the same pathologies (Figarella-Branger et al. 2010; Karsy et al. 2012). Several prospective studies reported different presentation of

biomarkers and genetic alterations in the same pathologies, which proposed to have causative, diagnostic or prognostic values (Figarella-Branger et al. 2010; Karsy et al. 2012; Matthew et al. 2012).

Nowadays, genetics are integrated into the classification of brain tumors and other cancers (Malzkorn and Reifenberger 2016; Figarella-Branger et al. 2010; Karsy et al. 2012; Matthew et al. 2012). They may summarize morphologic classifications or provide subgroups with different prognosis or treatment responses (Malzkorn and Reifenberger 2016; Figarella-Branger et al. 2010; Karsy et al. 2012; Matthew et al. 2012). Some of the biomarkers (gene protein products) or genetic mutations even overcome the treatment plans, regarding prognosis. IDH1 mutation, MGMT methylation status and 1p/19 q co-deletion are some of the most well-known and informative genetic changes that determine treatment planning of brain tumors, including extent of resection and adjuvant therapy regimen (Malzkorn and Reifenberger 2016). Even specific kits are designed which include up to one hundred of genetic mutations or biomarkers involved in a neoplasm, such as glioma. Therefore, beside routine pathology a very specific genetic profile of individualized tumor could be available, which would be used for individualized treatment of each patient as a part of personalized medicine (Karsy et al. 2012).

At World Health Organization (WHO) classification of brain tumors 2007, the known genetic alterations were not included in classification, due to the lack of equipment in developing and underdeveloped countries (Malzkorn and Reifenberger 2016). At present, Immunohistochemistry (IHC) could be available easily. Therefore, due to the enormous bulk of evidence provided since 2007, genetic based guidelines and integrated treatment algorithms are developed and the classification includes the most significant biomarkers and genetic mutations (Malzkorn and Reifenberger 2016).

The surgeons do not seem to be interested at such more complicated treatment planning. Furthermore, the costs of Next Generation Sequencing (NGS) are considerable and such facilities are not easily available, even in the academic centers (Malzkorn and Reifenberger 2016; Malekzadeh et al. 2009). Besides, the health insurances do not seem to have any interest in coverage of the costs (Malekzadeh et al. 2009).

Iran as a developing country is in epidemiological transition from communicable to non-communicable diseases (Malekzadeh et al. 2009). Although, cancer is the third cause of death in Iran, its mortality is on the rise during recent decades. As an example, the high frequency of positive family history of colorectal cancer in Iranian patients indicates that a significant number of colorectal cancers in Iran arise in family members and relatives of colorectal cancer patients (Malekzadeh et al. 2009). It is now clear that colorectal cancer develops as the result of genetic and epigenetic alterations that lead to malignant transformation of normal mucosa. In spite of these scientific progresses and the fact that screening can reduce the rate of death by detecting early cancer or premalignant polyps, the rate of screening is very low globally and negligible in Iran and many other developing countries which is due to cost, resistance by physicians, patients, and the healthcare system (Malekzadeh et al. 2009).

Laryngeal cancer is one of the most common malignant neoplasms of the head and neck and the most common malignant tumor in the upper aero-digestive tract (Saedi et al. 2009; Azarpira et al. 2011; Gan et al. 2013). Squamous cell carcinomas arising in the glottic region are the most common of all laryngeal cancers and more prevalent than the supraglottic ones (Saedi et al. 2009). But this pattern is reverse in some countries (Saedi et al. 2009; Azarpira et al. 2011). Tumors originating from different regions of the larynx have their own clinical behaviors and are also associated with different risk factors. Iranian population has the highest rates of esophageal squamous cell carcinoma in the world; poor diet, genetic susceptibility and opium were suggested as main risk factors (Saedi et al. 2009; Azarpira et al. 2011).

Recent discoveries in the molecular biology of Head and Neck Squamous Cell Carcinoma (HNSCC), and of laryngeal carcinoma in particular, can provide insight to understanding the molecular basis of these cancers, which can lead to the development of early diagnostic and targeted therapeutic strategies aimed to improve clinical outcomes, and possibly survival, in this patient population (Gan et al. 2013; Loyo and Pai 2008; Jaworowska et al. 2007). A genetic progression model can also guide the development of novel treatment strategies through gene therapy and the replacement of altered genes with their wild-type phenotype (Loyo and Pai 2008). Laryngeal cancer has a particular pattern of p53 mutations as compared with other regions of the head and neck (Gan et al. 2013; Loyo and Pai 2008; Jaworowska et al. 2007). The laryngeal pattern is more consistent with that of lung cancer, and it shows lower mutation indexes (35.4% vs. 60%) with different concentrated regions of p53 mutations (Loyo and Pai 2008).

Molecular diagnostics can detect abnormalities in lesions not yet appreciated histologically and may help in the detection of minimal residual disease or early recurrences (Loyo and Pai 2008). Specific molecular patterns can establish a tumor's behavior and help to guide patient management (Loyo and Pai 2008; Jaworowska et al. 2007). Patients who have less aggressive tumors may safely undergo treatment with organ preservation, whereas patients who have more aggressive tumors may benefit from extended surgical or chemo-radiation approaches (Gan et al. 2013; Loyo and Pai 2008; Jaworowska et al. 2007). With a better understanding of the molecular genetics and epigenetics of laryngeal cancer, novel targeted therapies for HNSCC can be translated into the clinical arena (Loyo and Pai 2008).

It is strongly suggested to use integrated classification in diagnosis and treatment planning of tumors. Genetic data should be provided beside routine pathology. Furthermore, the patients' knowledge is growing and a great amount of information is provided online. Hence, the patients expect receiving more information about the prognosis. There is no other option for surgeons, except in accepting genetic based protocols.

8.9 Psychosocial Interventions for Cancer Patients

Cancer can cause multiple impairments, limitation in activities, and participation restrictions. Cancer rehabilitation helps the patient to maintain maximum physical, social, psychological, and vocational functioning within the limits imposed by the disease and its treatment (Cromes 1978). There is empirical evidence suggesting that group psychotherapy is effective in ameliorating psychological stress. There is compelling evidence that group intervention is effective in reducing mood disturbance and pain, and in improving QOL.

The overall effectiveness of psychosocial interventions such as psychotherapy has been regarded with some degree of skepticism because of their non-acceptability in cancer patients and inconsistency that prevails in the reports.

Whether psychosocial interventions can improve health outcomes? In this arena, research remains tentative in its ability to provide a definite answer and conclusions reached so far fail in solving the dilemma (Nezu et al. 2003). Some studies have been well cited and were found to have positive effects of psychosocial interventions on survival rates of cancer patients initially (Fauzy et al. 1990; Spiegel et al. 1981) but were never replicated and it appears as if they are non—reproducible (Coyne et al. 2007).

Others have come up with still dismal results and state that it is premature to bring forward a concrete and conclusive statement about this issue (Smedslund and Ringdal 2004).

Again in another meta-analysis by another group of researchers it has been stated that psychosocial distress is associated with higher cancer incidence, poorer survival, and higher cancer mortality (Chida et al. 2008).

This latter study reflects that further research focused on biological and health outcomes secondary to psychological intervention is needed.

In another study, the researchers concluded that their intervention had positive effects in terms of improving health outcomes measured at 12-months assessment point (Andersen et al. 2007). In a similar study, data were collected in patients with recurring breast carcinoma and it showed that patients in the intervention group showed a reduced risk of death following recurrence coupled with significantly higher immune indices (Andersen et al. 2010). There appears to be a great degree of optimism in these findings but the exact impact of psychological intervention on the survival of patients with cancer needs further exploration. Some researchers have gone even further and questioned the methodology labeling it to be flawed (Coyne et al. 2007). Some are even pessimistic and doubt whether psychological intervention had any survival benefits (Coyne et al. 2009).

The question arises as to who should be subjected to psychotherapy because each patient is a different entity and furthermore different cancers have different prognosis and outcome. It has been suggested that the patient who is hopeless, overwhelmed, lethargic, and carries a flat affect should be considered for referral for psychotherapy. These patients may also benefit from anti-depressant medications (Tefler and Shepherd 1993).

In the literature, two studies are commonly cited which claim that psychotherapy promotes survival (Fawzy et al. 1993; Spiegel et al. 1989). But three meta- analysis have failed to find an overall effect of psychotherapy on survival (Smedslund and Ringdal 2004; Chow et al. 2004; Edwards et al. 2004). Such claims that psychotherapy extends survival in cancer patients could best be termed an illusion as they lack scientific credibility and an authoritative evidence. Based on the results of three meta-analyses, definite conclusion regarding the efficacy of psychosocial interventions in prolonging cancer survival seems premature (Smedslund and Ringdal 2004).

Having said that, it appears that the debate would continue and remain unabated. Where exactly do we stand at the moment? Shall we make a categorical statement that psychotherapy does not extend survival based on the body of existing evidence, or shall we keep our patients in the fond hope that psychotherapy in fact does prolong survival? With more than two decades since the initial studies were published and with the available evidence at hand, the time is ripe enough to provide a more authoritative statement about the efficacy of psychotherapy on survival and declare that it works or else state that psychotherapy has no role what so ever in extending survival in cancer patients. Extending survival is synonymous to death prevention and delaying death or preventing it by means of psycho interventions at least sounds like a big talk.

Another argument that brewed up among researchers centered on the premise that psychotherapy was deleterious for patients with cancer participating in such sessions or courses (Spiegel and Giese-Davis 2004). There also has been no systematic study to determine whether participation is in fact benign for all the participants (Spiegel et al. 1989). There has been some evidence of negative effects of participation among women with breast cancer, including declines in self-esteem, body image and increased preoccupation with cancer (Helgeson et al. 1999).

If no harm is inflicted, taking part in weekly sessions for a period of a year in itself is stressful (Goodwin et al. 2001). Such a stress on a patient who is counting the last days of his life and all the more when the entire ordeal is deceptively expected to increase survival when in fact it does not is perhaps alarming. How can patients be deceived that participation in such sessions would increase their survival when in reality no such thing is happening or in the offing? Despite an exhaustive research for almost two decades, the idea that psychotherapy prolongs the survival of patients with cancer is “inherently improbable” (Spiegel 2004). The impetus for this long research of two decades was provided in fact by two trials (Spiegel et al. 1989; Fawzy et al. 1993) in which the investigators claimed a strong effect on survival. There has been a great deal of skepticism about these two trials as they lack evidence for a mechanism by which psychotherapy should influence survival rate and secondly if psychotherapy were to improve survival, a great deal of pain and suffering could also have been ameliorated and avoided (Coyne et al. 2007). I very well remember a patient who was weary, befogged in mind and fatigued in body. He raised his voice in a wail of expostulation and dismay although under a full umbrella of psychotherapy. Should we start developing an erroneous concept that the mind and the brain are separate entities and not inseparable as being

claimed so far! If the patient experiences the worst form of fatigue despite attending sessions of psychotherapy, it reflects that the entire research about psychotherapy has ended up in a fiasco.

The two landmark publications (Fawzy et al. 1993; Spiegel et al. 1989) which claimed an improvement in survival with psychotherapy ignited an uproar among researchers and the medical community because it was hard to believe that mere attendance in classes of psychotherapy and group discussion could help in the longevity of life. As later research failed to replicate these findings, there prevailed skepticism and thus fingers were pointed towards methodological errors, selection bias, and type of interventions. At present, the debate has not abated as to whether psychosocial interventions prolong survival in cancer patients or not.

8.10 Mechanisms of Psychotherapy

If appears to be the least understood branch of psychotherapy and this is what the researchers are interested in and need extensive exploration. The possibility that psychotherapy could extend survival time for cancer patients has attracted attention among clinical investigators interested in the mind-body connection, among cancer patients seeking the best possible outcome and among the general public.

The inquisitive researcher is interested to find plausable answers to the following questions that are often being asked:

(1) Can emotional support affect the course of cancer or in other words bring about an improvement in survival in these patients?
(2) What physiological pathways are involved to mediate such an effect?
(3) Do psychosocial interventions inspire us to change the standard of care for cancer patients?

The notion held at present is rather pessimistic and the mechanisms of psychotherapy at the brain level are largely speculative. If psychotherapy is regarded as a form of learning, then the learning process that occurs in psychotherapy may produce alterations of the gene expression and thereby alter the strength of synaptic connections (Gobbard 2000). Rats groomed in an environment which requires complex, learning to survive have significantly greater number of synapses per neuron compared to rats raised in isolation (Greenough et al. 1987). It shows that the structure of the brain is dynamic and it possesses plasticity (Kandel 1998). Environmental factors affect gene expression, suggesting different interactions of children of the same family with their parents (Reiss et al. 1995).

The goal of psychological intervention is to help cancer patients and their families optimize health care and manage the psychological, behavioral, and social facets of cancer and to promote improved health. In this goal, there is no mention of increased survival or increasing the life span. Unfortunately the multiple complexities involved in conducting research with cancer populations have contributed to inconsistent reports about the overall effectiveness of psychosocial interventions.

Such extraordinary findings, in part, have led researchers to believe that psychological interventions targeting distress in cancer patients are ineffective, as well as not accepted by patients (Coyne and Lepore 2006; Coyne et al. 2006).

They contended that psychosocial interventions were ineffective and unaccepted by patients, pointing to confirmatory bias and methodological flaws of past decades (Coyne and Lepore 2006). In defense of psychosocial interventions, the opposing researchers pointed to various qualitative and quantitive reviews that found a sufficient "preponderance of evidence", citing in particular five Randomized Clinical Trials (RCTS) that demonstrated positive and sufficient results (Manne and Andrykowski 2006). While Coyne and colleagues (Coyne and Lepore 2006) recognized that an RCT by Nezu and colleagues (Nezu et al. 2003) was in fact one such methodologically sound study that did support the efficacy of a particular psychosocial intervention, they considered this to be a rare occurrence and labeled it a 'black swan'.

Since this debate, additional RCTS have been published that provide evidence for the effectiveness of psychosocial interventions. Several reviews and meta—analyses have examined overall effectiveness but continue to report varying sizes. Although researchers have improved the methodology of their studies to comply with CONSORT guidelines, criticism about effectiveness still remains (Coyne et al. 2009).

8.10.1 Psychoeducational Interventions

Psychoeducational interventions aim to reduce uncertainty, feelings of inadequacy, confusion, helplessness, and loss of control by supplying information about the disease, and resources available to cancer patients. Psychoeducational components have also been used in combination with other psychosocial interventions such as psychotherapy. Educational and informational interventions represent one fifth of past research (Moyr et al. 2009).

In their exhaustive review they (Jacobsen and Jim 2008) found out that psychoeducational interventions were effective in preventing or else relieving depression and anxiety in both newly diagnosed patients and in those undergoing surgery or chemotherapy. The potential benefits of psychoeducational interventions were perhaps attributed in curtailing the fear through preparation for treatment and information imported about the way in coping with the stress and treatment for cancer.

8.10.2 Cognitive Behavioral Interventions

These include relaxation, training, coping skills training, and cognitive restructuring. These interventions considerably ameliorate distress and promote well-being. Behavioral techniques such as distraction, desensitization, and hypnosis reduce

Anticipatory Nausea and Vomiting (ANV) in cancer patients (Holland and Alici 2010). ANV is an extremely unhappy feeling commonly seen in patients undergoing chemotherapy.

Both muscle relaxation and systemic desensitization procedures have been of considerable help in preventing and treating ANV (Hofman et al. 2007). Cancer patients experience great pain and Cognitive Behavioral Interventions (CBI) have been found to reduce pain to a certain degree (Tatrow and Montogomery 2006). CBI has also been found to improve emotional outcomes thus improving QOL (Jacobsen and Jim 2008). Fatigue is a very distressing symptom experienced by cancer patients especially those in whom the disease is advanced. Others also have found promising results with CBI (Kangas et al. 2008). Problem solving therapy alone was not efficacious, but combining it with anti-depressant medications improved the efficacy (Katon and Seelig 2008). During the terminal stages of cancer, the prospects of the patients are dismal and dreadful, and this you can easily conclude. The worn out faces of these patients lying in the wards and intensive care units fully index their ages, and indeed more than their ages. Some of these patients were in a state of tension which was indescribably more distressing than pain itself.

At times you could see the cloud and yet unable to see the storm. A pain and loss of hope of a great magnitude can usher in a multitude of events such as suicidal attempts and opting for voluntary euthanasia in the minds of these patients to bring an end to their perpetual and persistent sufferings and agonies. When the prospects are dismal and the hope for survival is minimal, the issues of do not resuscitate and euthanasia creep in and it is at such critical occasions that decisions are hard to be made (Khan 1995).

8.11 Immune-Stimulating Viral Therapy

Viruses are usually thought of as agents of disease. Scientists believe that virus can help thwart cancer, a development that could herald a new age of viral therapies. Lmlygic, a modified version of the herpes virus attacks the cancer and sparks the immune system into action against tumors. It has been approved by the Food and Drug Administration (FDA) and has been seen in some clinical trials in causing remission with few of the nasty side effects common to existing treatments. This field is in its very embryonic stage, and this would be another arrow in the quiver that oncologists use.

8.12 Future Trends

Having said that what appears above, it appears that the debate would continue and remain unabated. A lot of research findings do exist but they hardly provide us a clue as to which type of treatment approach is effective for a definite type of cancer,

stage of cancer or the nature and level of stress. Since these and a host of other variables vary from patient to patient and from one ethnic or social background to another, future research should preferably be targeted to answer these questions and many other questions that may sprout again as flaws in methodology are unravelled.

The research conducted so far has failed to provide an unequivocal answer to the effects of psychosocial interventions on survival of cancer patients. The literature so far has been indefinite in providing an unflinching answer to the often raised question as to whether psychosocial interventions do have an impact on the survival of cancer patients or not. Perhaps it would be hard to answer this question as it embodies a plethora of variables, nevertheless if answered in concrete and definite terms would save billions of dollars in terms of research savings and at the same provide a solace and appease the mind of those who are living in a world of utopia.

Unfortunately, a great number of research studies have obvious methodological limitations and as such are hard to be accepted on their face value. It is to be re-emphasized that future researches should try to improve the methodological rigor of the research. As a researcher you could be wrong in your conjecture although it may be placed on strong and forceful evidence. It appears that the deductions reached so far from contemporary research appears to be farfetched and exaggerated.

Moreover, many studies report an improvement but fail to pin-point as to which treatment component did bring about the actual improvement in symptoms.

Unless adequately highlighted and spotted, it would be exceedingly difficult or perhaps impossible to replicate or reproduce these studies. Recently, a novel gene-editing technique has been used to eliminate an infant's aggressive cancer, a small but significant step in the quest to treat deadly diseases by altering the human genome. When conventional treatments including chemotherapy and a bone-marrow transplant failed to be of any benefit, the girl was given just weeks or months to live. The doctors then used gene editing to make special cells that would destroy the cancer. A few weeks after the designer cells were injected, her leukaemia disappeared. Three months later, it remains at bay. This treatment has been tried on only one patient so far, with limited follow-up time.

In gene editing, certain molecules are used as tiny scissors to cut and fix a broken gene in a cell. The hope is to edit out faulty pieces of DNA and thereby treat a genetic disease.

This research has not yet been peer-reviewed or published in a journal but it passes on a message of optimism to researchers that gene editing is credited with eliminating an infant's aggressive cancer in the city of London.

In the end, I may add that we cannot understand everything at once and we cannot begin directly from perfection. We must first of all fail to understand a great many things (Khan 2014). This holds true for psychotherapy in cancer patients as well. We should not theorise before we have all the evidence because it biases our judgement. Lastly, as researchers we can't be acquitted of the blame in conducting a wrong research.

8.13 Conclusion

Many advances have been made in tackling the diabolical entity of cancer, and although scientists and researchers have made giant leaps in this regard, there remains a lot to still achieve in this apparently dark and fathomless area and domain of cancer.

8.14 Summary

Surgery, chemotherapy and radiotherapy have been regarded as the accepted modalities of treating cancer in general and cancer of the airway in particular. In the not very distant past, some studies promulgated the idea that psychosocial interventions including psychotherapy had a significant effect in promoting survival of cancer patients. Psychological interventions did produce or could produce favourable effects on some psychological outcomes such as anxiety, depression and mood disturbance, but there is no evidence so far that it was of help for an improvement in survival.

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Chapter 9
Female Reproductive System and Cancer

Natalia Jančárková

Abstract This chapter focuses on the main aspects of the issues relating to gynecological malignancies and their reflection in the psychosocial context. It gives an overview of gynecological cancers including epidemiological, histopathological, and clinical characteristics, as well as diagnostic methods and therapeutic approaches. Attention is given to the prevention of malignant tumours of the reproductive system on a general level, common for most of malignancies, at the same time focusing on some special options. The others mentioned above include HPV vaccination, cervical cancer screening with Pap smear tests, as well as genetic counseling of individuals with hereditary cancer susceptibility. Psychoneuroimmunological and psychosocial factors on cancer risk and survival are analyzed with regard to the specifics of women's health.

Keywords Psychosocial aspects of gynecological cancer · Pregnancy and cancer · Hereditary cancer susceptibility · Precancerous lesions and prevention

Abbreviations

ACOG	American College of Obstetricians and Gynecologists
AML	Acute myeloid leukaemia
ANS	Autonomic nervous system
BSO	Bilateral salpingo-oophorectomy
CIN	Cervical intraepithelial neoplasia
CIC	Cortical inclusion cyst
DIC	Disseminated intravascular coagulation
EGFR	Epidermal growth factor receptor
FAMM	Familial atypical multiple mole melanoma
HBOC	Hereditary breast and ovarian cancer syndrome
HCG	Human chorionic gonadotropin

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HG-CGIN	High-grade cervical glandular intraepithelial neoplasia
HLA	Human leukocyte antigen
HNPCC	Hereditary non-polyposis colon cancer syndrome
HPA	Hypothalamic-pituitary-adrenal
HPV	Human papillomavirus
HSIL	High-grade squamous intraepithelial lesion
IL	Interleukin
LOH	Loss of heterozygosity
LSIL	Low-grade squamous intraepithelial lesion
MMR	Mismatch repair
NHI	National Health Institute
NK	Natural killer
PNI	Psychoneuroimmunology
SCC	Squamous cell carcinoma
STIC	Serous tubal intraepithelial carcinoma
VAIN	Vaginal intraepithelial neoplasia
VEGF	Vascular endothelial growth factor
VIN	Vulvar intreapithelial neoplasia
VLP	Virus-like particle

9.1 Introduction

The gynecological malignant tumours, together with breast cancer are among the most frequently occurring malignant tumours, affecting women of all age groups. Of all disease, cancer is the one that has the most formidable psychological impact. It spells not only death and a progressive painful approach to it, but also mutilation. The threatened female identity concerning of the fertility role and life expectations is another aspect occurring in women suffering of malignancies of the reproductive system.

A general rise in the incidence of the malignant tumours becomes a typical characteristic of our contemporary world. The attitudes to cancer vary considerably in different parts of the world, depending on cultural, ethnic, social, economic and educational factors, among other. Cancer represents a large group of diseases, in which the treatment of the disease, as well as the disease itself, may result in profound disfigurement and disability. Self-image may be seriously altered. The individual is faced with the necessity to learn to live with the uncertainty.

There are considered many different reasons of cancer, especially environmental and civilizing factors, prolonging of human age, life style factors including geographic variations. In general, so called risk factor is anything that increases the chance of tumour development in the individual. The risk factors usually do not represent the direct cause of tumour development. However, their knowledge can help to understand etiopathogenesis of some tumours. They can be used in

diagnostic process and screening programs. Last but not least they can be useful in primary prevention care. The prognostic factors, on the other site, reflect biological signs of tumour (type, grading, molecular genetics, etc.), spreading of tumour (stage) and patient's ability (expressed as performance status) to cope with tumour and relevant treatment. We distinguish prognostic factors of tumour, of host and environment. The identification of the cause of a given cancer due to the complex nature and multistage process may not be sufficient to provide significant preventive measures. Early and accurate diagnosis of tumour disease is necessary for the effective individualized and targeted therapy, with in important impact on mortality and improvement of prognosis and quality of life of cancer patients (Hossfeld et al. 1990; Barakat et al. 2013).

The possibility that psychotherapy could extend survival time for cancer patients has attracted attention among clinical investigators interested in the mind-body connection, among cancer patients seeking the best possible outcome and among the general public. A small number of randomized trials have been conducted and they have produced conflicting results. No studies show an adverse effect of psychotherapy on cancer survival. Stress and support have been thought to be related to cancer risk and progression, but evidence has been mixed. Depression is a natural co-morbid condition with cancer. It has not been clear how stress and support could physiologically affect the rate of cancer progression. Immune function was not thought to have much relevance to cancer progression. Few other physiological mechanisms linking stress to cancer progression were known. There is evidence indicating that effective psychosocial support improves quantity as well as quality of life with cancer. There is evidence that chronic depression predicts poorer prognosis with cancer. Dysregulated circadian cortisol patterns predict more rapid cancer progression. Inflammatory processes affect cancer growth and progression. Sympathetic nervous system activity, telomere length, telomerase activity, and oncogene expression are affected by stress and can affect cancer growth (Spiegel 2002, 2014). There is a need for investigating the interactions between the medical, psychosocial and health behavior components of intervention programs, as several published studies indicate reduced mortality among patients who engage in physical activity and change to a healthier diet (Boesen and Johansen 2008).

9.2 Epidemiology, Etiology, Clinical Symptoms, Diagnostics, Histopathology

9.2.1 Malignant Tumours of the Vulva

Vulvar cancer is a rare genital neoplasm occurring predominantly in postmenopausal women. Malignant tumours of the vulva account for 3% of all female genital cancers and approximately 1% of all malignancies in women. It mainly includes two different types of carcinomas. Keratinizing carcinomas predominate in

older women and have little association with human papillomavirus (HPV) infection. Conversely, the majority of warty and basaloid carcinomas are caused by HPV (Prat et al. 2014). Although the incidence rate of vulvar precancerous lesions (high-grade squamous intraepithelial lesion—HSIL, lichen sclerosus) is increasing, that of squamous cell carcinoma of the vulva—the most frequent type of vulvar cancer is not following this trend, reflecting earlier detection and more successful treatment. In addition to human papillomavirus infection, cigarette smoking and sexual transmitted diseases are a putative risk factor for vulvar squamous carcinoma. The squamous cell carcinoma associated with lichen sclerosus involving vulva are usually of the keratinizing type and is not with human papillomavirus (HPV) associated. Other important epithelial malignancies of the vulva as Paget disease and Bartholin gland carcinoma are much less common than squamous lesions. Prominent non-epithelial tumours are malignant melanoma and embryonal rhabdomyosarcoma, metastatic tumours are very rare. The risk factors and the specific etiology of most vulvar epithelial tumours are largely unknown. Important role in prognosis of patients with vulvar cancer has performance status and their age, staging and grading of the tumour and optimal treatment modality. The worth prognosis is described in non-HPV associated tumours (Hoskins et al. 2000; Tavassoli and Devilee 2003; Kurman et al. 2014). The directed biopsy of identified lesions is a method of primary diagnosis. Imaging studies including ultrasound, computer tomography or magnetic resonance are employed to evaluate pelvic and paraaortic lymph nodes. The detection of the sentinel lymph node may reveal micrometastases. Rectoscopy or cystoscopy are additional methods which are useful according the location of the tumour.

Squamous cell carcinoma is usually solitary and may present as an ulcer nodule, macule or mass. An ulcerated area is the most common findings, bilateral or even symmetrical lesions are not rare. About two thirds of patients complain of vulvar pruritus, which quite often occurs several years before the onset of cancer and is associated with precancerous lesions. Pain, bleeding, discharge are other symptoms. Adenopathy in the case of involved regional lymphonodes may be presenting symptom, though this is rare. About fifty percent of vulvar cancers are preceded by states that are more or less multifocal precancerous lesions. Squamous cell carcinoma—over 90% of all vulvar carcinomas—is an invasive neoplasm composed of squamous cells of varying degrees of differentiation. Several morphological variants have been described: keratinizing, non-keratinizing, basaloid, warty, verrucous. Basal-cell carcinoma is a slowly growing, locally invasive but rarely metastazing tumour. Squamous cell carcinoma has an increasing incidence with age. One group of tumours is linked to high risk HPV, cigarette smoking and high-grade intraepithelial lesions–HSIL (vulvar intraepithelial neoplasia 2/3), the second one to chronic vulvar inflammatory disorders (lichen sclerosus, lichen planus). Up to 6% of patients with clinical lichen sclerosus develop carcinoma and this risk is higher in symptomatic postmenopausal women. A third group, verrucous carcinoma, is occasionally linked to low-risk HPV types 6 and 11, but most are HPV negative.

Bartholin gland carcinoma presents as an enlargement in the Bartholin gland area that may clinically resemble Bartholin duct cyst. The tumour is typically solid,

various types of carcinoma have been described as adenocarcinoma, adenosquamous carcinoma, adenoid cystic carcinoma, transitional cell carcinoma. Vulvar Paget disease, adenocarcinoma of Skene gland origin, and phyllodes tumour with mammary-like epithelium are the other vulvar *gland carcinomas*.

Embryonal rhabdomyosarcoma (sarcoma botryoides) occurs almost exclusively in children younger than 10 years. Other *soft-tissues tumours* (liposarcoma, leiomyosarcoma, dermatofibrosarcoma protuberans) are extremely rare.

High-grade neuroendocrine carcinoma is a rare tumour exhibiting neuroendocrine differentiation, its incidence increases with age. The occurrence is significantly higher in immunosuppressed individuals (HIV-positive or those who have had organ transplantation). *Germ cell, neuroectodermal tumours (Ewing's sarcoma), lymphoid and secondary tumours* occasionally spread to the vulva.

Malignant melanoma, a malignant tumour arising from melanocytes, is the second most common malignant neoplasm of the vulva and accounts for 5–10% of all vulvar malignancies. Approximately 3% of all melanomas in women arise in the female genital tract. Melanomas of the vulva are likely to arise via an ultraviolet radiation-independent pathway. Symptoms include vulvar bleeding, pruritus and dysuria. Although vulvar malignant melanoma usually presents as pigmented mass, one third are non-pigmented ones. Tumour presents as a nodule or polypoid mass. Three histological types of melanoma are identified: superficial spreading, nodular and mucosa/acral lentiginous. Approximately 25% of cases are unclassifiable. Genetic alterations in RAS-related pathways are present in most acral/mucosal lentiginous melanomas. Late stage melanomas overexpress epidermal growth factor receptor (EGFR). Patients with familial atypical multiple mole melanoma (FAMMM) syndrome have 9p21 deletions centered on CDKN2A, the familial melanoma gene Advanced clinical stage of disease is an adverse prognostic factor (Hoskins et al. 2000; Tavassoli and Devilee 2003; Kurman et al. 2014).

9.2.2 Malignant Tumours of the Vagina

Cancer of the vagina is a rare condition occurring predominantly in elderly women, approximately 1–2% of all malignant tumours of the female genital tract. High-grade squamous intraepithelial lesion–HSIL (vaginal intraepithelial neoplasia–VAIN grade 2/3) is considered a typical, though not obligatory precursor lesion of squamous cell carcinoma. Persistent infection with high-risk human papillomavirus (HPV) is probably a major etiological factor. Low-grade squamous intraepithelial lesion–LSIL—may progress to HSIL, particularly if associated with high-risk HPV infection. Prior pelvic irradiation, immunosuppression, simultaneous or prior preinvasive or invasive disease elsewhere in the lower genital tract, are observed in women with vaginal malignant tumours. The occurrence of cases of vaginal clear cell adenocarcinoma in younger women is associated with in utero exposure to diethylstilbestrol. Prognosis depends on staging and grading of tumour, age and performance status of patient, however the clinical stage is the most

significant prognostic factor. The commonest symptom is a bloody vaginal discharge. However, the patient may be completely asymptomatic. The urinary tract symptoms, painless bleeding or postcoital bleeding can occur. Pelvic pain and dysuria usually signify advanced disease. The lesion arises usually in the upper third of the posterior wall, involving the rectovaginal septum early. The directed biopsy of identified lesions is a method of primary diagnosis. Imaging studies including ultrasound, computer tomography or magnetic resonance are employed to evaluate pelvic spread of the tumour and lymph nodes involvement (Hoskins et al. 2000; Tavassoli and Devilee 2003; Kurman et al. 2014).

Squamous cell carcinomas present the great majority of vaginal malignancies, the tumour is ulcerative in half of cases, exophytic in a third and annular and constricting in the remainder. The majority of vaginal squamous cell carcinoma of all histological types is associated with high-risk HPV. HPV-negative pathway may exist especially in the lower vagina. HPV-positive squamous cell carcinomas are associated with high-grade squamous intraepithelial lesion–HSIL (vaginal intraepithelial neoplasia–VAIN grade 2/3) and are p16 immunopositive.

Adenocarcinomas developing from the remnants of Gartner's duct may be found. *Clear cell carcinoma* of the vagina has an appearance to those tumours arising in the cervix, endometrium and ovary. Primary mucinous or endometrioid adenocarcinoma, mesonephric carcinomas are rare. In young girl, *sarcomas* (embryonal rhabdomyosarcoma) may be found. Primary malignant melanomas developing in vaginal mucosa have been recorded. The vaginal wall, especially the lower third, is a common site of metastases from endometrial and ovarian malignancies, as well as lymphoid tumours (Hoskins et al. 2000; Tavassoli and Devilee 2003; Kurman et al. 2014).

9.2.3 Malignant Tumours of the Uterine Cervix

Cancer of the uterine cervix is the fourth most common cancer in women worldwide, with major geographical variations in incidence. An overwhelming majority of the cases is occurring in developing countries and it is caused by human papillomavirus (HPV) infection (Prat et al. 2014). The average age of women with invasive cervical cancer varies from 48 to 52 years. The incidence of cervical cancer, which is predominantly of the squamous cell type (over 90%) has markedly declined in many developed countries in the past decades, mainly due to cytological screening programs, coupled with infrastructure of clinically managed precursor lesions. Today, more than 80% of women dying from cervical cancer live in developing countries. Incidence rates of cervical cancer are inversely related to socioeconomic status—the incidence is higher in the low-income groups than in the high-income groups of a given population.

Sexually transmitted virus, human papillomavirus (HPV) is the major etiological factor. Nearly all cervical cancers are caused by persistent infections with one of around 15 carcinogenic types of human papillomavirus (HPV), the most frequently occurring types are HPV16 and HPV18. The etiology of cervical cancer can be

divided into multiple phases such as HPV acquisition, HPV persistence and progression to cervical cancer precursors. Data suggest that the final step, progression from HSIL to invasion, is slowest, though variable among women. Several host and environmental factors contribute, however, to enhance the probability and progression to cervical neoplasia. Among the more consistent and significant are age of first intercourse, number of sexual partners, number of children, and the number of sexual partners of the husband (high-risk male). A history of cigarette smoking, sexual transmitted infections, such as chlamydia trachomatis, herpes simplex virus infection or veneral warts has also been associated with an increased risk of cervical cancer. An aggregation of tobacco-related cancers and cancers linked with HPV and immunosuppression was found in families with familial clustering in cervical carcinoma. Thus, familial predisposition for cervical cancer is likely to imply genes which modulate immune response, e.g. human leukocyte antigen (HLA) haplotypes and/or shared sexual or lifestyle factors in family members. The high rate of spontaneous regression of HPV-induced lesions implies that altered immunity or other disease-promoting factors are required for persistent active or latent infection and conversion to malignancy. It is estimated that all cervical squamous cell carcinomas are high-risk HPV positive. A very high fraction of cervical carcinoma is p16 positive by immunohistochemistry. TP53 mutations are relatively rare, lost of heterozygosity (LOH) has been detected in multiple chromosomal regions in invasive carcinoma (1q, 3p, 3q, 6p, 6q, 11q, 17p, 18q). Some of these chromosomal abnormalities are being tested as potential biomarkers of progression risk. It has not been found sufficient evidence for any of these (except p16) to be used currently in routine clinical practice. When exfoliative cytology was introduced some seven decades ago for cervical cancer screening, it offered the hope of eliminating death from this malignancy in an adequately screened population, because it was capable of detecting occult cancer and, even more important, precancerous conditions. The incidence of preinvasive lesions (squamous intraepithelial neoplasia, dysplasia, cervical intraepithelial neoplasia,) relative to that of invasive cancer is surprisingly large. Not all lesions from the group of high-grade squamous intraepithelial lesions (HSIL) will progress to invasive tumours, on the other side since even annual screening does not assure absolutely that a cervical cancer precursor will be picked-up before an invasive cancer develop (Hoskins et al. 2000; Tavassoli and Devilee 2003; Kurman et al. 2014).

Early invasive cancers can be asymptomatic. As the tumour grows and becomes exophytic, vaginal bleeding and discharge are the most common symptoms. With lateral growth into the parametrium the ureters become obstructed. Pelvic sidewall involvement can cause pain and, less common, lymphoedema in law extremities. Anterior tumour growth in advanced stage disease causes urinary frequency, bladder pain and haematuria. Direct extension to the bladder may cause urinary retention from bladder outlet obstruction and eventually vesicovaginal fistula. Posterior expansion leads to low back pain, tenesmus and rectovaginal fistula. On examination cervical cancer may appear as red, friable, exophytic or ulcerated lesion. Rectovaginal palpation can detect induration or nodularity of the parametria in advanced lesions. Common errors consist of treating patients for menstrual

disorders, complications of pregnancy, or vaginal infection without a pelvic examination and placing too much reliance on Pap smears. The major differential diagnoses are dysfunctional bleeding, "erosions", cervicitis, condylomata accuminata, herpetic ulcer. The abnormal cervix needs a biopsy diagnosis, not just a Pap smear.

Cervical cancer is the only gynecological cancer that is clinically staged by physical examination, imaging methods, chest X-ray, cystoscopy and proctoscopy. The bioptic verification (minibiopsy or diagnostic conisation) is necessary. Magnetic resonance or expert ultrasound examination is used to clarify the extent of the disease. Tumour marker—SCC correlates with the stage of disease. Computer tomography is useful in estimating of lymph node involvement and distant metastases especially in recurrent disease. Squamous carcinoma of the cervix typically arises at the active squamocolumnar junction from a preexisting precancerous lesion, however sometimes invasion may occur even before HSIL (high squamous intraepithelial lesion) develops. No single or combination of biomarkers has been convincingly found to predict definitely whether a given lesion will persist, progress or regress. No biomarkers are yet proven to be clinically reliable for segregating the HSIL that needs treatment from the HSIL that can be safely followed up, also considering the clinical and colposcopic characteristics of the lesion and the patient as a whole. Protein p16 imunohistochemistry can be extremely helpful in the assessment of HSIL's and distinction between precancerous lesion and atrophy. Once a basal membrane is breached and stromal invasion occurs, the process is generally regarded as irreversible (Hoskins et al. 2000; Tavassoli and Devilee 2003; Kurman et al. 2014).

Squamous cell carcinoma is an invasive carcinoma composed of squamous cells of varying degrees of differentiations. A variety of histological types of squamous cell carcinoma have been described: *keratinizing, non-keratinizing, basaloid, verrucous, warty, papillary, lymphoepithelioma-like.*

Another histological type occurring on the cervix is adenocarcinoma—a carcinoma that shows glandular differentiation. We distinguish *mucinous, endometrioid, serous, viloglandular, clear cell and mesonephric carcinoma, and adenocarcinoma* in situ. Quite rare are *adenosquamous carcinoma, glassy cell carcinoma variant, adenoid cystic and adenoid basal carcinoma. Small cell carcinoma* and *undifferentiated carcinoma* are rare tumours too, with worse prognosis. Adenocarcinoma in situ is an intraepithelial lesion containing malignant-appearing glandular epithelium that carries a significant risk of invasive adenocarcinoma if not treated.

Among the extremely rare tumours of the uterine cervix have been described *mesenchymal tumours—leiomyosarcoma, endometrioid sarcoma, angiosarcoma, mixed epithelial and mesenchymal tumours—carcinosarcoma, adenosarcoma*, *neuroendocrine tumours*. *Malignant melanoma* of the cervix is considerably less common than vulvar or vaginal melanoma. The tumour occurs in adults and approximately one-half had spreads beyond cervix at the time of presentation. The prognosis for patients with cervical melanoma is dismal. Involvement of the cervix by *lymphoma or leukaemia* may rarely be primary but is more commonly part of systemic disease with no specific symptoms referable to the cervix. The majority of

clinically significant *secondary tumours* of the cervix originate in the female genital system (endometrium, ovary, vagina and fallopian tube in that order). Metastases from extragenital primary tumours may be suspected based on the submucosal location of tumours cells with a normal overlying cervical epithelium. The distinction of primary cervical adenocarcinoma from secondary involvement may be difficult in some case (Hoskins et al. 2000; Tavassoli and Devilee 2003; Kurman et al. 2014).

9.2.4 Malignant Tumours of the Uterine Corpus

The uterine corpus represents the second most common site for malignancy of the female genital system. Cancer of the corpus uteri (endometrial cancer) is the sixth most common cancer in women (almost 5% of all cancers in women) (Prat et al. 2014). There is a negative correlation at the international level between the incidence of cervical and endometrial cancer. The former is common in developing countries and the latter in affluent westernized societies. It is uncertain how much of it is due to screening and other methods of early detection and medical care of cervical cancer, which are rare in extensive areas where cervical cancer is highly prevalent. Patient age at onset is much higher than is in the case with cervical cancer, the overwhelming majority of patients with endometrial cancer being postmenopausal.

The wide variations in incidence indicate that environmental factors play a part in the development of this disease. This hypothesis is supported by a number of findings. The incidence of endometrial cancer, in contrast to the situation with cervical cancer, is higher among higher-income groups and among nulliparous women or women with history of infertility. There is the evidence of a frequent association between endometrial cancer and metabolic disorders such as obesity, hypertension, and diabetes. Premenopausal estrogen therapy is an etiological factor. Worldwide, the variable incidence of endometrial cancer is most strongly associated with total fat consumption.

No satisfactory technique is available for the routine screening of the large populations of women for endometrial carcinoma and its precursors. The prominent symptom of endometrial carcinoma is postmenopausal bleeding. Approximately three-quarters of the cases occur in this age group, and in more than 90% of them the initial complaint is vaginal bleeding. The importance of this symptom is recognized by most women and they usually seek medical consultation within the three months after the first episode. The other important initial symptoms are a purulent, sometimes blood-tinged discharge and pain. Endometrial carcinoma is diagnosed in 1–5% of the cases while the patient is asymptomatic. Women with endometrial carcinoma who are premenopausal invariably have abnormal uterine bleeding often characterized as menometrorrhagia or oligomenorrhoea.

Two distinctly pathogenetic forms of endometrial carcinoma are percepted. One is associated with excessive, prolonged estrogen exposure, either exogenous or

endogenous, and has a more favorable income. The estrogen-associated lesion (type I) is further characterized by coexisting endometrial hyperplasia (hyperplasia without atypia, atypical hyperplasia/endometrioid intraepithelial neoplasia) as the result of unopposed estrogenic stimulation), better differentiation, less myometrial invasion, earlier stage, and a higher hormone-receptor content than non-estrogen-related malignancy. Type II is not estrogen-dependent and develops independently of endometrial hyperplasia. It occurs in older women and is more aggressive (Hoskins et al. 2000; Tavassoli and Devilee 2003; Kurman et al. 2014). Endometrial carcinoma is a primary malignant epithelial tumor, usually with glandular differentiation, arising in the endometrium that has the potential to invade into the myometrium and to spread to distant sites. The diagnosis of endometrial cancer needs to be determined by endometrial sampling. Hysteroscopy is a reliable method for diagnosis of intrauterine pathology. Transvaginal ultrasound is the imaging technique for the assessment of the endometrium in symptomatic women. More accurate is magnetic resonance, especially in estimating of invasion to myometrium. The ultrasound presence of abnormal thickness of endometrium in postmenopausal symptomatic women is, however, non specific finding. Non invasive imaging, as ultrasound or magnetic resonance, are utilized in distinguish suspicious ovarian and uterine tumours.

The most frequent epithelial cancer of the uterus, which presents about 75% of all uterine malignancies, is *endometrioid carcinoma*, which may exhibit a variety of differentiated epithelial types including secretory type, type with squamous differentiation, mucionous, ciliated cell and viloglandular type. About 35% of tumours display microsatellite instability. In sporadic endometrioid carcinoma, microsatellite instability is most often due to hypermethylation of the MLH1 gene promoter. The most frequent alterations include mutation or inactivation of PTEN, mutation in PIK3A, PIK3R1, ARID1A, KRAS and TP53. The most common cause of familial endometrial carcinoma is Lynch syndrome which is due to germline transmission of defective DNA mismatch repair genes (MSH2, MLH1, MSH6 and PMS2) resulting in an autosomal-dominant inheritance pattern. Cowden syndrome is a germline mutation of PTEN. Less common epithelial tumour of the uterine corpus is *mucinous adenocarcinoma*, which is low grade tumor type I. The *serous adenocarcinoma* presents about 5–10% of all uterine carcinomas, it is typical tumour of type II, without association with hyperestrinism. Quite rarely, in 1–5% of all uterine adenocarcinomas the *clear cell adenocarcinoma* is diagnosed. Squamous cell carcinoma, transitional cell or small cell carcinoma may occur, too.

Uterine *mesenchymal tumours* are derived from the mesenchyme of the corpus, consisting of endometrial stroma, smooth muscle and blood vessels or admixture of these. Quite rarely these tumours may show mesenchymal differentiation that is foreign to the uterus. The most common presentation for mesenchymal tumours is uterine enlargement, then uterine bleeding or pelvic pain. *Endometrial stromal sarcomas* have traditionally divided into low and high grade types based on mitotic count. Distinction based on the features such as nuclear pleomorphism and necrosis, distinguish low grade and undifferentiated endometrial sarcoma, which has important implications regarding prognosis. Low grade endometrial stromal

sarcoma is indolent tumour with a propensity for local recurrence, usually many years after hysterectomy. Distant metastases are less common. In contrast, undifferentiated endometrial sarcoma is highly aggressive tumour with the majority of patients presenting with extrauterine disease at the time of diagnosis and dying within two years of diagnosis. *Leiomyosarcoma* is a highly malignant tumour and represents the most common pure uterine sarcoma and comprimes slightly over 1% of all uterine malignancies. The risk factors are not known to relate to leiomyosarcoma. *Carcinosarcoma* is the most common neoplasm of the group of mixed epithelial and mesenchymal tumours, although there is increasing evidence that these tumours are monoclonal and should be considered subsets of endometrioid carcinoma. The clinical course of uterine carcinosarcoma is generally aggressive with a poor prognosis, considerably worse than that of a poorly differentiated endometrioid carcinoma. *Adenosarcoma* is a biphasic neoplasm containing a benign epithelial component and a sarcomatous mesenchymal component. *Choriocarcinoma,* a rare malignancy of trophoblastic origin associated with pregnancy, is arising from the embryonic chorion, both layer of the trophoblastic epithelium being involved. The uterus is the most common, but not the only site of the primary growth. About fifty percent of choriocarcinomas are preceded by a mole, 25% by abortion, about 22% by normal delivery, and the rest by ectopic pregnancy. The most important feature of choriocarcinoma is that the tumour secrets chorionic gonadotropin (HCG) which can be detected in the serum. Thank to this tumour marker it is possible to make diagnosis, access response to treatment and follow up the patient with the great deal of confidence and accuracy.

Among the rare tumours sex cord-like and neuroectodermal tumour occur as well as lymphomas and leukaemia. *Secondary tumours* of the uterine corpus can be divided into two groups: tumours of the genital and extragenital organs. Mammary lobular carcinoma, gastric signet-ring cell carcinoma and colonic carcinoma are the most frequently reported extragenital primary tumours (Hoskins et al. 2000; Tavassoli and Devilee 2003; Kurman et al. 2014).

9.2.5 Malignant Tumours of the Fallopian Tube

Tumours of the fallopian tube are much less common than the corresponding ovarian neoplasms; however, histologically the same surface epithelial-stromal tumour subtypes are recognized. Serous tubal intraepithelial carcinoma, a non-invasive serous carcinoma is defined as an epithelial precursor lesion. Serous borderline tumours (atypical proliferative serous tumours) are fallopian tube neoplasms that resemble their ovarian counterpart. The risk factors appear similar to those of epithelial ovarian cancer. Serous adenocarcinoma is the most frequent tumour of the fallopian tube; mucionous, endometrioid, clear cell and undifferentiated carcinoma are less common. Sex cord-stromal and germ cell tumours are rare Metastatic tumours involving the tube usually are the result of secondary spread from carcinomas of ovary or endometrium. Blood-borne metastases from breast

carcinomas may also occur. The gestational choriocarcinoma is uncommon complication of tubal ectopic pregnancy (Hoskins et al. 2000; Tavassoli and Devilee 2003; Kurman et al. 2014).

9.2.6 *Malignant Tumours of the Ovary and Peritoneum*

Ovarian cancer is the seventh most common cancer in women, representing 4% of all cancers in women. The fatality rate of ovarian cancer tends to be rather high relative to other cancers of the female reproductive organs (Prat et al. 2014). Tumours of the ovary represent about 25–30% of all cancers of the female genital system. It is the most frequent cause of death from gynecological malignancy.

The economically advanced countries show the highest rate of incidence. Two factors consistently associated with a reduced risk of the disease are high parity and the use of oral contraceptives. On the other side, an increased risk of ovarian cancer was recorded in several studies in postmenopausal women treated with high-dose estrogen replacement therapy for more than 10 years. Very little is known of the etiology of non-familial cases. The protective effect of pregnancies, breast feeding, and of oral contraception suggests a direct role for ovulation in causing the disease, but no convincing mechanism linking the risk factors with malignant transformation has been proposed. Several dietary factors have been related to ovarian cancer. There is emerging evidence that the Western lifestyle, in particular, obesity, is associated with an increased risk. Numerous epidemiological investigations of ovarian cancer have attempted to quantify the risks associated with a positive family history. Approximately 5–10% of ovarian cancers are hereditary cancers caused by mutations in specific genes. Women with ovarian cancer have a poor prognosis. This unfavorable outcome is largely ascribed to a lack of early warning symptoms and a lack of diagnostic tests that allow early detection. As a result, approximately 75% of patients present when this cancer is in an advanced stage, i.e. it has metastasized to the upper abdomen or beyond the abdominal cavity. It is recognized that the overwhelming majority of women diagnosed with ovarian cancer actually have symptoms, but they are subtle and easily confused with those of various benign entities, particularly those related to the gastrointestinal tract. Ovarian cancers spread mainly by local extension, by intra-abdominal dissemination and by lymphatic dissemination, but rarely through the blood stream. The extent of the tumour at the time of diagnosis is one of the most important variables influencing the prognosis in ovarian carcinoma. The likely origin of ovarian surface epithelial-stromal tumours is the mesothelial surface lining of the ovaries and/or invaginations of this lining into the superficial ovarian cortex that form inclusion cysts. The tendency to implant in the peritoneal cavity is a characteristic feature of malignant ovarian tumours, and results in ascites. Ovarian tumours are mostly asymptomatic, they present with abdominal enlargement, which is confirmed by imaging methods, mainly by ultrasound. Ultrasound presents exceptionally accurate staging method utilizing special sonomorphological signs of tumour. Imaging

methods (ultrasound and computer tomography) may give valuable information on the size and extent of the lesion prior to operation. Laparotomy with biopsy is therefore the most important diagnostic procedure, revealing the extent and nature of the growth and indicating treatment.

Epithelial tumours of the ovary account the most important part of all ovarian cancers. They demonstrate a broad variation in biologic behavior that correlates rather well with the degree of histologic differentiation. The cell type of the epithelial component can usually be identified as *serous* (the most frequent type) and *mucionous* adenocarcinoma. Both have a tendency to develop bilaterally and to form implants in the peritoneal cavity. *Endometrioid, mesonephroid*, *clear cell carcinoma* and *Brenner tumors* are less common. Due to recent developments the serous ovarian neoplasia is considered to represent two separate diseases: low-grade and high-grade serous carcinoma. The prototypic type I tumour is low-grade serous carcinoma which has a high frequency of KRAS and BRAF mutations but no TP53 mutations. The prototypic type II is high-grade serous carcinoma, which is characterized by a high level of genetic instability and harbours TP53 in nearly all cases. The tumours in the two categories develop via separate pathways. The precursor of low-grade serous carcinoma is a borderline serous tumour/atypical proliferative tumour. In contrast, high-grade serous carcinomas harbor mutations in TP53 and are, chromosomally, highly instable. High-grade serous cancers are usually rapidly growing, highly aggressive neoplasms that are often diagnosed at an advanced stage. Although low-grade serous carcinoma may rarely progress to high-grade serous carcinoma, the majority of low-grade serous carcinomas develop along a pathway that is distinct from implicated in the development of their high-grade counterparts. Conventionally, it has been assumed that the majority of serous epithelial tumours arise primarily from metaplastic changes of the surface mesothelium, despite the apparent rarity of putative precursor lesions. In recent years, an alternative tubal origin has emerged as an important source of high-grade serous carcinomas. The non-invasive intraepithelial lesions have been designated serous tubal intraepithelial carcinoma or high-grade serous tubal intraepithelial neoplasia. They have cytological features identical to high-grade serous ovarian cancer and also show TP53 mutation and aberrant p53 protein expression, high-proliferation indices and marked genomic instability. It has been shown, that serous tubal intraepithelial carcinoma is present in the fallopian tube up to 60% of women with high-grade serous cancers that would have been considered to be ovarian or primary peritoneal tumours based on conventional criteria. The possibility of a field change within native or metaplastic tube-type epithelium, resulting in multifocal lesions must also be considered as a plausible alternative pathogenetic hypothesis. Although high-grade, extra-uterine serous carcinomas may arise from the fallopian tube, ovary or rarely from the peritoneum, the site of origin is often obscured by bulky disease at the time of diagnosis. Nonetheless, these tumours are characterized by common epidemiological features and clinical behavior. Accordingly, tumour diagnosed as ovarian high-grade serous carcinoma might be viewed as an amalgamation of primary (extra-uterine) “pelvic high-grade serous carcinoma” (Kurman et al. 2014).

Borderline epithelial tumours have histologic and biologic features occupying a position between those of the clearly benign and frankly malignant ovarian epithelial neoplasms. Clinically, these tumours are characterized by a predominantly early stage at diagnosis, infrequent and late recurrence, and long survival with residual or recurrent malignancy. The average age of women with the borderline serous and mucinous tumours is between that of women with frankly malignant ovarian carcinomas and benign cystomas. Fatality from serous borderline tumours results from progression to low grade serous carcinoma in approximately 5%.

Sex cord-stromal tumours are composed of granulose cells, theka cells, Sertolli cells, Leidig cells and fibroblasts of stromal origin, singly or in various combinations. Overall, sex cord-stromal tumours account for about 8% of ovarian tumours. The etiology of these tumours is unknown. *Germ cell tumours* (dysgerminoma, embryonal carcinoma, yolk sac tumour, non-gestational choriocarcinoma, immature teratoma) originate at different stages of development from germ cells that colonize the ovary. Malignant germ cell tumours are the most common ovarian cancer among children and adolescent females. *Mixed germ cell-sex cord-stromal tumours* (gonadoblastoma) typically is identified in children or young adults.

Secondary tumours of the ovary—metastatic tumours to the ovary are common and occur in approximately 30% of women dying of cancer. The term Krukenberg tumor refers to a metastatic mucinous/signe-ring cell adenocarcinoma of the ovaries which typically originates from primary tumours of the gastro-intestinal tract, most often colon and stomach. Above mentioned carcinoma, as well as carcinoma of breast, lymphomas and leukaemias account for the vast majority of cases.

Peritoneal tumours (peritoneal malignant mesothelioma) are quite rare, highly aggressive neoplasms with primary manifestation in the abdominal cavity in the absence of a visceral site of origin. They spread primary by exfoliation of cancer cells from the primary site of origin. The patients with this tumour present with non-specific manifestations including abdominal discomfort and distension, digestive disturbances and ascites (Hoskins et al. 2000; Tavassoli and Devilee 2003; Kurman et al. 2014).

9.3 Treatment Modalities in Gynecological Malignancies

Human neoplasms form a large group of diseases of varying frequency, site, anatomical extent, pathology, clinical course and prognosis, suitability for surgery, and responsiveness to ionizing radiation, chemotherapeutic agents, and hormones. The variation in methods used for cancer diagnosis and treatment call for a collective and multidisciplinary team approach to all problems connected with the clinical management of cancer. While cancers are chronic disease, the decisions made about treatment following the initial diagnosis often determine whether the outcome will be the best possible. The treatment of malignant tumours of the female reproductive system is applied by all major oncological treatment modalities i.e.

surgery, radiotherapy and pharmacotherapy, which includes chemotherapy, hormonal therapy and biological therapy.

Surgery was the only method of treatment of cancer for centuries and remains today the primary treatment for the vast majority of patients who are cured. It is locoregional therapy, and it can cure that disease which is localized to the tissue of origin and its draining lymph nodes. Surgery is the most important and fundamental treatment modality in all pelvic gynecological tumours, too, in spite of introducing of the new effective cytostatics, the advances of radiotherapy and possibilities of the new biological substances. We can recognize curative surgery, preventive surgery, diagnostic surgery, palliative surgery, reductive surgery, surgery for recurrences and surgery for reconstruction. Especially in the early stage of tumours the surgical approach presents the best outcomes. Surgery is the only curative method in ovarian tumours. Last but not least are plastic and reconstruction procedures as relocation of skin parts after removing of local advanced vulvar tumours or urine and faeces derivation after pelvic exenterations. All surgical procedures were described several decades ago, in spite of this fact, surgical treatment at present time is passing through a dynamic development—indications are changing, extension of procedure are clarifying, new technologies are introducing. Individualization and tailored approach based on evaluating of a number of prognostic parameters is the main stream of the last years.

Radiotherapy can be first choice in special cases of the other tumours, when surgical procedure is contraindicated, or in addition to the therapeutic importance of surgery, which is a crucial method for dignosis and staging of the tumour. In advanced tumour it works rarely as curative method, but quite often refines diagnosis, leads to improvement of quality of life and prolonging disease-free survival or overall survival. In the case of pain, profuse bleeding or intestinal disorders caused by obstruction surgery has a palliative function, with the goal to improve quality of life. Radiation therapy is locoregional therapy, introduced to the treatment of gynecological malignancies more than a century ago. It is applied in gynecological tumours as primary treatment, adjuvant or neoadjuvant treatment and palliative method. In order to improve antitumour effect of cytostatics concomitant approaches are used. Radiotherapy is applied with a curative goal in malignant tumours of uterine cervix, uterine corpus and vulva. Whole pelvis external beam radiotherapy and intravaginal brachytherapy are widely used in gynecological oncology.

The use of systemic *chemotherapy* in treatment of human cancer has relatively recent origins with first report of the employment of an alkylating agent in the late forties in the treatment of patients with Hodgkin's disease. Since these initial efforts, both number of the active drugs available and knowledge of the proper use of these agents had increased dramatically. As the systemic therapy may affect cancer cells throughout the body, it is needed in large proportion of cancer patients. Chemotherapy as adjuvant, neoadjuvant and palliative approach is applied practically in all gynecological malignancies. Another forms are concomitant (combination with radiotherapy in order to enhance effect of radiation), and consolidation (treatment given after induction therapy in order to enhance the likelihood of a durable complete remission).

Hormonal treatment is used in gynecological oncology more often than in the treatment of other solid tumours. The hormonal manipulations can favorably influence neoplastic growth arising in hormone responsive organs. There are several ways of producing hormonal alterations in the organism: ablative hormone treatment with removal of hormone-producing organs or destruction of their capacity to function. Another type is additive hormone therapy which exerts its effects by administrating hormones and hormone-like substances which interfere with hormone-induced growth mechanism.

Immunotherapy and targeted biological treatment are recently introduced pharmacological approaches. Optimal therapeutic use will involve combination use with existing treatment modalities (Barakat et al. 2013).

Surgery is the primary treatment approach in *vulvar cancer*. For patients with early stage of carcinoma, with small risk of recurrence, the therapy is usually local excision with sufficient margin of normal tissue. Inguinofemoral lymph node dissection is usually unnecessary. For greater tumours partial or total deep vulvectomy with ipsilateral or bilateral inguinofemoral lymph node resection may be required. If superficial lymph nodes contain tumour, radiotherapy to the deep pelvic nodes or chemoradiation may be necessary. Primary radiation is the method of choice in the cases, when surgery is contraindicated. Chemotherapy is applied in the palliative indications.

Radiation is the preferred treatment for most cases of *vaginal carcinoma*. Stage I disease located in the upper part of the vagina, a radical hysterectomy, pelvic lymphadenectomy and partial vaginectomy may be considered. Otherwise, radiation therapy given as intracavitary therapy, interstitial implants and/or external pelvic/inguinal radiation, often in combination, is the most frequently adopted modality. In tumours of the middle or lower third of the vagina the external radiation field should include the inguinal and femoral lymph nodes.

The clinical factors that influence prognosis in invasive *cervical cancer* are age, stage of disease, volume, lymphatic spread and vascular invasion. Radiotherapy and surgery produce similar results for early invasive cancer. Fertility sparing procedures are method of choice in younger women. More advanced lesions are treated with combination of external radiotherapy and intracavitary radiation. A significant overall and disease free survival advantage for cisplatin-based chemotherapy given concurrently with radiotherapy was confirmed. A significant benefit of chemoradiation on both local and distant recurrence has been observed and it is emerging as the standard of care for advanced cervical cancer.

The main procedures for treating *endometrial cancer* are surgery—hysterectomy with bilateral salpingo-oophorectomy, lymphadenectomy in indicated cases. Combined surgery and postoperative radiotherapy or radiotherapy alone is used in special cases. Hormonal therapy with progestational agents may be useful in palliation in advanced cases of some tumours and often may hold metastatic disease in check several years. Chemotherapy has a limited place in the palliative approach.

Choriocarcinoma is an exceptional malignancy to be cured by chemotherapy alone and remains as an outstanding example of a cancer curable by drug.

Surgery is the treatment of choice in *ovarian cancer*. The surgical procedure, however, is not solely for treatment, but it is decisive for diagnosis and staging. Surgical approach is different in early and advanced stages. The main goal is debulking and removing the maximum amount of tumour mass, without residual tumour tissue. Ovarian cancer is chemosensitive, bur not chemocurable. Chemotherapy has a wide and important use in adjuvant, neoadjuvant and palliative treatment of ovarian cancer. The most frequent agents are paclitaxel and platina derivates, cyclophosphamid, epirubicin, doxorubicin, docetaxel, gemcitabin and etoposid. Supportive care is necessary considering high toxicity and side effect of the cytostactic. Hormonal therapy, immunotherapy and targeted molecular biological therapy are involved in the treatment protocols of advanced ovarian tumors (Barakat et al. 2013).

9.4 Pregnancy and Cancer

Malignant tumours in pregnant women are not longer a rare diagnosis, affecting between 0.05 and 0.1% of all pregnancies (approximately 1 in 1000 pregnancies). They present severe and specific complication, considering not only medical aspects (Weisz et al. 2001, 2004; Timur et al. 2016). The incidence is expected to rise with the concomitant increasing age of childbearing and postponing of fertility plans to a higher age category. An increase in the average pregnancy age has increased the co-occurrence of pregnancy and cancer on one side, and the occurrence of subsequent pregnancies after cancer treatment on the other side. The most common malignancies associated with pregnancy include cervical and breast carcinomas, malignant melanoma, lymphomas and leukaemia, cancer of the thyroid, colon and ovary. Vulvar cancer in pregnancy is extremely rare finding (Pavlidis 2002; Han et al. 2013).

Malignant disease during pregnancy presents an extreme stress to both patient and physician, and its occurrence raises a conflict between optimal maternal therapy and fetal well-being. The concurrence of cancer and pregnancy complicates treatment in each stage, including diagnostic, treatment, as well as delivery, postpartum and neonatal period. The goal of treating cancer-complicated pregnancy is to reach as close to the standard prognosis as possible. Treatment should be similar to non-pregnant cancer patients whenever possible (Woo et al. 2012; Han et al. 2013). Many reports have noted that pregnant patients tend to have a worse outcome when compared with non-pregnant women. Some have postulated that the altered hormonal environment of pregnancy may adversely influence the course of the disease. The immunosuppressive effect of pregnancy may facilitate tumours dissemination. However, based on contemporary well-controlled studies, it seems that pregnancy per se should not be considered as a poor prognostic factor. On the other side it is necessary to admit that the gestational status might interfere with correct diagnosis and treatment, whereby the result might be an inferior outcome (Weisz et al. 2001, 2004). Treatment management, which may include surgery, radiotherapy,

chemotherapy, treatment with targeted agents, and immunosuppressives in mono- or poly-therapy, are potential teratogenic. However, one must consider that even in healthy, unexposed pregnancies, women have an inherent risk for aversive outcomes, with baseline risks of miscarriage (up to 15%), stillbirths (0.5%) or major fetal malformations (1–3%) (Moran et al. 2007). The diagnosis of cancer in pregnancy is a dramatic event that poses difficult dilemmas for the pregnant patient, her family and treating physicians. Therefore, the importance of psychological support for those who are undergoing or have undergone cancer-complicated pregnancies should not be underestimated. Improvements in patients' coping skills, social interactions, social adjustment as well as stress and anxiety management were observed in those with psychological intervention (Merckaert et al. 2010). Staging procedures are performed as in non-pregnant women and are important as far as they will alter and determine appropriate therapeutic procedures that optimally treat the mother while maximally protecting the fetus, with additional concern for preventing an accumulation of low-dose radiation. Ultrasonography and magnetic resonance imaging technique are preferred, although ionizing examinations of distant parts of the maternal body expose the fetus to low doses of radiation, the accumulation of which may harm the fetus. The general rule when performing radiologic and nuclear medicine examinations during pregnancy is that the radiation doses should be kept as low as reasonably achievable if not avoided (McCollough et al. 2007). Most patients with a pregnancy-associated malignancy, who choose to continue pregnancy, are treated with the chemotherapy. Many chemotherapeutic agents possess high teratogenic or abortifacient effects in the first trimester. The rate of fetal malformations following the first trimester treatment with drugs in combination is about 25% versus 17% for single agent use. The recent information supports the previous evidence that chemotherapy after the first trimester is not associated with the increased rates of birth defects above the rate in the general population (3%). Although, intrauterine growth restriction and transient myelosupression have been reported, and need to be investigated at birth (Cardonick et al. 2010). Major concerns arise over the possible effects of chemotherapy on the developing brain, which is potentially vulnerable to damage through the entire gestation. Recently, a disharmonic intelligence profile was reported, but an important bias by prematurity was suggested. These findings support the hypothesis that more subtle changes in neurodevelopment are possible and need to be prospectively investigated (Amant et al. 2012a, b). Radiotherapy of cancers of the abdomen and pelvic region is contraindicated due to the possibility of serious adverse effects to the fetus. Therapeutic doses of radiation can induce microcephaly, mental retardation, microphtalmia, iridal defect, skeletal anomalies and fetal death (Weisz et al. 2004, Moran et al. 2007). Surgery is an essential part of oncological treatment, particularly for gynecological cancers. Surgery during pregnancy is not rare, with approximately 0.5–2% of pregnant women undergoing non-obstetric surgery. Surgery methodology and outcome is dependent on gestational age and the stage of fetal development. If the expected delivery date is forthcoming, one could consider delaying surgery until postpartum. In specific cases operations could

directly follow caesarean section. Primary surgical treatment should be performed whenever is indicated (Allaert et al. 2007).

A major problem is the assessment of the optimal time of delivery. For all patients, a term delivery is preferred. However, deterioration of maternal condition or the need for radiotherapy may indicate a need for preterm delivery. A natural vaginal birth is normally preferred whenever possible in the absence of complicating factors. Caesarean section is indicated in case of cancer metastases to the long bones, in the presence of cervical cancer. The duration of gestation, the maternal disease and the toxicity of treatment in conjunction with the timing of the interventions are factor that may affect early postnatal and long term outcomes of children born to mothers with cancer (Han et al. 2014; Amant et al. 2015).

9.4.1 *The Most Common Malignant Tumours Occurring in Pregnancy*

Cervical cancer. Invasive cervical cancer is one of the most common malignancies occurring in pregnancy. The incidence varies between 0.02 and 0.9%. The diagnosis of invasive cervical cancer in pregnancy is sometimes delayed, because bleeding could be interpreted as pregnancy-related complication (Pavlidis 2002). Abnormal bleeding is the symptom that leads to examination and diagnosis in 55% of women, while 45% are asymptomatic but show abnormal cervicovaginal cytological tests (39%) or abnormal finding at vaginal examination (6%). Any abnormal cytological test should always be followed by colposcopy and punch biopsy. Conisation should be left for the second trimester and in cases where the diagnosis of invasive cancer cannot be made otherwise. Surgery and radiation are the treatments of choice for non-metastatic non-recurrent invasive cervical cancer. Both forms of treatment will cause termination of pregnancy. There is no convincing evidence whether delay in therapy to allow fetal maturation may adversely affect maternal outcome. However, in some studies an unfavorable impact on maternal prognosis was found when treatment was delayed from the second trimester onwards (Oduncu et al. 2003; Han et al. 2013).

Breast cancer. The incidence of breast cancer during pregnancy is approximately 1 in 3000–10,000 pregnancies. Three percent of all breast malignant tumours is associated with pregnancy i.e. tumours diagnosed during pregnancy or within one year postpartum (Pavlidis 2002). During pregnancy the woman undergoes several hormonal changes. Since receptors for each of these hormones are found on breast cancer cells, some studies have indicated a poor prognosis because of stimulation of tumour growth. However, there is no epidemiological, clinical or prognostic evidence that pregnancy or its termination will alter the natural history of breast cancer. Due to substantial physiological changes including enlargement of the breast it might be more difficult to notice suspicious nodes that forewarn of cancer. Due to the mentioned above, the pregnant women are at a higher risk of

presenting with more advanced disease. Surgery alone or in combination with other modalities is feasible in most cases. Radio- and chemotherapy are best delayed after first trimester (Pavlidis 2002; Oduncu et al. 2003). The evidence increasingly supports administration of chemotherapy from 14 weeks of gestation onward. New breast cancer treatments might be applicable to pregnant patients, but tamoxifen and trastuzumab are contraindicated during pregnancy. Cancer treatment during pregnancy will decrease the need for early delivery and thus prematurity, which is a major concern in management of breast cancer in pregnancy (Amant et al. 2012b).

Malignant melanoma. Generally, it is postulated that melanoma accounts for about 8% of all malignant tumours arising during gestation, it occurs in 2–3 of every 1000 pregnancies (Pavlidis 2002; Oduncu et al. 2003). Pregnant women are diagnosed at a later stage and thus show a worse prognosis. This may be because of a delay in diagnosis, since benign nevi may darken and/or enlarge during pregnancy. The early detection and treatment of malignant melanoma is highly important. The metastatic melanoma still remains very resistant to chemo- and radiotherapy. Nevertheless, there is no difference in the disease-free interval and overall survival between pregnant and non pregnant patients (Oduncu et al. 2003).

Malignant lymphoma. The incidence of Hodgkin's disease ranges from 1:1000 to 1:6000 delivery. Most of the patients are diagnosed at stage II–IV on average at 22 weeks of gestation. Thus the pregnant women have not more advanced disease than non-pregnant women, and overall survival in both groups is identical. Non-Hodgkin's lymphoma occurring in pregnancy is rarer. There is no evidence of teratogenic effects using standard chemo- or radiotherapy during the second and third trimester of pregnancy (Pavlidis 2002; Han et al. 2014).

Leukaemia. The real incidence of leukaemia during gestation is not well known. It is estimated that it is a rare disease with an estimated incidence of 1per 75,000 to 100,000 pregnancies annually. Acute leukaemias are more often diagnosed, among them acute myeloid leukaemia is diagnosed twice as often as lymphatic laukaemia. From existing data of the literature it seems that leukaemia can affect both the pregnancy and the fetus. Special attention should be paid to acute promyelocytic leukaemia, a subset of acute myeloid leukaemia (AML), characterized by the specific chromosomal translocation t(15;17). This form of AML is associated with life-threatening disseminated intravascular coagulation (DIC). The treatment of acute leukaemia should be started immediately after diagnosis. The therapeutic management of pregnant women with acute leukaemia is very difficult and the final decision should be taken between the hematooncologist, the patient and the family. Abortion should be recommended during the first trimester of pregnancy.

Ovarian cancer. The incidence of ovarian malignancies during pregnancy is very low. Around 40% of these tumours are germ-cell tumours. Epithelial ovarian tumours are usually of low stage and low grade. Due to frequent ultrasound examinations applied in pregnant women, the detection rate of early-stage ovarian malignancies increases. These tumours are in general associated with a better maternal prognosis. *Thyroid cancer* seems to be rare during pregnancy. No endocrine association between maternal hormonal changes and thyroid cancer was found. *Colorectal cancer* is another very rare tumor diagnosed in pregnancy. The

delay in diagnosis is resulting in the presentation of more advanced stages and poorer prognosis. Vertical transmission of cancer is exceptionally rare, although maternal cells do reach fetus. The most common tumour metastazing to the placenta or fetus is malignant melanoma followed by leukaemia and lymphoma (Pavlidis 2002; Han et al. 2014).

Cancer is not an acute emergency; multidisciplinary deliberation and a second opinion or referral to an expert center is recommended to compose the optimal treatment plan. The management of gynecological malignancies during pregnancy involves careful consideration of several factors. These include the type of cancer, disease stage, maternal age, dose and duration of treatment, and fetal risks such as treatment-related teratogenicity, all of which are dependent upon the gestational age of the pregnancy. Despite limited evidence-based information, cancer treatment during pregnancy can succeed. State-of-art treatment should be provided for this vulnerable population to preserve maternal and fetal prognosis. To maximize the maternal outcome, cancer treatment should follow a standard treatment protocol as for non-pregnant patients, iatrogenic prematurity should be avoided. Individualization of treatment and effective psychological support is imperative to provide throughout the pregnancy period. Moreover, adequate counseling and respecting the patient's wishes is an essential part of respect for patient's autonomy (Amant et al. 2014; Han et al. 2014).

9.5 Hereditary Syndromes and Genetic Susceptibility of Gynecological Tumours

Inherited cancer susceptibility is recognized as a significant risk for cancer of the breast and female genitals organs. For many inherited tumour syndromes, the underlying germline mutations have been identified. This allows genetic testing and counseling of at risk family members and to estimate the associated disease burden. The genetic basis involves mutational inactivation of tumour suppressor and DNA repair genes. Additional familial aggregations have been observed but the responsible genes have not yet been identified and may involve multigenic traits including epigenetic ones. Inherited genetic mutations play a major role in about 5–10% of all cancers. Researchers have associated mutations in specific genes with more than 50 hereditary cancer syndromes, which are disorders that may predispose individuals to developing certain cancers. Genetic changes that increase cancer risk can be inherited from the parents, if the changes are present in germ cells, which are the reproductive cells of the body (eggs and sperm). Such changes called germline changes are found in every cell of the offspring. Cancer causing genetic changes can also be acquired during one's lifetime, as the result of errors that occur as cells divide during a person's lifetime or exposure to substances such as certain chemicals in tobacco smoke and radiation such as ultraviolet rays from the sun, that damage DNA (Garber and Offit 2005; Lindor et al. 2008; Committee

Opinion ACOG 2015). Hereditary breast and ovarian cancer syndromes (HBOC) and hereditary non-polyposis colon cancer syndrome (HNPCC) are the two most important and known syndromes responsible for inherited malignant tumours in gynecology.

9.5.1 Hereditary Breast and Ovarian Cancer Syndrome

Ovarian cancer represents the leading cause of cancer death among gynecological malignancies. The lack of effective early detection strategies and an unfavorable anatomy are associated with the advanced stage at diagnosis and poor prognosis often found in ovarian cancer patients. Overwhelming majority of ovarian tumours is sporadic, without a positive family history. Malignant transformation is caused by somatic mutations. For both sporadic and hereditary tumours are typical accumulations of genetic transformations and high degree of genetic alterations. The recent development and implementation of next-generation sequencing technologies have provided the opportunity to analyze multiple cancer susceptibility genes and to optimize the molecular diagnosis of hereditary tumours. More than one-fifth of ovarian tumours are supposed to have hereditary susceptibility and, in about 65–85% of them, the genetic abnormality is a germline mutation in tumour suppressor BRCA genes (Toss et al. 2015). Nevertheless, several other suppressor genes and oncogenes have been associated with hereditary ovarian cancers, including mismatch repair (MMR) genes in Lynch syndrome, the tumour suppressor gene TP53 in Li-Fraumeni syndrome, and several other involved in the double-strand breaks repair system, such as CHEK2, RAD51, BRIP1, and PALB2. To date, at least 16 genes are known to be involved in the mechanism of hereditary ovarian carcinogenesis, but several mutation still remain unknown and cannot be detected by specific tests (Toss et al. 2015).

The breast cancer -associated genes BRCA1 on chromosome 17q and BRCA2 on chromosome 13q are the most well-known cancer susceptibility genes, responsible for HBOC syndrome. HBOC syndrome is an autosomal dominantly inherited disease characterized by a young age of onset, more than one synchronous or metachronous tumour, and a family history of first and second degree relatives with similar cancers. Women with HBOC syndrome are diagnosed at a younger age (approximately 10–15 years earlier than women with sporadic tumours) and result in an increased lifetime risk for developing breast, ovarian and other cancers. Women carrying a BRCA1 or BRCA2 genetic mutations have 60–80 and 20–40% lifetime risk of developing breast cancer and ovarian cancer, respectively (Kobayashi et al. 2013). More modest increase in risk of other cancers had been noted: additional sites include stomach, pancreas, prostate and colon. BRCA1 and BRCA2-related invasive epithelial ovarian cancers have better 5-years overall survival compared with sporadic ovarian cancer. The 5-year overall survival was better in BRCA2 carriers compared to BRCA1 carriers (36% for non-carriers, 44% for BRCA1 carrier and 52% for BRCA2 carriers (Kobayashi et al. 2013). The

spectrum of mutations is different depending on the race. The mutations are detected in 10–12% of Ashkenazi Jewish women diagnosed with breast cancer. Ashkenazi Jewish subjects are observed at increased frequency compared to other Caucasians, because this population harbors ancient BRCA1 and BRCA2 mutant alleles. The carrier frequency of hereditary breast and ovarian cancer syndrome is approximately 1 in 500 individuals in the general population, but it has a prevalence of 1 in 40 individuals in Ashkenazi Jewish population (Whittemore et al. 1997). Therefore, it is important to inquire about maternal or paternal ancestry in male and female relatives. Hereditary breast and ovarian cancer syndrome, as well as many other hereditary cancer syndromes, displays incomplete penetrance (meaning that not everyone with a gene mutation will develop cancer). The mutations include partial or complete gene deletions, duplications, large insertions, splice alterations, frameshifts, coding silent as well as missense and nonsense mutations. Deletions or insertions usually lead to abnormal structure and function. Germline mutations are usually pathogenetic point mutations, and are scattered throughout their coding regions. The potential hot-spot mutations within BRCA1 and BRCA2 are uncommon (Kobayashi et al. 2013). Ovarian cancer is a heterogenous disease that includes different biological behaviors at the clinical and molecular level. More than 90% of ovarian cancers are epithelial, while about 1% of ovarian cancers develop from germ cells or granulose-theca cells. Epithelial tumours may arise from the ovarian surface epithelium but also may arise from fallopian tube, foci of endometriosis, or the peritoneum. Two novel hypotheses for the pathogenesis of serous ovarian cancer have been recently proposed. According to the first mechanism, precursors of ovarian cancer develop in the fimbria from occult serous tubal intraepithelial carcinoma (STIC), and only subsequently do they involve the ovary. The second theory supports the implantation of normal epithelium from the fimbria onto the ovarian surface during ovulation, which results in a cortical inclusion cyst (CICs) where malignant transformation can arise (Kurman 2013). At the molecular level, sporadic ovarian cancer is characterized by a wide genetic instability linked to the modulation of several genes. According to clinical behavior and molecular genetic abnormalities, ovarian cancer can be classified into two different types. Type I tumours include low-grade serous carcinomas, borderline serous tumours, low-grade endometrioid, and mucinous and clear cell carcinoma. These kinds of tumors are relatively genetically stable, and the most frequent mutations involve KRAS, BRAF, ERB2, PTEN, PIK3CA genes, b-catenin gene (CTNNB1), ARID1A, and PPP2R1A. On the other hand, type II ovarian cancer includes high-grade serous carcinomas, carcinosarcomas, and undifferentiated cancers. Type II tumours, which comprise almost 70% of all epithelial tumours, are aggressive ones and present in advanced stage. At the time of presentation, they exhibit high genomic instability and, in up to 95% of patients, the gene mutated is TP53. Moreover, this type of tumor is characteristic of BRCA1 and BRCA2 mutation carriers and mostly arises from serous tubal intraepithelial carcinoma (STICs) (Kurman and Shih 2008; Kurman 2013). There were no significant differences in ovarian cancer morphology between BRCA1 and BRCA2 carriers. Ovarian cancer patients with BRCA mutations were associated with an increased

chemosensitivity and improved overall survival, but some investigators failed to confirm improved survival (Kobayashi et al. 2013).

9.5.2 Lynch Syndrome

Lynch syndrome, also known as hereditary non-polyposis colon cancer (HNPCC), is a highly penetrant autosomal dominant hereditary cancer syndrome caused by defects in the DNA mismatch repair (MMR) system. This syndrome predisposes young people (mean age 45) not affected by adenomatous colonic polyp, to develop colorectal cancer (lifetime cancer risk, 70–80%). Members of these families are also prone to excess of extracolonic cancers, including carcinomas of endometrium (50–60%), ovary (9–14%), stomach cancer (13–19%), small bowel, hepatobiliary tract, pancreas, brain, as well as transitional cell carcinoma of ureters and renal pelvis (Kobayashi et al. 2013; Toss et al. 2015).

Mutations in four MMR genes (MLH1, MSH2, MSH6 and PMS2) are associated with Lynch syndrome and account for another 10% of hereditary ovarian cancer. An average age of onset of ovarian cancer is 51 years in families associated with MLH1 mutations and 45 years in families associated with MSH2 mutations. From the clinical point of view, ovarian cancers in Lynch syndrome are mostly endometrioid or clear cell ones, and the tumours are less advanced at the time of diagnosis, showing strikingly high stage-specific survival rates. The most frequent extracolonic cancer is endometrial cancer. HNPCC (hereditary non-polyposis colorectal cancer) -associated endometrial cancer is diagnosed approximately 10 years earlier than in the general population, overall survival rate is similar in both groups. Germline mutations associated with Lynch syndrome has been described in 2–3% of patients diagnosed with endometrial cancer. Among Lynch syndrome-related cancer, endometrial cancer is riskier than colorectal cancer in terms of estimated lifetime cumulative risk (Meyer et al. 2009). The overall 5-year survival rate for endometrial (88% vs. 82%) or ovarian cancer (64% vs. 58%) was not significantly different between patients with endometrial or ovarian cancer that are associated with Lynch syndrome and the controls with sporadic cases (Masuda et al. 2011).

9.5.3 Other Hereditary Syndromes Associated with Gynecological Malignancies

Mutations in BRCA1 and BRCA2 as well as defects in MMR genes do not account for all cases of hereditary gynecologic tumours. The remaining cases can be attributed to the involvement of other susceptibility genes. Other genes, including Fanconi anemia (FA) cluster (FANCD2, FANCA, and FANCC), DNA checkpoint cluster (ATM, ATR, and CHK1/2), and tumour-suppressor cluster (TP53, SKT11,

and PTEN) have been associated with increased risk of breast and ovarian cancer as part of other cancer syndromes. There is an increasing understanding that the interrelationship between BRCA gene cluster and Fanconi anemia, mismatch repair and DNA repair gene status plays a key role in the pathogenesis of cancer predisposition syndromes (Kobayshi et al. 2013).

Li-Fraumeni syndrome is a rare autosomal dominant condition with increased risk of multiple primary neoplasms especially in children and young adults, with a predominance of soft tissue sarcomas, osteosarcomas, breast cancer, and an increased incidence of brain tumours, leukaemia and adrenocortical carcinoma. Less frequent malignancies associated with Li-Frumeni syndrome include ovarian cancer too. These tumours occur at earlier than expected median age at diagnosis. Particularly, for ovarian cancer the median age is 39.5, compared with 64.3 years for sporadic cases. The majority of Li-Fraumeni cases are caused by a TP53 germline mutation. In general, tumours associated with a TP53 germline mutation develop earlier than their sporadic counterparts, but there are marked organ-specific differences. The TP53 gene, located on chromosome 17p13.1, belongs to a family of growth suppressors and is frequently mutated in most forms of sporadic cancers, with prevalence that range from a few percent in cervical cancer and in malignant melanomas to over 50% in invasive carcinomas of the aero-digestive tract. Because of its comprehensive role as a cancer suppressor gene, TP53 is also defined as the "guardian of the genome" (Lane 1992). The most common mutations observed in germline and sporadic cases is the missense mutation (about 75%), resulting in a defective transcriptional activity. Tumours developed from acquired TP53 mutations are characterized by worse survival rates, increased resistance to chemotherapy and radiation, and elevated relapse rates (Olivier et al. 2003; Toss et al. 2015).

Ataxia telangiectasia syndrome is a rare progressive neurological disorder that manifests at the toddler stage. The disease is characterized by cerebellar degeneration (ataxia), dilated blood vessels in the eyes and skin (telangiectasia), immunodeficiency, chromosomal instability, increasing sensitivity to ionizing radiation and a predisposition to cancer, in particular leukaemia and lymphomas, cancers of the breast, stomach, ovary and melanoma. Germline mutations in the ATM gene are the cause of this autosomal recessive disorder. ATM gene is located on human chromosome 11q22-23. Heterozygous carriers of ATM mutations have a higher mortality rate and an earlier age at death from cancer and ischemic heart disease than non-carriers. Significant loss of heterozygosity in sporadic breast tumours across chromosome 11q22-23 where the ATM gene is located has been reported (Tavassoli and Devilee 2003).

Cowden syndrome is an autosomal dominant disorder caused by germline mutations of the tumour suppressor PTEN gene, which resides on chromosome 10q23.3. The mutations comprise loss-of-function mutations including missense, nonsense, frameshift and slice site mutations. This syndrome is characterized by multiple hamartomas involving organs derived from all three germ cell layers and a high lifetime risk of breast cancer (25–50%), uterine cancer (6–10%) and non-medullary thyroid cancer (Tavassoli and Devilee 2003).

Peutz-Jeghers syndrome is an autosomal dominant condition caused by mutation in the serine/threonine kinase 11 (STK1) gene. This syndrome is associated with an increased risk of breast cancer, ovarian cancer, cervical cancer (especially histologic diagnosis of adenoma malignum), uterine cancer, pancreatic cancer, lung cancer, stomach cancer, gastric cancer and colon cancer, as well as ovarian sex cord tumors. Women with Peutz-Jeghers syndrome have a 50% lifetime risk of developing breast cancer as well as an increased risk of ovarian cancer, uterine cancer, and cervical cancer (McGarrity 2016).

Defects in genes involved in the repair of double stranded breaks, other than BRCA1 and BRCA2, represent alternative mechanism of hereditary ovarian carcinogenesis. The double-strand breaks are repaired by homologous and non-homologous end joining. Several proteins are widely involved in the homologous repair system, including BRCA1/2, ATM, CHEK2, RAD51, and Fanconi anemia proteins BRIP and PALB. The mentioned above proteins interact and cooperate with BRCA1 and BRCA2 proteins in the DNA repair process, and therefore, in the maintenance of genomic stability. It has been hypothesized that genes coding for these proteins would be alternative candidates for ovarian cancer susceptibility. Particularly, tumours with a defect in the homologous repair system other than BRCA express the BRCAness profile. These tumours present a specific phenotype with feature and behavior similar to BRCA-related ovarian cancers, including sensitivity to DNA-damaging agents (i.e. platinum), improved disease-free intervals and survival rates, and high-grade serous histology. Interestingly, these BRCAness patients are at increased risk for both ovarian and breast cancers, similar to BRCA carriers. The main genes involved in the BRCAness syndrome in ovarian cancer are RAD51, PALB2, CHEK2, BARD1 genes (Toss et al. 2015). Between 50 and 80% of HBOC syndrome can be explained by defective germline mutation in BRCA1 and BRCA2 as well as, to a lesser degree, other genes described above, but in the remaining families the factor driving susceptibility remain unknown (Chen et al. 2006; Kobayashi et al. 2013).

Approximately one third of the HBOC families do not have evidence of the germline mutations in BRCA1 and BRCA2. The loss of BRCA function might be due to either germline/somatic mutation or epigenetic silencing. Since little is known about the contribution of epimutations or to the remaining BRCA1/2 mutation-negative cases, epigenetic silencing has been explored in HBOC syndrome. The activities of tumour suppressor genes and cancer susceptibility genes could be influenced by genetic and epigenetic alterations. Decreased expression of cancer susceptibility genes has been observed in sporadic breast and ovarian cancer where it is often associated with the aberrant epimutations or hypermethylation of the BRCA1 and BRCA2 genes. The loss of BRCA1 function due to hypermethylation explained about 10% of sporadic breast cancer cases (Kobayashi et al. 2013). Genetic or epigenetic loss-of-function mutations of genes that are known to be involved in the repair of DNA damage might lead to an increased risk of developing a broad spectrum of breast and ovarian cancers.

The identification of mutations in ovarian cancer susceptibility genes has a fundamental role both in the preventive setting and after the diagnosis of ovarian

cancer, in the selection of treatment. In healthy mutation carriers, the presence of one of these mutations may justify more intensive surveillance, chemopreventive approaches and/or prophylactic surgeries that would not otherwise be justified by family history alone. In this particular setting, the candidates for genetic testing should be identified according to their personal and family history of ovarian cancer. On the other hand, in already affected patients, the identification of a mutation in susceptibility genes may guide treatment decision-making by providing potential targets for biologic agents or by helping to select treatment strategies, that is avoiding radiotherapy in patients with Li-Fraumeni syndrome (Toss et al. 2012; Cortesi et al. 2014). Currently, the following avenues are being explored to improve the prognosis of women with a BRCA1 or BRCA2 mutations all aiming for either early detection or prevention of breast cancer and/or ovarian cancer: (i) regular surveillance, (ii) prophylactic surgery, and (iii) chemoprevention.

9.6 General Principles of Cancer Prevention. Precancerous Lesions. HPV Vaccination. Genetic Counseling

Cancer occurs everywhere in the world, and in virtually every part of the body. One in three individuals is likely to be affected by the disease at some point during the lifetime. But there is a great hope—the latest research confirmed that cancer is to a certain extent largely a preventable disease. Cancer is a complex group of diseases, all of which arise as a result of the uncontrolled reproduction of a damaged cell within a specific type of tissue or organ, to form a mass of cells or tumour. A wide range of factors, including genetics, and the environment including lifestyle, most of all play a key role in their development. The fact is, genetic make-up might increase the susceptibility to cancer, but it does not automatically mean that it will occur. Most cancers arise from environmental factors, evidence of which can be easily seen in studies of people who migrate from one part of the world to another. Such studies have shown that migrants rapidly acquire the cancer risk profile of their adoptive country rather than keeping that of the country of origin. And as rates of cancer at different body sites vary worldwide, it is clear that cancer risk is not determined solely by inherited factors.

Combination of genetic, behavioral, and environmental factors is involved in starting the chain reaction that turns a normal cell into an abnormal cell, and an abnormal cell into cancer. The detail biology of cancer is still poorly understood, but we do have an increasing understanding of the factors which are involved in the cancer process. Early detection of cancer greatly increases the chances of successful treatment and recovery. That is why after taking steps towards prevention, the other next important line of defense against cancer is to get to know the own body, and be aware of possible warning symptoms. Cancer cannot be eradicated completely, but healthy lifestyle can dramatically reduce the risk of many cancers, as well as other

diseases. Although great advances have been made in the detection, diagnosis and treatment of cancer, by far the greatest hope lies in its prevention. Oncopreventive measures present several levels.

Primary prevention is aimed at reduction of incidence of malignant tumours by eliminating or decreasing of the main risk factors, identified in epidemiologic studies, especially those associated with the lifestyle. It includes fight against tobacco smoking, alcohol drinking, skin protection. Very important are dietary choices, then a regular physical activity, and normal body weight. To the primary prevention measures we include elimination of potential carcinogenic compounds found in commercial products, as well as the vaccination against human papillomavirus. It can be summarized that the main risk factors that may be considered in primary prevention are: tobacco, alcohol, diet, obesity, sunlight, radiation, cancer-causing substances as well as age, chronic inflammation, immunosuppression and infectious agents. No less importance lies in detection of precancerous conditions and chemopreventive measures in order to influence development of precancerous conditions to cancer.

The aim of secondary prevention is to reveal and detect tumours in early and curable stadium. The secondary prevention programs include screening of breast cancer, screening of cervical cancer, and colorectal cancer screening. Special campaigns are aimed on detection of melanotic morphs, field-specific precancerous lesions and early carcinoma in specific group with high oncological risk. The specialized care and support should be provided to individuals with increased hereditary risk of cancer arising from genetic testing or family histories.

Tertiary prevention is aimed at recognition and diagnostics of tumour recurrence, and it leads to avoid misinterpretations of different analysis and incorrect assessment of further treatment possibilities. It is necessary to concentrate not only at previously treated disease sites, but to provide complex oncological preventive care to the individuals, in which the risk of occurrence of another tumour of different provenience is in general higher than in normal population.

Quaternary prevention must be aimed at anticipation and prevention of consequences of tumor disease progression with its adverse impact on quality of life. I must cover social, mental and somatic aspects as well.

A significant increase in the incidence of cancer is associated with physical and emotional problems of individuals, and their families. We cannot ignore the economic impact of this chronic disease both on individuals as on society. The economic burden of cancers is substantial worldwide. By preventing cancer the number of cancer patients can decrease as the number of deaths.

Except above mentioned general principles, there are several evidence-based intervention available to reduce just the gynecologic cancer incidence and mortality:

(a) routine vaccination against the HPV virus in young females and males as nearly all of cervical and 40–70% of vaginal and vulvar cancers are associated with the human papillomavirus (HPV) (De Vuyst et al. 2009; Forman et al. 2012),

(b) routine cervical screening for women of all age groups as the Pap test is able to detect precancerous lesions and cervical cancer at early stages,
(c) availability of the specialized and professional genetic testing and genetic counseling as some of the ovarian and uterine cancers are linked to genetic syndromes,
(d) concentration of patients with gynecological malignancies to specialized centers as high professional oncological care in these specialized oncological centers is associated with better survival among these patients. Studies have consistently demonstrated that gynecologic oncologists, subspecialists specifically trained to perform gynecologic cancer surgery and administer chemotherapy, more often adhere to standard treatment guidelines resulting in increased survival from gynecological cancers. The chance of receipt this standardized care for all women suffering from gynecologic tumours is a significant factor in better disease prognosis (Chan et al. 2011; Stewart et al. 2013).

9.6.1 HPV Vaccination

The appropriate fraction of cancers and other conditions caused by HPV is based on a variety of information, including epidemiological data and studies evaluating biopsy specimens for human papillomavirus (HPV). HPV, specifically HPV-16 and HPV-18, is responsible for a considerable burden of cervical and other anogenital cancers, oropharyngeal cancers, and cervical, vaginal, and vulvar intraepithelial neoplasias. The available data for the contribution of HPV, and HPV-16, and HPV-18 to cervical cancer and precancers are substantial. More than 70% of cervical cancers are caused by HPV-16 and/or HPV-18 worldwide, and other HPV types, including HPV-31, HPV-33, HPV-39, HPV-45, HPV-52, and HPV-58, contribute to the majority of the remaining cervical cancers. The contribution of HPV types other than HPV-16/HPV-18 to cervical cancers varies geographically. HPV infection is the necessary but not sufficient cause of cervical cancer, because not all individuals with infection develop cancer. Approximately 50% of high-grade squamous intraepithelial lesions (HSIL), which are considered a precursor to cervical cancer, are caused by HPV-16 and/orHPV-18 worldwide. Recent data suggest an important role for HPV as a cause of vaginal and vulvar precancerous lesions (Dunne et al. 2008).

The prevalence of genital HPV differs by population, sampling, and laboratory methods. The prevalence of infection has been found to be high, although this does not indicate persistent infection. Although most sexually active women acquire HPV infection, and many have persistent infection detected, available studies demonstrate that, by 2 years, more than 90% of women do not have detectable virus (Woodman et al. 2001). Some women develop persistent infection with high-risk HPV types, and these women are at the greatest risk for developing precancerous

lesions, such as HSIL of the cervix. Factors such as smoking, older age, HPV type, duration of infection, and suppressed immunity have been identified as predictors of persistent infections.

Two prophylactic HPV vaccines have been developed first: one is a quadrivalent HPV vaccine (HPV-6, HPV-11, HPV-16, and HPV-18), and the other is a bivalent HPV vaccine (HPV-16 and HPV-17). Although both vaccines are L1 VLP (virus-like particle) vaccines, they differ in the HPV type VLPs and adjuvans. The quadrivalent HPV vaccine (Gardasil) was licensed for use in the US in June 2006, the bilavalent HPV vaccine (Cervarix) in March 2007. Both vaccines are licensed in multiple countries worldwide (Dunne et al. 2008).

The third vaccine available newly—Gardasil 9—widens the scope of the quadrivalent vaccine. All three vaccines prevent infections with HPV types 16 and 18, the two high-risk HPV that cause about 70% of cervical cancers and an even higher percentage of some of the other HPV-associated cancers. Gardasil also prevents infection with HPV types 6 and 11, which cause 90% of genital warts. Gardasil 9 prevents infection with the same four HPV types plus five additional high-risk HPV types (31, 33, 45, 52 and 58) and is therefore called nonavalent, or 9-valent vaccine. All three vaccines are given though a series of three injections into muscle over a 6-month period. The FDA approved Gardasil and Gardasil 9 for use in female ages 9 through 26 for the prevention of HPV-caused cervical, vulvar, vaginal, and anal cancers; precancerous cervical, vulvar, vaginal, and anal lesion; and genital wart. Gardasil and Gardasil 9 are also approved for use in males for the prevention of HPV-caused anal cancer, precancerous anal lesions, and genital wart. Gardasil is approved for use in male ages 9 through 26 and Gardasil 9 is approved for use in males ages 9 through 15. Females and males who have previously received Gardasil may be also received Gardasil 9. The Cervarix vaccine targets two HPV types 16 and 18 and is called a bivalent vaccine. The FDA has approved Cervarix for use in females ages 9 through 25 for the prevention of cervical cancer caused by HPV. In addition to providing protection against the HPV types included in these vaccines, the vaccines have been found to provide partial protection against a few additional HPV types that can cause cancer, a phenomenon called cross-protection. The vaccines do not prevent other sexually transmitted diseases, nor do they treat existing HPV infections or HPV-caused disease. Because currently available HPV vaccines do not protect against all HPV infections that cause cancer, it is important for vaccinated women to continue to undergo cervical cancer screening. There could some future changes in recommendations for vaccinated women (https://www.cancer.gov/human Papillomavirus(HPV) Vaccines-NHI). The HPV vaccines have been identified as highly effective in preventing infection and disease associated with types included in the vaccines. Vaccination is not a substitute for routine cervical cancer screening, and vaccinated individuals should have cervical cancer screening as currently recommended. The widespread use of prophylactic HPV vaccine could reduce cancer precursor lesions and cancers associated with HPV types (Dunne et al. 2008).

9.6.2 Precancerous Lesions

When exfoliative cytology was introduced some 70 years ago for cervical cancer screening, it offered the hope of eliminating deaths from this malignancy in an adequately screened population, because it was capable of detecting occult cancer and, even more important, precancerous conditions. Successful programs have been reported from many countries. In general, the incidence and mortality rates for cervical cancer have been reduced, and the proportion of early cases has increased. Most of the patients in the target populations who develop cancer have not been screened. Despite the relative ease of the procedure, even when it is available free of cost, women fail to report for repeating testing during their years at risk. On the other hand, the lower-risk group tends to be overscreened. The incidence of preinvasive lesions (squamous intraepithelial lesion, high (H SIL) or low (L SIL), older terms dysplasia, cervical intraepithelial neoplasia) relative to those of invasive cancer is surprisingly large. There is any clearly convincingly mean to distinguish the lesions which will progress from those which will not. This, of course, greatly increases the cost of preventive care. Cervical cytology has also proved to be less accurate than it was anticipated. New advantages are expected from liquid base cytology and high rate HPV contesting, in comparison with conventional cytological smears. Cervical cancer prevention is at a transition from cytology based screening programs to HPV-based prevention. With primary prevention using vaccines and secondary prevention using a highly sensitive HPV DNA test wit long-term negative predictive value at hand, extending screening intervals will be crucial for these programs to work. New biomarkers will be important to decide who among the HPV-positive women needs to be referred for further evaluation or treatment. Disease specific biomarkers such as p16, HPV E6/E7 mRNA, or novel methylation essays may serve as secondary markers after a positive HPV DNA test to identify women with prevalent precancers who require immediate colposcopy or treatment. The identification of prognostic biomarkers that can predict progression to invasive cancers is an important but challenging area of biomarkers research. Candidate biomarkers will be increasingly available for clinical validation through new technologies. Markers of viral and host methylation changes, markers demonstrating chromosomal imbalances, miRNA, and proteomics markers are some of the most likely candidates that may progress further in the developmental pipeline to clinical correlative studies and may play an important role in the therapy decisions (Sahasrabuddhe et al. 2011).

A disturbing aspect of cervical cancer screening is the frequency with which invasive carcinoma is diagnosed in women who have had normal Pap smear. It is expected that invasive squamous carcinoma nearly always arise from a precursory lesion and the transit time to invasive lesion would take several years. Thus, even with long intervals between Pap smears, it was expected that the time for the detecting precursor lesion would be satisfactory. Unfortunately, the failures of this system are numerous. There is, for example an inherent false negative rate for Pap smear, than a fact that some of the tests are misinterpreted. Some of the women with

abnormal Pap test dot not return for follow-up evaluation until years later. There is, however, evidence that some small amount of squamous carcinoma evolve from cytological normal epithelium during a 2–5 year time period. Incorporation of the HPV testing into cervical screening is aimed to establish the less-frequent but more effective method. HPV testing is more sensitive than cytology while cytology is more specific. A promising future strategy is: HPV test first, then cytology. Several studies confirmed the superior sensitivity of HPV testing for detection of CIN (cervical intraepithelial neoplasia) 2+. In addition, the novel biomarkers for HPV oncogenic activity (p16 and Ki-67) may eventually play a greater role in primary screening. The unscreened and underscreened population carries a higher burden of cervical cancer and of death from cervical cancer (Jin et al. 2013).

Premalignant disorders of the lower genital tract include the mentioned cervical precancerous lesions and also preinvasive lesions of vagina and vulva. These findings are quite rare in comparison with cervical lesions. The main risk factors are similar both for cervical as for vulvar and vaginal intraepithelial neoplasia.

The previous three-tier terminology (cervical intraepithelial neoplasia CIN1, 2, 3, vulvar intraepithelial neoplasia VIN1, 2, 3, and vaginal intraeptihelial neoplasia VAIN 1, 2, 3) was replaced with a two-tier system of low- and high-grade intraepithelial lesions (LSIL and HSIL respectively). The rationale for this change is that the two-tier system is more biologically and clinically relevant and histologically more reproducible. High-grade squamous intraepithelial lesions are classified as precancerous conditions, meaning they carry a significant risk if invasive cancer development if untreated. Low-grade intraepithelial lesions of squamous epithelium represent the clinical and morphological manifestation of a productive HPV infection and refer to the associated low risk of concurrent or future cancer. High-grade intraepithelial lesions of squamous epithelium of uterine cervix exhibit aneuploidy more frequently than polyploidy, a reflection of genetic instability. In addition they show relatively more frequent HPV DNA integration compared to LSIL. Similar biological concept is applied in terminology of glandular cervical precancers. Adenocarcinoma in situ or high-grade cervical glandular intraepithelial neoplasia (HG-CGIN) carries a significant risk of invasive adenocarcinoma if not treated. The most common presentation of adenocarcinoma in situ is abnormal cervical cytology that shows atypical endocervical glandular cells, often associated with high-grade squamous intraepithelial lesions (HSIL).

Invasive cancer of the vulva is derived from two different entities. The more common, keratinizing carcinoma, seen in older women, usually arise, in a background of lichen sclerosus and/or squamous hyperplasia, so called non neoplastic disorders of vulva or differentiated-type vulvar intraepithelial neoplasia. The lesion is HPV negative. The risk of cancer increases with age and duration of chronic anogenital inflammatory skin disease. In younger women the carcinoma is related to human papillomavirus (HPV)-associated warty/basaloid vulvar high-grade squamous intraepithelial lesion, with high-grade malignant potential. Many women with invasive vulvar cancer have a long history of vulvar symptoms and have had inadequate investigation and treatment of precursor lesions. The worldwide increasing incidence of vulvar neoplasms in young women demands increased

awareness of vulvar symptoms. HSIL has a significant invasive potential, and treatment should aim to eliminate the condition without causing mutilation (Joura et al. 2000). Human papillomavirus is thought to be a major etiologic factor in a rare occurring vaginal, as well as in cervical and vulvar, high-grade squamous intraepithelial lesion, sometimes so called low genital tract neoplasia syndrome. The most common location of vaginal HSIL is the upper third of the vagina. The lesions are best evaluated with colposcopy. The posthysterctomy patient must be considered to appearance of this mostly multifocal affection. The low-grade squamous intraepithelial lesions (LSIL) of vulva or vagina refer to low risk of concurrent or future cancer.

Precancerous conditions of endometrium have a slightly increasing incidence. Serous endometrial intraepithelial carcinoma is non-invasive, immediate precursor of invasive uterine serous carcinoma. The unique feature of serous endometrial intraepithelial carcinoma is that, although it does not invade the endometrium, it is frequently associated with disseminated pelvic serous carcinoma. It occurs predominantly in elder women, may be in association with a cumulation of mutations due to higher age. Precursors of endometrioid carcinoma are hyperplasia without atypia (progression to well-differentiated endometrioid carcinoma occurs in 1–3% of women with hyperplasia) and atypical hyperplasia/endometrioid intraepithelial neoplasia. The second one condition coexists with carcinoma in approximately 25–40% of women. It occurs more frequently in perimenopausal women, it is estrogen-related. The preventive measures are the same as in endometrial carcinoma, as well as symptoms. Ultrasound thickness of endometrium may be a warning sign, without biopsy it cannot be distinguished from disorderly proliferative endometrium. If bleeding does occur, endometrial sampling may be performed and a diagnosis established.

Currently screening techniques utilizing serum CA-125 levels, pelvic ultrasound, and frequent pelvic examination are not likely to have the necessary sensitivity nor the specificity to markedly increase the number of patients diagnosed with ovarian cancer at an earlier stage. Consequently, the optimal management for women in the high-risk familial ovarian cancer syndromes remains controversial. If prophylactic oophorectomy is recommended at a minimum, women should be counseled that such a procedure is not always protective and they may be at risk for the development of intra-abdominal carcinomatosis, even when histologically normal ovaries are removed. Precisely defined precancerous conditions of ovarian cancer are not known. The preventive measures do arise from knowledge of common risk factors described in the epidemiological studies (Kurman 2013).

9.6.3 Genetic Counseling

Women with personal and family histories consistent with gynecologic cancer-associated hereditary cancer susceptibility disorders should be referred for genetic risk assessment and counseling. Genetic counseling facilitates informed

medical decision making regarding genetic testing, screening, and treatment, including chemoprevention and risk-reducing surgery. Because of limitations of ovarian cancer screening, hereditary breast and ovarian cancer-affected women are offered risk-reducing bilateral salpingo-oophorectomy (BSO) between ages 35 and 40 years, or when childbearing is complete. Women with documented Lynch syndrome, associated with mutations in mismatch repair genes, should be screened at a young age and provided prevention options, including consideration of risk-reducing total abdominal hysterectomy and bilateral salpingo-oophorectomy, as well as intensive gastrointestinal screening. Clinicians caring for high-risk women must consider the potential adverse ethical, legal, and social issues associated with hereditary cancer risk assessment and testing. Additionally, at-risk-family members should be alerted to their cancer risks, as well as the availability of risk assessment, counseling, and treatment services (Miesfeldt et al. 2013).

Cancer risk associated with hereditary cancer syndromes are significantly elevated in comparison to general population risks, and there is both as increased risk of developing more than one primary cancer, and of having an earlier age of onset than is typical. Consequently, for unaffected, at-risk individuals, cancer screening is usually indicated at an earlier age, and may include different and/or more frequent screening tests than those used in average risk population. Individuals with cancer syndromes who are diagnosed with cancer may also be offered different surgical treatment options. Therefore, identification of individuals at increased risk for hereditary cancer has implications for screening and clinical management (Riley et al. 2012). Genetic testing and risk assessment is the process of identifying and counseling individuals at increased risk of developing cancer, and distinguishing between those at high risk (highly penetrant hereditary cancer syndrome), those at a modestly increased risk (multifactorial etiology) or low penetrance allele), and those at average risk. Using a combination of pedigree analysis, genetic testing, risk modeling, biochemical tests and imaging, and sometimes consideration of physical features, potential hereditary syndromes are identified and cancer risk are quantified for patients and their biological relatives. The information is then used to develop a management plan for cancer screening, prevention, and risk-reduction as well as notification of at-risk family members. Genetic counseling also includes patient education about hereditary cancer syndromes and assistance coping with the psychological responses that can occur in families at increased cancer risk (Trepanier et al. 2004).

The recommendations of the National Society of Genetic Counselors concerning counseling for hereditary breast and ovarian cancer are summarized in the practice guidelines and include following steps: gathering personal medical and family history data, psychosocial assessment, discussion of cancer and mutation risk and how personalized risk estimates are derived, facilitation of the informed consent process through discussion of the risks, benefits, limitations, and likelihood of identifying a mutation with genetic susceptibility testing results disclosure (if applicable), discussion of medical management options, review of issues related to genetic discrimination (Trepanier et al. 2004). Genetics professionals are uniquely

suited to facilitating clients' understanding of the genetics of cancer, personalized risk calculations, and the potential psychological, social, and medical implications associated with cancer risk assessment and genetic testing. Genetic professionals are also adepts at identifying clients who may need additional support, and providing a referral to appropriate mental health care professionals (Berliner et al. 2013).

9.7 Psychosocial Aspects of the Gynecological Tumours

Understanding of the psychosocial consequences of cancer has increased during the past few decades. In response, supportive psychosocial intervention strategies have been developed and are tailored to the problems that cancer patients face during the course of their disease.

9.7.1 Personality Traits and Cancer

Psychosocial factors such as *personality traits* and depression seem to alter immune and endocrine function, with possible effects on cancer incidence and survival. Although these factors have been extensively studied as risk and prognostic factors for cancer, the association remains unclear. The findings do not support the hypotheses that personality traits and depression are direct risk factors for cancer and cancer survival (Nakaya 2014). Personality traits have long been hypothesized to have a causal role in cancer development and progression. In 1962, Kissen and Eysenck conducted one of the first modern studies on the association between personality traits and cancer and reported that, as compared with hospital controls, patients with lung cancer were more likely to be extraverted and less likely to be neurotic. It could be interpreted to indicate that extraverts are at increased risk of cancer because they seek stimulation and thus experience high levels of stress, whereas individuals with low levels of neuroticism could be at increased risk of cancer because they tend to have fewer emotional outlets and therefore accumulate emotional stress (Eysenck 1988; Nakaya 2014). Since that, several well-conducted prospective studies found no direct association between personality traits (e.g. extraversion, neuroticism, and trait anxiety) and cancer risk. The association between neuroticism and prevalent cancer may be a consequence rather than a cause of cancer diagnosis and symptoms.

The hypothesis regarding cancer survival could also be interpreted as being related to stress. Accumulated repression of emotions may cause stress, which could affect cancer progression by influencing immune and endocrine function (Kiecolt-Glaser and Glaser 1999; Antoni et al. 2006). Patients with low extraversion and high neuroticism are believed to repress their emotions, which are considered one of the most important aspects of the type C personality (Eysenck 1990).

It has been hypothesized that depression, quite common among cancer patients, affects mortality risk in cancer patients through endocrine and immunologic pathways. Another possible explanation for the increased mortality observed among cancer patients with depression is that depression may simply reflect poor clinical status, which by itself would be associated with increased cancer mortality (Nakaya 2014).

It is suggested that serious psychosocial problems may develop among *partners of cancer patients.* The effect of cancer on the psychological well-being of partners could increase the risk of several psychiatric disorders related to stressfull life events. The mechanism underlying this hypothesis implies the existence of not only psychological distress from caregiving and grief but also a shared unhealthy lifestyle. The mechanism of these effects may involve several interacting pathways: the event may cause stress in the partner; it may deprive the partner of emotional, social, and economic support; and it can influence the daily life and behavior of partner (House et al. 1988; Nakaya 2014).

9.7.2 Psychoneuroimmuological Aspects

Notwithstanding the above observations, there is the substantial evidence from both healthy population under stress as well as individuals with cancer-associated psychological stress with *immune down-regulation*. Cancer is comprised of a heterogenous group of diseases with multiple etiologies, and immunological involvement varies across different cancers. Those cancers that are induced by chemical carcinogens may be less influenced by psychological, behavioral, and immunological factors than cancers that are associated with a virus. A significant role in malignant process belongs to natural killer (NK) cell. NK cells play an important role in a variety of immune functions, including defense against viral infection and surveillance of tumour cells. Cytokines such as recombinant interferon-gamma and recombinant interleukin-2 can enhance NK cell and lymphocyte-activated killer cell cytotoxicity. NK cell cytotoxicity can be down-regulated by stress, presumably through neuroendocrine mechanisms. In addition, distress or depression is also associated with two important processes in carcinogenesis: poorer repair of damaged DNA, and alteration in apoptosis (Kiecolt-Glaser and Glaser 1999).

Across a broad number of studies, stressors are associated with dysregulation of the immune system and in particular, decreased lymphocyte proliferation and reduced NK cell cytotoxicity are consistently observed. These studies demonstrate that stress can dysregulate NK cells function, including depressing the stimulatory response of NK cells to cytokines. Psychosocial factors may also act in concert with other risk factors for cancer to promote immune dysregulation, For instance, depression and smoking had synergic effects on reduced NK cells. It is therefore, possible that stress could alter potentially important defenses against malignant disease (Zorrilla et al. 2001; Kiecolt-Glaser et al. 2002).

Psychological stress can be considered to be a factor that may affect ability of NK cells to function properly, and thereby has an impact on one aspect of how the immune system defends the body against the spread of tumour cells. Stress may also have a direct effect on the initiation and/or production of abnormal cells independent of the immune system. Most carcinogens appear to induce tumours by damaging cellular DNA producing abnormal cells. The processes for repair or destruction of damaged DNA are critical, since faulty DNA repair is associated with an increased incidence of cancer. It is important to consider the stress-related DNA repair deficits in light of discussed stress-related decrements in NK activity. Taken together, these data suggest that stress might have direct effects on carcinogenesis through alterations in DNA repair, as well as indirect effects through the poorer destruction or elimination of abnormal cells. The alterations in apoptosis provide additional evidence of pathways through which psychological stress could contribute to increased cancer risk by modifying cell responses to environmental factors such as tumour promoters and oncogenic viruses. Apoptosis is a process of genetically programmed alterations in cell structure that leads to failure of proliferation and differentiation and eventual cell death. Psychological distress could ultimately lead to progressive accumulation of errors within the cell genome, reduced immune competence and increased risk of environmentally associated malignant and infectious diseases. The mechanism linking the physiological changes in DNA repair, apoptosis and sister chromatid exchange is not known. Since the hypothalamic-adrenal-pituitary axis and the autonomic nervous system are activated by stress, it is possible that one or more "stress" hormones may mediate theses responses. The possibility that psychological interventions may enhance immune function and survival among cancer patients does the evidence suggesting that social support may be a key psychological mediator (Kiecolt-Glaser and Glaser 1999; Kiecolt-Glaser et al. 2002).

The brain, as an adaptive and dynamic synthesizer of experiential and perceptual processes, can participate in the complex regulation of signaling systems used by the diverse array of cells and structures to enable carcinogenesis. Experimental and clinical studies suggest that downstream activation of the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis exerts selective physiologic pressures that initiate molecular signaling pathways involved in DNA repair, angiogenesis, cell survival, inflammation, invasion, metastasis, and resistance to therapy (Antoni et al. 2006; Cole and Sood 2012). Immune-to-brain communication cascades are thought to undergird cancer and treatment-related symptoms such as fatigue, depression, cognitive dysfunction, and sleep disturbances (Bower et al. 2011; Dantzer et al. 2012).

The links between psychosocial factors and the development of malignancy is assumed, however there is much stronger evidence that psychological factors play an important role in cancer progression and mortality (Penninx et al. 1998; Ross 2008; Lutgendorf et al. 2010). One likely mechanism linking psychological outcomes to cancer progression is dysregulated immune function: stress can suppress cellular immune function and enhance inflammation. The autonomic nervous system (ANS) and hypothalamic-pituitary-adrenal (HPA) axis compose two major

pathways by which stress dysregulates immune functions. Lymphocytes, macrophages, and granulocytes have receptors for products secreted by ANS and HPA axes. Biochemical mediators released through these pathways (e.g. cortisol from HPA axis and epinephrine from sympathetic-adreanal-medullary axis) increase the release of certain signaling molecules of the immune system, notably pro-inflammatory cytokines, such as interleukin-1 (IL-1) and intereleukin-6 (IL-6). Continued exposure to high concentrations of stress mediators causes decreased expression of glucocorticoid receptors, thereby leading to cortisol resistance, which ultimately inhibits cortisol from exerting its downregulating action on the HPA axis. Normal cortisol regulation of the immune system response is lost, leading to a pro-inflammatory state and immune system dysregulation (Glaser and Kiecolt-Glacer 2005; Cohen et al. 2007; Webster Marketon and Glaser 2008). Norepinephrine and epinephrine, catecholamines that are released by the sympathetic nervous system during stress, can promote tumours cell proliferation (Webster Marketon and Glaser 2008; Fagundes et al. 2013). Catecholamines and psychological factors can also modulate vascular endothelial growth factor (VEGF) which is an important angiogenesis promotion agent. When VEGF activates endothelial cell, they produce matrix metalloproteinase (MMPs) enzymes, a family of matrix-degrading enzymes that contribute to angiogenesis by promoting endothelial migration (Lutgendorf et al. 2008). Proinflammatory cytokines such as interleukin 6 (IL-6) and interleukin-8 (IL-8) also promote angiogenesis, their higher levels were found in patients with lower social support (Costanzo et al. 2005). Stress alters natural killer (NK) cell activity. Biobehavioral factors are important in tumour gene expression. Higher levels of depression and lower social support were associated with the upregulation of over 200 gene transcripts involved in tumour growth and progression. These findings suggest that psychological factors can impact cellular functioning, even at the molecular level (Lutgendorf et al. 2009). Circadian rhythm and cortisol production can be disrupted by psychological stress as well as sleep disruption, with impact on cancer progression (Sephton et al. 2000). Psychological stress and depression can drive latent virus reactivation or replication by impairing the ability of the cellular immune system to control viral latency. Stress hormones can impact a variety of cell-mediated immune responses affecting both the recognition of tumour and immunological defense against them. HPV's initiate tumour-supporting genetic and immunological changes when activated by glucocorticoides (Antoni et al. 2006; Fagundes et al. 2013).

Except the above mentioned interactions of psychosocial factors with the immune system, the interaction of immune system with brain must be considered in contribution to the quality of life of cancer survivors. Fatigue and depression can be side effects of long term and persistent low-grade inflammation. Proinflammatory cytokines can access the brain through a variety of key pathways and act on the brain to facilitate sickness behaviors by reducing connectivity of brain areas associated with lethargy. Alterations in immune regulatory systems that are linked to inflammation may play an important role in fatigue and depression (Raison et al. 2006; Bower 2007). Dysregulation of the immune system has critical consequences for the cancer population, such as increasing susceptibility to infection and the

progression of cancer, thereby increasing the likelihood of cancer recurrence and development of secondary cancers (Thaker et al 2007; Antoni and Lutgendorf 2007).

9.7.3 Psychosocial Support Approaches

Advances in prevention, detection, and treatment continue to yield significant declines in the incidence of most cancers and death rates for all cancers combined. These trends, combined with overall increase in life expectancy, have created a "booming [aging] cancer survivors population" (Parry et al. 2011). These survivors are at risk for recurrence and subsequent malignancies, as well as cardiac, vascular and other complications. Their need in psychosocial support is more urgent and justified compared with other population. Equally relevant needs are recorded in the group of individuals with hereditary susceptibility to cancer (Eijzenga et al. 2014).

A variety of psychosocial therapies for managing acute and chronic stress have been developed for patients with cancer, based on the scientific framework of psychoneuroimmunology (PNI). These psychosocial therapies, also referred to as psychosocial interventions, involve a wide range of activities, such as educating patients, enhancing coping skills, and providing supportive care to cancer patients (Fawzy 1999; Newell et al. 2002).

Although studies supporting a relationship between psychological stress and adverse health outocomes have been published since the 1940s, substantial progress in psychomeuroimmunological (PNI) research occurred only after 1980. A study published by Spiegel et al. (1989) drew considerable attention to psychosocial intervention research, as it reported an increased survival rate in breast cancer patients who participated in a group-therapy psychosocial intervention. Though the study by Spiegel et al. was controversial and replication studies of their group-therapy intervention failed to demonstrate increased survival, researchers became interested to explore the neuroendocrine-immune effects of a variety of psychosocial therapies, such as cognitive-behavioral stress management and supportive therapy in persons with cancer (McGregor and Antoni 2009; Boesen and Johansen 2008; Kissane 2007). The major types of therapies include cognitive-behavioral therapies, and complementary medical therapy. The cognitive-behavioral therapy consists of intervention that emphasizes cognitive and behavioral activities and approaches such as cognitive restructuring, psycho-education, and coping skill training. Complementary medical therapies involve activities such as yoga, meditation, qigong, mind-fullness-based stress reduction and massage. Remarkable advances have occurred in innovative medical technologies and therapies for patients with cancer such as targeted chemotherapy, robotic surgery and positron imaging tomography/positron emission tomography scans for radio-imaging. Past experiences suggest that some psychological therapies have the potential to influence PNI-based outcomes. However, psychosocial therapies have not witnessed the same advancement in scientific support and

technological delivery (Subnis et al. 2014). Further coming studies of psychosocial therapies should attempt to demonstrate changes in psychosocial outcome measures to predict changes or show associations in neuroendocrine-immune biomarkers over time to provide evidence for their PNI mechanism of action. While it appears likely that the inflammatory mechanism is a major contributor toward a tumour-promoting environment that may also involve cellular transformation, the proliferation and survival of malignant cells, development of angiogenesis and mestastases, and reduction of adaptive immune response, direct causation between inflammation and tumour has not been yet established. Some data suggest that psychological factors such as major depression, chronic and daily life stress and anger suppression may trigger an inflammatory response. Unregulated, and often aggravated by the contribution of behavioral factors (dietary, obesity, smoking, sedentary life-style), such immunological response often develops into chronic disease. Although there is no evidence to support a direct effect of psychological distress on the development of malignancies, psychosocial factors should be a target of critical importance in clinical settings as they are often modifiable and such intervention may alter or even prevent the course of chronic disease associated with cancer development (Fagundes et al. 2013).

The effectiveness of psychological intervention including the group therapy for cancer survivors, interventions for couples coping with cancer, implementation of methods of "positive psychology" in cancer care as well as psychological consequences of predictive genetic testing present still topics for further research and development.

9.7.4 Psychological Aspects of Genetic Testing

Predictive genetic testing are being offered for an increasing number of conditions, including single gene conditions (Huntington's disease), and multifactorial condition such as hereditary cancers and familial hypercholesterolemia (Broadstock et al. 2000). Prenatal testing and carrier testing were among the first services offered, affording an opportunity for individuals to learn whether they had transmitted an altered gene to their offspring. As these tests provided information about the risk to the fetus, the focus of counseling tended to be on reproductive decision making. On the other side, genetic testing being applied to detect personal susceptibility to disease is shifting the focus counseling to personal risk reduction. The hope is that awareness of genetic risk will enhance informed medical decision making by physician and patients alike. However they may also be psychological and social risks of genetic testing that should be considered, regardless of the potential medical benefits provided by testing. Across all genetic-testing domains, a common theme is that participants' decision about testing are influenced less by their actual risk status than by subjective risk and emotional factors (Lerman et al. 2002). Individuals form families with a known hereditary cancer syndrome and individuals with familial occurrence of cancer may carry a germline mutation. Over 50 hereditary cancer

syndromes have been identified. Individuals who carry a germline mutations or one of these cancers syndrome have a significant higher risk of development cancer compared to the general population. Proven carriers or individuals at high risk of carrying mutations may benefit of screening options and possible other treatment options (Lindor et al. 2008; Eijzenga et al. 2014). The main themes identified in reported studies concerning genetic testing on cancer syndromes are following: coping with cancer risk, practical problems (health or life insurance), family-related problems, children-related problems, living with cancer and fear of developing of cancer, emotion reaction reflecting uncertainty about the future (Eijzenga et al. 2014).

Only a minority of individuals who undergo cancer genetic counseling experience heightened levels of psychological distress, but many more experience a range of cancer genetic-specific psychosocial problems. It is supposed the potential importance of asking counseling about the specific psychosocial problems of clients at the time of cancer genetic counseling, prior to undergoing DNA testing and receiving the DNA results. Studies of the routine use of patient-reported outcome measures in daily clinical practice have demonstrated their value in enhancing communication between patients and their health care providers (Broadstock et al. 2000). The individuals undergoing predictive genetic testing do not experience adverse psychological consequences. Pre-test emotional state is predictive of subsequent distress in a minor part of tested patients. It is suggested that testing protocols should include a pre-test of emotional state so that post-test counseling can be targeted at those more distressed before testing (Broadstock et al. 2000).

Women who are counseled and tested for mutation in BRCA1/2 genes and which have minor-age children confront difficult decisions about, when, and how to share hereditary breast/ovarian cancer risk information with their children. These choices are often seemingly influenced by how mothers anticipate the emotional burden they and their children will experience in response to test results (Tercyak et al. 2013; O'Neill et al. 2015). It was estimated, that maternal anxiety/depression surrounding genetic counseling have long been tied to poorer emotional and behavioral outcomes among those seeking BRCA1/2 testing. Whether this finding reflects women's accurate understanding of their tendency to experience negative affect and/or reflects biased forecasting as noted above is unclear. The general psychological well-being should be screened at the time of the pretest genetic education and counseling session to identify those who are more susceptible to adverse emotional reactions following testing (O'Neill et al. 2015).

Concurrently, the growing field of integrative cancer care which seeks to synthesize evidence-based therapies that mutually address the physical and psychosocial-spiritual needs of cancer patients, has been generating an extensive evidence base (Block 2002; Geffen 2010). Multiple signaling pathways by which "macroenvironment" can influence the tumour microenvironment are identified, but many unanswered questions remain. For example, numerous effects of catecholaminergic and glucocorticoid signaling on tumour growth and progression have begun to be mapped, but it is likely that there are multiple downstream effects on tumour growth processes, many of which have not been identified. These include

sustaining proliferative signaling; evading growth suppressors; avoiding immune destruction; enabling replicative immoratality; tumour-promoting inflammation; activation invasion and metastasis; inducing angiogenesis; genome instability and mutation; resisting cell death; and deregulating cellular energetic. In addition to effects on the tumour and microenvironment, there are likely multiple upstream biobehaviorally modulated pathways that may affect tumour growth. These include the role of the parasympathetic nervous system, of biobehaviorally sensitive neuropeptides and hormones such as oxytocin, prolactin, growth hormone, and prostaglandins, as well as a variety of metabolic mediators (e.g. insulin growth factor-1, leptin, and ghrelin) that are sensitive to biobehavioral pathways. Biobehavioral mediators seldom work alone. However, to understand the relevannt mechanism, it will be important to understand downstream effects of interconnected pathways. Along with understanding how biobehavioral pathways regulate tumour growth, the effects of biobehavioral pathways on recovery from specific cancer treatment are important. Future research holds promise for discovery of novel biobehavioral signaling pathways that are relevant to cancer and greater understanding of behavioral, pharmacological, and complementary interventions that target these mechanisms (McDonald et al. 2013).

9.7.5 Specific Psychosocial Aspects of the Gynecological Oncology

Although cancer in general affects an aged population, a significant number of women develop cancer at childbearing age. Long-term survival rates after gynecological cancer, especially in young patients are increasing. Consequently, all quality-of-life aspects, including preservation of fertility have become of major relevance. Indications for fertility-sparing surgery are in general restricted to women presenting with a well differentiated low-grade tumour in its early stages or with low malignant potential. Several current fertility-sparing cancer treatments may result in subfertility and in those cases assisted reproductive techniques are indicated (Feichtinger and Rodriguez-Wallberg 2016).

The morbid conditions associated with prior anticancer treatments including chemotherapy, radiotherapy, surgery, and/or hematopoetic stem-cell transplantation may induce not only obstetric and neonatal complications but also long-term effects on offspring. Whereas some risks are predominantly evidenced in untreated women others are observed in both treated and untreated women. This risk may be superimposed on those induced by current women's trend in western societies to postpone maternity. Most female childless cancer survivors may feel cancer-related infertility as an emotionally distressing and devastating health problem. This distress, however, may be attenuated if female cancer patients were properly informed about the risks to fertility of anticancer therapies and offered fertility preservation options prior starting any treatment (Tarin et al. 2016).

9.8 Summary

The chapter focuses on the main aspects of the issues relating to gynecological malignancies and their reflection in the psychosocial context. It gives an overview of gynecological cancers including epidemiological, histopathological, and clinical characteristics, as well as diagnostic methods and therapeutic approaches. This applies to cancers of the vulva, vagina, uterine cervix, uterine corpus, fallopian tubes, and ovaries. Attention is given to the prevention of malignant tumours of the reproductive system on a general level, common for most of malignancies, at the same time focusing on some special options. The others mentioned above include HPV vaccination, cervical cancer screening with Pap smear tests, management of the detection and treating of precancerous conditions, as well as the genetic counseling of individuals with hereditary cancer susceptibility. The identification of mutations in cancer susceptibility genes plays a role both in the affected individuals and in the healthy mutations carriers. It may influence the selection of treatment in the first mentioned group, as well as it may justify more intensive surveillance, chemopreventive steps and/or prophylactic surgeries in the second group. Another part of the chapter deals with the relationship between pregnancy and cancer. The most common malignant tumours occurring in pregnancy are discussed, as well as the aspects of pregnancies of women with prior anticancer therapy.

A special attention is paid to the psychoneuroimmunological and psychosocial aspects of cancer development and the relationship between personality traits and cancer. The psychosocial support approaches, psychological aspects of genetic testing are discussed with regard to the specifics of women's health associated with the fertility of the women with cancer.

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Chapter 10
Cancers of the Endocrine System

Mohammad Amin Tabatabaiefar, Abbas Moridnia and Laleh Shariati

Abstract *Endocrine cancers*: Endocrine cancers are an important category of cancers. The frequencies of different types of endocrine cancers vary greatly. While thyroid cancer subtypes constitute approximately 1% of new cancer cases in the United States each year, prostate cancer assumes a top rank after cutaneous cancers; one in six white men and one in five African-American men are diagnosed with prostate cancer. This chapter reviews the updates on different aspects of endocrine cancers including pathophysiology, classification, genetics, genotype-phenotype correlations, screening, surveillance, genetic counseling, testing strategies, management and individualized medicine of Type 1 multiple endocrine neoplasia (MEN1), Type 2 multiple endocrine neoplasia (MEN2), different types of thyroid cancer, and prostate cancer. *Thyroid cancer*: This section describes the thyroid cancer. A general pathophysiology and classification will be detailed on solitary thyroid nodule, papillary carcinoma, follicular carcinoma, Hürthle cell carcinomas, medullary thyroid carcinoma and Anaplastic carcinoma as well as "other" thyroid carcinomas. Genetics, genotype-phenotype correlations, screening and surveillance, genetic counseling and testing strategies and management and individualized medicine are discussed separately for each cancer type.

Keywords Endocrine cancer · Multiple endocrine neoplasia · MEN1 · MEN2 · Thyroid cancer · Solitary thyroid nodule · Papillary thyroid cancer · Hürthle Cell Carcinoma · Follicular thyroid cancer · Medullary thyroid cancer · Anaplastic carcinoma · Prosttae cancer

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Abbreviations

ATA	The American Thyroid Association
ATC	Anaplastic thyroid cancer
CT	Computerized tomography
DNA	Deoxyribonucleic acid
DRE	Digital rectal examination
DTC	Differentiated thyroid cancer
FAP	Familial adenomatous polyposis
FMTC	Familial medullary thyroid cancer
FNA	Fine-needle aspiration
HCC	Hürthle cell carcinoma
MAPK	Mitogen activated protein kinase
MEN	Multiple endocrine neoplasias
MIFC	Minimally invasive follicular cancer
MRI	Magnetic resonance imaging
MTC	Medullary thyroid cancer
NCCN	National Comprehensive Cancer Network
NGS	Next-generation sequencing
PHEO	Pheochromocytoma
PHPT	Primary hyperparathyroidism
PTC	Papillary thyroid cancer
PTH	Parathyroid hormone
RNA	Ribonucleic acid
TGCA	The Cancer Genome Atlas
TK	Tyrosine kinase
TKI	Tyrosine kinase inhibitor
TM	Transmembrane domain
TSH	Thyroid stimulating hormone
VEGFR	Vascular endothelial growth factor receptor
WIFC	Widely invasive follicular cancer

10.1 Endocrine Cancers

10.1.1 Wermer Syndrome (MEN Type 1)

10.1.1.1 Introduction

Multiple endocrine neoplasia (MEN) syndromes have 2 categories: MEN type 1 (MEN1) and MEN type 2 (MEN2). MEN2 has been subcategorized into MEN2-A, MEN2-B. Some new variants were introduced but were later reclassified in the current categories: MEN4 is now considered to be a variant of MEN1 and the

familial medullary thyroid cancer (FMTC) is regarded as a variant of MEN2A (Kloos et al. 2009). MEN1 is characterized by parathyroid tumors, pancreatic islet cell tumors, and anterior pituitary tumors. MEN1 often follows the autosomal dominant mode of inheritance. However, sporadic cases have been reported as a result of de Novo mutations. Untreated MEN1 leads to decrease in life expectancy (up to 50% probability of death by the age of 50 years) (Thakker et al. 2012).

10.1.1.2 Pathophysiology and Classification

Common clinical findings in MEN1 include Primary hyperparathyroidism (about 90% of the cases) (Brandi et al. 2001a), Pancreatic islet cell tumors (30–80% of the cases), and Pituitary tumors.

1. Primary hyperparathyroidism: Clinical manifestations of primary hyperparathyroidism typically include hypercalcemia (and the resultant phenotypes such as polydipsia, polyuria, constipation), nephrolithiasis, and bone abnormalities. Notably, compared to non-MEN1 primary hyperparathyroidism, the MEN1 associated primary hyperparathyroidism is more likely to occur at an earlier age and could include multiple glands (Eller-Vainicher et al. 2009).
2. Pancreatic islet cell tumors (neuroendocrine tumors) constitute the second most common clinical finding of MEN1 (30–80% of cases). They could include gastrinomas, insulinomas, glucagonomas, vasoactive intestinal polypeptidomas (VIPomas), and pancreatic polypeptidomas (PPomas) which produce peptides and biogenic amines (Thakker et al. 2012). These tumors may become malignant later in the life course. Malignant (non-functioning) pancreatic neuroendocrine tumors are the most common cause of death in people with MEN1 (Triponez et al. 2006). They have been underdiagnosed in the past since they typically have no clinical features other than minor elevations in pancreatic hormones. Sensitive radiological screening methods have improved their diagnosis (Anlauf et al. 2005; Thakker et al. 2012).
3. Pituitary tumors: MEN1-associated pituitary tumors could be secretory of prolactin (60%), growthhormone (25%), corticotropin (5%). The remaining tumors are nonfunctional(Thakker et al. 2012). Compared to other pituitary tumors, MEN1-associated pituitary tumors could be larger (macroadenomas) and have more aggressive potential. They tend to respond poorly to therapy (Fernandez et al. 2010).

Other tumors associated with MEN1

Many other clinical findings may be associated with MEN1; carcinoid tumors in the bronchi, gastrointestinal tract, pancreas, and thymus can occur in patients with MEN1 (Ferolla et al. 2005). Cutaneous symptoms represent one of the most common presentations of MEN1. Subcutaneous lipomas have been reported from one third of MEN1 patients. When a mutation is identified, phenotypic findings such as facial angiofibromas and collagenomas could be presymptomatic presentation of MEN1 in relatives of the MEN1 patient (Thakker et al. 2012).

10.1.1.3 Genetics

The *MEN1* gene, localized to 11q13, codes for the menin protein with a significant role in the regulation of transcription and maintenance of genome stability. Loss of heterozygosity for this genomic region has been linked to MEN1, suggesting it to be a tumor suppressor. Germline mutations are dominantly inherited. However, one mutated copy of the gene is not sufficient for the occurrence of cancer in an individual and requires a somatic mutation of the second copy before a tumor development process starts. Less frequently, both copies of the gene can be mutated somatically leading to sporadic cases of MEN1 (Andrew 2017).

10.1.1.4 Genotype-Phenotype Correlations

Variable expression is usually observed among MEN1 patients sharing the same genetic defect which advocates the lack of the genotype-phenotype correlation. This could be due to background genetic and epigenetic events. On the other hand, founder mutations have been associated with characteristic phenotypes. For example, a variant of the MEN1 syndrome known as MEN1-Burin or "the prolactinoma variant", is caused by a founder nonsense mutation in the *MEN1* gene which is associated with a characteristic phenotype including a higher incidence of carcinoid and pituitary tumors, of prolactinoma type, low frequent pancreatic endocrine tumors, and a late onset Primary hyperparathyroidism (PHPT) (Romei et al. 2012).

10.1.1.5 Screening and Surveillance

Routine surveillance including biochemical assay and imaging is suggested for asymptomatic individuals with *MEN1* pathogenic variants and high risk individuals. This should be started from early childhood. Such screening can detect the disease about ten years before symptoms appear which provide an opportunity for the disease control (Bassett et al. 1998).

10.1.1.6 Genetic Counseling and Testing Strategies

MEN1 syndrome follows an autosomal dominant mode of inheritance. About 90% of MEN1 patients have an affected parent. While, approximately 10% have de novo mutations in the *MEN1* gene. Parents of a proband with possible de novo mutation should be investigated by molecular genetic testing. When a mutation is identified, they should be monitored for phenotypic findings such as facial angiofibromas and collagenomas (Thakker et al. 2012).

If a parent of the proband is affected or has a pathogenic variant, the risk to the sibs is 50%. However, If the pathogenic variant found in the proband is not found in the DNA of either parent, germline mosaicism in a parent or a de novo mutation in the proband

could be the reason. Although germline mosaicism has not been reported, it remains a possibility. Children of the affected person or carrier of a pathogenic mutation have a 50% risk of inheriting the *MEN1* pathogenic variant (Giusti et al. 2015).

MEN1 has ten exons of which exon 1 and 10 are non-coding. Sequence analysis of the *MEN1* gene is the best way for determining the carrier status. Testing should be done in an index case suspected of MEN1 (2 or more endocrine tumors, or parathyroid adenomas in an individual younger than 30 years or a history or gastrinoma or multiple pancreatic neuroendocrine tumors at any age), in asymptomatic relative with a known MEN1 mutation; in first-degree relatives of a MEN1 mutation carrier with clinical findings, and biochemical or radiological evidence of *MEN1* (Thakker et al. 2012).

Next-generation sequencing (NGS) has been shown to be of high accuracy and very good sensitivity for molecular diagnosis of endocrine cancers. For example, an NGS panel has been validated including 9 causative genes for pheochromocytoma and paraganglioma (Rattenberry et al. 2013). More recently, another targeted NGS panel has been introduced for the mutation analysis of pheochromocytoma and paraganglioma, including 14 different susceptibility genes. A similar NGS-targeted approach has been used for molecular diagnostics of thyroid cancer (ThyroSeq panel including 12 cancer genes with 284 mutational hot spots) (Nikiforova et al. 2013).

10.1.1.7 Management and Individualized Medicine

Generally, genetic diagnosis has not been proved to significantly improve morbidity and mortality in MEN1 in contrast to genetic testing of MEN2. However, clinical practice guidelines on the management of assymptomic MEN1 gene carriers have recently been published (Thakker et al. 2012). In summary, the following biochemical screening should be performed annually: (1) PHPT: intact parathyroid hormone and albumin-corrected total serum calcium or ionized serum calcium by age 8. (2) Pituitary tumors: serum prolactin and insulin growth factor 1 (IGF-1) by age 5. (3) Insulinoma: serum fasting glucose and insulin by age 5. (4) Gastrinoma: gastrin, gastric acid output, and secreting stimulated gastrin. (5) Other neuroendocrine tumors: proinsulin, glucagon, and plasma chromogranin A before the age of 10 years. Diagnostic imaging are recommended for detecting pituitary tumors (MRI every 3 years), neuroendocrine tumors (with the exception of gastrinoma and insulinoma) (MRI, CT, or EUS annually), adrenal lesions (MRI or CT annually), thymic and bronchial carcinoids (CT or MRI every 1–2 years) (Romei et al. 2012).

10.1.2 Type 2 Multiple Endocrine Neoplasia

10.1.2.1 Introduction

Multiple endocrine neoplasia type 2 (MEN2) is an autosomal dominant disorder with the prevalence of about 1 per 30,000. MEN2 has two distinct types: types 2A

(MEN2A) and 2B (MEN2B). For MEN2A, four different clinical variants have been described: the classical MEN2A, MEN2A with cutaneous lichen amyloidosis (CLA), MEN2A with Hirschsprung disease (HD), and familial medullary thyroid cancer (FMTC) (Wells et al. 2015). Activating mutations in the *RET* gene, on chromosome 10 coding for a proto-oncogene, is the cause of MEN2A variants. In both MEN2A and MEN2B multifocal tumors emerge in the tissues where *RET* is expressed.

10.1.2.2 Pathophysiology and Classification

MEN2 is described by co-occurrence of series of benign and malignant endocrine neoplasia with certain non-endocrine diseases. The medullary thyroid cancer (MTC), originating from C cells, is the constant part of MEN2 syndromes. Pheochromocytoma (PHEO) and/or adenomatosis of parathyroid glands together with hyperparathyroidism (PHPT) are among other findings. The association of MEN2 with or without other non-endocrine diseases gives rise to 3 different syndromes: MEN 2A, MEN 2B, and FMTC. The latter syndrome is regarded to be a variant of MEN2A (Rodriguez et al. 2008).

MEN2A syndrome is the most frequent MEN2 syndrome. MTC is the first manifestation of MEN2A (developing between 5 and 25 years of age) which is usually bilateral, multifocal, of C-cell origin. PHEO is the second phenotype occurring simultaneously or after MTC; although sometimes it could be observed earlier than MTC. In some MEN2A patients, an associated Hirschsprung's disease is reported (Rodriguez et al. 2008).

MEN2B syndrome is the rarest (5–10% of all cases) but the most aggressive form of MEN 2. Due to the metastatic rapid developing MTC tumors, patients rarely reach adulthood. Typically, MEN2B also includes mucosal neuromas, bumpy lips, ganglioneuromatosis of the alimentary canal, and Marfanoid habitus.

Familial MTC (FMTC) is considered to be the mildest variant of MEN2 which is rarely associated with other clinical findings of MEN2A. It comprises 35–40% of all MEN patients (Romei et al. 2012).

10.1.2.3 Genetics

The MEN2 responsible gene, known as *RET*, has been mapped to 10q11.2. It is composed of 21 exons. It codes for a tyrosine kinase receptor proto-oncogene. The receptor is composed of an extracellular domain, with a distal cadherin-like motif and a juxtamembrane cystein-rich region, a transmembrane domain (TM) and an intracellular tyrosine-kinase (TK) domain. After interaction with its ligand, RET is dimerized and activated (Romei et al. 2012).

Activating germline point mutations of the *RET* gene cause MEN2A, MEN2B, and FMTC. Both MEN2A and MEN2B follow autosomal dominant pattern of

inheritance and have a very high penetrance. Tumor formation can occur in all organs where the *RET* gene is expressed especially the thyroid, parathyroid, and adrenal glands (Frank-Raue et al. 2010).

10.1.2.4 Genotype-Phenotype Correlations

Activating germline point mutations of *RET* lead to MEN2A, MEN2B, and FMTC. *RET* mutations are widely distributed in the gene. The non-cysteine mutations are generally associated with FMTC (Romei et al. 2011; Verrienti et al. 2015). Early diagnosis of sporadic and hereditary MTCs helps decrease mortality and morbidity (Frank-Raue et al. 2010).

For MEN2 syndromes, there is a strong genotype-phenotype correlation and certain *RET* mutations are associated with distinct phenotype:

1. Almost 98% of families with MEN2A have a germline *RET* mutation in exon 10 or 11 (Verrienti et al. 2015). Mutations at codon 634 (exon 11) is the most common mutation in typical MEN2A patients (87%). Typical phenotypes include endocrinopathies such as MTC, PHEO and PHPT. Mutations of cysteine residues are less frequently associated with the co-occurrence of the three aforementioned endocrinopathies. Based on previous experiments, different population-based mutation frequencies and phenotype correlations might be expected for a typical gene (Jalilian et al. 2017; Masbi et al. 2014; Tabatabaiefar et al. 2011; Yazdanpanahi et al. 2015).
2. Germline *RET* mutations account for 95% of FMTC cases. These mutations are usually positioned in the non-cysteine codons of exons 5, 8, 13, 14 and 15. Again, population-specific mutation frequency and phenotype correlation may exist (Masbi et al. 2014).
3. About 95% of MEN2B patients have a single point mutation in the tyrosine kinase domain of *RET* (M918T); a threonine is substituted for methionine at codon 918 in exon 16.

Different mutations might be associated with different degrees of penetrance and differences in aggressiveness of MTC. The American Thyroid Association (ATA) has recently issued a revised guideline for classification of the RET mutations into different levels of risk (including, highest risk, high risk and moderate risk); totally, 67 evidence-based recommendations have been put forth to assist clinicians in the care of patients with MTC to adopt therapeutic and follow-up strategies (Romei et al. 2012; Treglia et al. 2016).

10.1.2.5 Screening and Surveillance

For MTC, there has been reported an excellent prognosis if it is diagnosed at the earliest stage (Frank-Raue et al. 2010). Total thyroidectomy and neck nodal

dissections have been recommended in these cases. Since normal plasma calcitonin concentrations have been observed in some individuals with a *RET* pathogenic variant, unremitting monitoring for residual or recurrent MTC is recommended after thyroidectomy. This involves an annual measurement of serum calcitonin. If residual disease is detected, more frequent follow up would be necessary. Individuals negative for pheochromocytoma upon screening should be subjected to annual biochemical screening. If the latter results are abnormal, MRI and/or CT follow-up is recommended (Marquard and Eng 2015).

MEN 2 women should be screened for pheochromocytoma before family planning (Marquard and Eng 2015).

10.1.2.6 Genetic Counseling and Testing Strategies

MEN2 subtypes are inherited in an autosomal dominant mode of inheritance. About 95% of MEN2A patients have an affected parent. About 5% of cases are caused by a de novo germline pathogenic variant (Schuffenecker et al. 1997). About 50% of MEN2B patients have an inherited germline mutation while the remaining cases are as the result of a de novo germline RET mutation (Carlson et al. 1994). The genetic counseling process of MEN2 might be difficult because of reduced penetrance, expressivity and, or late onset phenotypes in the affected patients. Thus, appropriate clinical evaluation and/or molecular genetic testing are necessary. Notably, during genetic evaluation, for patients who develop bilateral pheochromocytomas, assessment should be done for MEN2.

If a parent has a germline mutation, whether clinically affected or unaffected, the risk to the progeny is 50%. In case, no pathogenic mutation is detected in the whole blood extracted DNA of either parent, the risk to the progeny would be low but still greater than that of the general population, as there is a possibility of germline mosaicism.

Molecular testing includes DNA sequencing of selected exons, whole gene sequencing, and NGS-based gene panel. Since most of the pathogenic variants have been reported in exons 10, 11, and 13–16, and even for some populations up to 98% of families with MEN2A have a germline *RET* mutation in exon 10 or 11 (Verrienti et al. 2015), a selected number of exons could be prioritized for sequencing of the *RET* gene. Alternatively, NGS-based gene panel involving *RET* as well as other related genes may also be considered, especially in isolated or familial pheochromocytoma cases (Marquard and Eng 2015).

10.1.2.7 Management and Individualized Medicine

Management of MEN2 includes referral to an endocrinologist and a clinical geneticist. Biochemical and molecular genetic testing are necessary for diagnosis

and monitoring of the disease status in individuals with MTC. The standard surgical removal of the thyroid and lymph node dissection are considered to be the standard procedure (Kloos et al. 2009). Thyroid hormone replacement therapy is essential in such cases.

When pheochromocytomas are detected by biochemical testing and radionuclide imaging, adrenalectomy would be offered. Although some experts recommend total bilateral adenectomy, others point to the resultant Addisonian crisis following bilateral adrenalectomy. Therefore, unilateral adrenalectomy in unilateral tumors followed by intensive monitoring of the remaining adrenal gland is recommended. Although in most MEN2A patients, hyperparathyroidism is diagnosed long after thyroidectomy, its monitoring is important for ensuring a reduced morbidity (Kloos et al. 2009).

Different mutations might be associated with different degrees of penetrance and differences in aggressiveness of MTC. The American Thyroid Association has recently issued a revised guideline for classification of the RET mutations into different levels of risk; totally, 67 evidence-based recommendations have been suggested which will assist clinicians in the care of MTC patients to adopt proper individualized therapeutic and follow-up strategies (Romei et al. 2012; Treglia et al. 2016).

High-risk MEN2A and MEN2B genotypes include RET mutations on codons 883, 918, and 922. They should be offered for surgical intervention during infancy (Brandi et al. 2001b). RET genotypes of intermediate risk include codons 611, 618, 620, and 634. They should be subjected to surgery before 5 years of age. RET genotypes of low risk include mutations in codons 609, 630, 649, 768, 790, 791, 804, and 891 (Brandi et al. 2001b). Patients with mutations of codon 609 of *RET* may delay thyroidectomy until 10–15 years old. The 2009 American Thyroid Association (ATA) has recommended prophylactic thyroidectomy in the low-risk group (ATA-A) when the calcitonin levels increase or the patient is between 5 and 10 years old (Brandi et al. 2001b).

10.2 Thyroid Cancer

10.2.1 Introduction

Thyroid cancers constitute about 1% of new cancer cases in the United States each year (Weir et al. 2015). Thyroid malignancies are classified into papillary carcinomas (80%), follicular carcinomas (10%), medullary thyroid carcinomas (5–10%), anaplastic carcinomas (1–2%), primary thyroid lymphomas (rare), and primary thyroid sarcomas (rare) (Morris et al. 2013).

Hürthle cell carcinoma is a rare type of differentiated thyroid cancer and is responsible for 3% of all thyroid malignancies. Although it has been considered to

be a variant of follicular thyroid carcinoma, the genomic alterations favor the fact that it is a distinct class of thyroid malignancy (Petric et al. 2016).

10.2.2 Pathophysiology and Classification

Thyroid cancers are of different cellular origins, features and prognoses. Follicular thyroid ectodermal cells and neuroendocrine-derived calcitonin-producing C cell (also known as parafollicular C cells) are two types of endocrine thyroid cells from which thyroid cancers are derived. Papillary thyroid cancer (PTC), follicular thyroid cancer (FTC), poorly differentiated thyroid cancer (PDTC) and anaplastic thyroid cancer (ATC) originate from follicular thyroid cells and constitute the majority of thyroid malignancies. PTC and FTC are grouped as differentiated thyroid cancer (DTC). Medullary thyroid cancer (MTC) arises from parafollicular C cells and is very rare. The aberrant activation of RET signaling, caused by *RET* mutations, account for the tumorigenesis. This molecular etiology is not present in follicular thyroid cell-derived tumors (Xing 2013). There are two other tumor types with yet different origin: thyroid lymphomas which are derived from intra-thyroid lymphoid tissue, and thyroid sarcomas which arise from connective tissue of the thyroid gland. Radiation exposure significantly increases the risk for thyroid malignancies, chiefly papillary thyroid carcinoma (Le et al. 2016).

10.2.3 Solitary Thyroid Nodule

10.2.3.1 Introduction

Most thyroid nodules are felt as a lump in front of the neck upon self-examination. A discrete swelling in one lobe of the thyroid gland with no palpable anomaly in other parts of the gland is called a solitary (isolated) nodule. About 70% of them are clinically isolated. The prevalence of solitary tumors depends on many variables such as age, sex, diet, and iodine deficiency and radiation exposure. They have an increased risk of neoplasia compared with other thyroid swellings. About 15% of them can be malignant and about 30–40% are follicular adenomas. The remaining cases are non-neoplastic such as thyroiditis and cysts (Haridas 2017).

10.2.3.2 Pathophysiology and Classification

A palpable mass may be solid, cystic, or mixed in nature. Benign cysts usually have no recurrence. A true cyst has a very low risk of malignancy. However, the presence of a cyst does not rule out the possibility of neoplasia, especially when the mass is mixed. In this regard, half of the patients with thyroid carcinoma have been reported to have a cystic component in their tumor (Andre and Hoffman 2017).

10.2.3.3 Genetics

Most patients with indeterminate cytology at Fine-needle aspiration (FNA) biopsy are subjected to diagnostic surgery for making a histopathological diagnosis. However, only 10–40% of them would show malignancies (Baloch et al. 2008). Therefore, most surgeries are unnecessary. The assessment of genes associated with thyroid carcinomas (such as *PTC, RAS, RET/PTC, BRAF, FTC, PAX8/PPARc1* etc.) in the specimen, in combination with clinical and cytological features, can improve preoperative diagnosis of thyroid nodules (Nikiforov et al. 2009).

At the present time, there are 2 commercially available genetic that can be done on FNA biopsy samples. Veracyte Afirma Gene Expression Classifier, developed by Genzyme (Cambridge, MA, USA), assesses mRNA expression levels of 142 genes. It has been shown to have a negative predictive value of 96% when evaluated in samples with indeterminate cytology, thus will help avoid unnecessary surgeries. As an alternative platform, miRInform Thyroid, provided by Asuragen (Austin, TX, USA), a panel of 7 genes involved in thyroid cancers are tested (*BRAF, KRAS, HRAS, NRAS, RET/PTC1, RET/PTC3, PAX8/PPARγ*) (Li et al. 2011). The latest version of the test covers 17 genes engaged in thyroid cancer.

Next-generation sequencing (NGS) based tests are currently in development and are evaluated in thyroid cancer.

10.2.3.4 Genotype-Phenotype Correlations

Currently, no genotype-phenotype correlation has been revealed for solitary thyroid tumors. Especially of interest, mutations of some of the genes related to thyroid cancers are also involved in other types of tumors (Asl et al. 2014; Soltani et al. 2017)

10.2.3.5 Screening and Surveillance

Most patients presenting with a solitary thyroid nodule are euthyroid, and the simplest way to verify this is a serum thyrotropin (TSH) level. If below normal, the workup proceeds with measuring total or free thyroxine (T4) and total triiodothyronine (T3) to better evaluate the hyperthyroid state. This result occurs in approximately 10% of patients with a solitary thyroid nodule and is suggestive of a benign hyperfunctioning adenoma (Bomeli et al. 2010).

10.2.3.6 Genetic Counseling and Testing Strategies

Currently, there are 2 commercially available assays that provide molecular testing of the thyroid cytologic specimens from FNA biopsy. Veracyte Afirma Gene Expression Classifier, promoted by Genzyme (Cambridge, MA, USA), evaluates messenger RNA (mRNA) expression levels for 142 genes. It has a negative predictive value of

96% when evaluated in samples with indeterminate cytology, thus helping patients with benign lesions to avoid unnecessary surgeries. A cost-effectiveness analysis by Li and colleagues (Li et al. 2011) predicts that routine application of the gene expression classifier lowers the rate of surgeries for benign nodules from 57% (with current practice) to 14%. miRInform Thyroid is another commercially available assay provided by Asuragen (Austin, TX, USA), which analyzes a panel of 7 molecular markers most commonly encountered in thyroid cancers (*BRAF, KRAS, HRAS, NRAS, RET/PTC1, RET/PTC3, PAX8/PPARc*). In contrast to the Veracyte product, it is designed to improve the preoperative cytologic diagnosis of indeterminate thyroid nodules by predicting which nodules are most likely to be malignant. Its clinical validation still needs to be determined, but the analytical specificity was found to be 99%, and the sensitivity 95% (Popoveniuc and Jonklaas 2012).

10.2.3.7 Management and Individualized Medicine

Although benign tumors are the most common cause of thyroid nodules in children (because of the higher rates of malignancy in this population), we should consider the possibility of neoplasia in the presence of a solitary thyroid nodule. Missed malignancy is tragic, but the prospect of lifetime hormone replacement therapy in the absence of pathological need is frustrating, making accurate diagnosis much more vital in the pediatric population.

Surgical treatment is recommended for nodules causing compressive symptoms, and can be considered for toxic nodular disease and thyroid cysts. T4 suppressive therapy is controversial: it is associated with the risks of iatrogenic hyperthyroidism, but may prevent new nodule formation. Most benign thyroid nodules do not require any specific intervention, unless there are local compressive symptoms from significant enlargement, such as dysphagia, choking, shortness of breath, hoarseness, or pain, in which case thyroidectomy should be performed.

Other indications for surgery in benign nodules include the presence of a single toxic nodule, or a toxic multinodular goiter. Radioiodine (131-I) therapy is another option for treatment of toxic nodular goiters, but they are usually more radioresistant than toxic diffuse goiter and radioiodine is not the first-line therapy if compressive symptoms are present.

Aspiration is the treatment of choice in thyroid cysts, but the recurrence rates are high (60–90% of patients), particularly with repeated aspirations and large-volume cysts. Percutaneous ethanol injection (PEI) has been studied in several large randomized controlled studies, with reported success in 82–85% of the cases after an average of 2 sessions, with a volume reduction of more than 85% from baseline size.

Levothyroxine (T4) therapy for benign thyroid nodules has been proposed with the aim of achieving nodule shrinkage and preventing further appearance of new nodules through TSH suppression. Although several randomized control trials and meta-analyses have demonstrated nodule shrinkage in patients from areas of iodine deficiency, 85–88 a clinically significant decrease in nodule volume is achieved only in a minority of patients with sufficient iodine intake (Popoveniuc and Jonklaas 2012).

10.2.4 Papillary Carcinoma

10.2.4.1 Introduction

Cancers of follicular thyroid ectodermal cells include papillary thyroid cancer (PTC), follicular thyroid cancer (FTC), poorly differentiated thyroid cancer (PDTC) and anaplastic thyroid cancer (ATC). As mentioned before, papillary and follicular cancers are regarded as differentiated thyroid cancers (DTC). Interestingly, similar treatment strategies have been applied to these cancers although they are mechanistically different. PTC is the most frequent thyroid cancer with the prevalence of about 80%, and is 3 times more frequent among women than men. PTC can be clustered in families or be seen as sporadic. Furthermore, it can occur as isolated, with no associated clinical finding, or in associated with Gardner syndrome (Michael Tuttle et al. 2017b).

10.2.4.2 Pathophysiology and Classification

Typically, PTCs are not covered by capsules and may be partly cystic in nature. Most have papillae with one or two layers of tumor cells encompassing a distinct fibro-vascular core; No follicles and colloid are typically present. PTC is often multifocal and can be of different clonal origins. There are several variant forms of PTC including the follicular variant, tall cell, insular, and diffuse sclerosing variants (Michael Tuttle et al. 2017b). PTC often follows multifactorial inheritance. Exposure to radiation is associated with increased risk of PTCs. Other risk factors include iodine deficiency, oral contraceptives, benign thyroid nodules, late menarche, and advanced age upon first maternity. familial adenomatous polyposis (FAP), Gardner's syndrome, and Cowden's disease may be associated with thyroid papillary tumors (Keith and Baldwin 2016a).

10.2.4.3 Genetics

The Cancer Genome Atlas (TGCA) has included *BRAF*, *NRAS*, *HRAS*, *KRAS*, and *EIF1AX* as potential *PTC* cancer genes (Cancer Genome Atlas Research Network 2014).

10.2.4.4 Genotype-Phenotype Correlations

Activating mutations in the components of RET/PTC-RAS-BRAF signaling pathway plays a fundamental role in pathogenesis of PTC. Interestingly, mutation in one of these components drive the pathway and together with mutations elsewhere could lead to PTC. However, there are genotype-phenotype correlations: for example, *BRAF* is associated with classic PTC and tall cell carcinoma while *RAS* is associated with the follicular variant (Nikiforova et al. 2003).

10.2.4.5 Screening and Surveillance

Surgery is the main therapeutic strategy for DTCs (such as papillary and follicular). Ultrasound imaging should be performed pre-operatively. For detection of post-operational persistence or recurrence of disease neck ultrasound monitoring and measuring TSH, and serum Tg levels are important necessary steps. The type and timing of testing for detection of recurrent disease is according to ATA initial risk stratification and evaluations of response to therapy (Michael Tuttle et al. 2017a).

10.2.4.6 Genetic Counseling and Testing Strategies

PTCs are commonly caused by activation of mitogen activated protein kinase (MAPK) signaling pathway through rearrangements of *RET* and *NTRK1* tyrosine kinases, and activation of *BRAF*, or RAS. Typically, any given PTC would only have one mutation in one of the above named genes (Malchoff et al. 2017).

10.2.4.7 Management and Individualized Medicine

After thyroid surgery, almost all of the patients need degrees of postoperative thyroid hormone therapy [T4 (levothyroxine)]. This can have an additional benefit of preventing the tumor. Thyroid-stimulating hormone (TSH) levels should also be monitored. For high-risk and some intermediate-risk patients radioiodine should be administered (Michael Tuttle et al. 2017a). The most useful drugs for the treatment of PTC include levothyroxine and radioiodine. For metastases, chemotherapeutic agents such as cisplatin and doxorubicin may be valuable (Keith and Baldwin 2016b).

In the field of target therapy, Vemurafenib, targeting V600E BRAF, appears to have a promising outcome in patients with metastatic PTC. Small molecules targeting the vascular endothelial growth factor receptor (VEGFR) have been evaluated in clinical trials and have great potential to be standard of care for patients with progressive, radioiodine-resistant disease (Kim et al. 2013).

10.2.5 Follicular Carcinoma

10.2.5.1 Introduction

Follicular carcinomas are the most common forms of thyroid cancer and also endocrine malignancies. These neoplasms (such as adenoma, follicular, and papillary carcinomas) have common clinical and cytological features. Pathological and microscopy investigations might be necessary to distinguish between the malignant and benign tumors.

Follicular thyroid carcinoma (FTC) is similar to the normal microscopic pattern of the thyroid. In term of prevalence, it ranks second after papillary carcinoma (Luigi 2016).

10.2.5.2 Pathophysiology and Classification

FTC is a well-differentiated thyroid tumor. It is the second most prevalent thyroid cancer after papillary carcinoma. Unlike PTC, metastasis to the neck region is a rare event. However, metastasis to distant sites (ex. Lung and bones) is considerably increased (approximately 20%). As compared to the PTC, FTC is more prevalent in iodine-deficient regions and normally afflicts the older population. The histological study could, to some extent, clarify the prognosis: normal, well-differentiated thyroid tissue with follicle and colloid suggest good prognosis, while poorly-differentiated solid growth with absence of follicles and invasion to vascular or capsular invasion are suggestive of poor prognosis. In this regard, two sort of capsular invasion can be defined: the first one is known as minimally invasive follicular cancer (MIFC) with low degree of microscopic penetration into the capsule and with no vascular invasion, while the second type includes widely invasive follicular cancer (WIFC) which penetrates beyond the capsule into blood vessels and into the neighboring thyroid parenchyma. Newer category of tumor categorization is developing (Ross 2017).

10.2.5.3 Genetics

Point mutations of *RAS*, encoding a proto-oncogene, is found in about 40% of FTC cases (Medema and Bos 1993). Mutations in *NRAS*, *HRAS* and *KRAS* more frequently occur in FTC than in follicular adenomas (Ross 2017; Zhu et al. 2003). The fusion gene PAX8-PPAR gamma 1 caused by a rearrangement is found in follicular adenoma (10%) and FTC (40%) (Fagin 2000). Mutations of other genes such as *TP53* (encoding p53), *MYC* (encoding c-myc), *FOS* (encoding c-fos) and *TSHR* (encoding thyroid-stimulating hormone receptor) have also been reported in FTC cases (Ross 2017; Nikiforov 2004).

10.2.5.4 Genotype-Phenotype Correlations

RAS mutations in FTC are associated with a more aggressive cancer and of poor prognosis (Xing 2016). Moreover, *RAS* mutations have also been identified in both poorly differentiated and anaplastic thyroid cancers (Garcia-Rostan et al. 2003). The presence of the PAX8-PPAR gamma 1 fusion gene prevents thiazolidinedione activation by PPAR gamma, leading to bypassing the cell cycle checkpoints (Martelli et al. 2002; Wartofsky and Van Nostrand 2016).

10.2.5.5 Screening and Surveillance

Papillary and follicular carcinomas are usually treated by total thyroidectomy. Post-operatively, radioiodine scanning should be performed for the patient followed by radioablation of any residual disease. Poorer outcomes have been reported with increasing tumor size and stage, node positivity and more advanced age of the patient (Barney et al. 2011). Patients with minimal disease invasion have very good prognosis and do not need to undergo nodal surgery (Asari et al. 2009).

10.2.5.6 Genetic Counseling and Testing Strategies

NGS-based testing is currently being evaluated for thyroid cancer. The initial results have been promising (Cha and Koo 2016).

ThyroSeq® has been developed to investigate thyroid cancer related genes in FNA and tissue samples. ThyroSeq v.2 offers detection of 14 thyroid cancer-related genes and for 42 types of gene fusions. It is used to further narrow the indeterminate category defined by cytology for thyroid nodules thus preventing unnecessary surgery in the patient (Alsina et al. 2016).

10.2.5.7 Management and Individualized Medicine

According to the guideline by the National Comprehensive Cancer Network, TSH measurement and ultrasound of the thyroid and central neck should be performed; Patients with thyroid nodules and a low TSH level should be subjected to radioiodine imaging: in case a functioning nodule is shown, thyrotoxicosis should be considered. However, for hypofunctional nodules with a normal or elevated TSH level, FNA biopsy may be performed. Distinction of benign from the malignant counterpart of a given tumor might be difficult and would need detailed pathological assessment. Polymerase chain reaction (PCR)—based genotyping of *RAS* should be helpful in the clinical and histological reassessment of these tumors. Measuring the serum level of carcinoembryonic antigen (CEA) may also be valuable; although CEA elevation is not specific to FTCs (National Comprehensive Cancer Network 2015).

10.2.6 Hürthle Cell Carcinomas

10.2.6.1 Introduction

Hürthle cell carcinoma (HCC) of the thyroid makes up about 3% of thyroid cancers. They are generally categorized in the same group as FTC. However, genomic and functional studies favor the idea that they should be regarded as a

distinct group. As compared to FTC, HCC tends to be multifocal and more aggressive and less likely to take up iodine. HCCs are oxyphilic; they have a number of mitochondria, leading to their larger size and their granular appearance (Cibas and Alli 2009).

10.2.6.2 Pathophysiology and Classification

Although FTC and HCC are grouped together, some evidence would suggest a separate category for HCC. An adenoma-to-carcinoma conversion has been suggested in some cases. However, a follicular carcinoma in situ is not generally accepted. As a general rule, the advanced forms of HCCs have more somatic mutations in cancer genes. Most FTCs as well as HCCs are thought to have monoclonal origin (Aytug and Harris 2016).

10.2.6.3 Genetics

RAS mutations (point mutation and translocation involving *RAS*) and large genomic gains on chromosomes 5, 7, 12, and 17 have been reported in HCC tumor samples (Ahmadi and Stang 2016).

10.2.6.4 Genotype-Phenotype Correlations

Point mutation in *RAS* or translocation involving *RAS* (MAPK pathway) is a common finding (about 40%) in both follicular adenomas, FTC and HCC suggesting its involvement in early steps of tumorigenesis.

Overexpression of the *TP53* gene product (p53) has been found in a subset of HCC. Some Hürthle cell adenomas and carcinomas have *RET* gene arrangement, which is more unique to PTC. Defects of cytochrome c oxidase and mitochondrial DNA deletions are frequently observed in HCC (Aytug and Harris 2016).

In a recent study, p27 and cyclin D3 proteins were found to be overexpressed in HC cell lines and clinical samples (Aytug and Harris 2016). *ATPase 6* gene mutations were significantly more frequent in tumor simples of HCC patients than in those of non-HCC patients (Maximo et al. 2002).

Genomic studies of HCC have suggested it could be a distinct type of malignancy. Activation of signaling pathways including PIK3CA-Akt-mTOR and Wnt/β-catenin differentiate Hürthle cell adenomas from the invasive Hürthle cell carcinomas. Thus, they could provide possible targets for the treatment HCC (Ganly et al. 2013).

10.2.6.5 Screening and Surveillance

At the present time, HCC is still categorized by the WHO as a histopathologic variant of follicular carcinoma. The same holds true in the American Thyroid Association (ATA) and National Comprehensive Cancer Network (NCCN) treatment guidelines. Thus, the risk stratification for HCC is the same as that for FTC. According the ATA guideline, intrathyroidal encapsulated tumors with minimal capsular or vascular invasion (<4 foci) or ≤ 5 metastatic lymph nodes with the foci of metastases less than 0.2 cm are categorized as low risk. Intermediate risk have vascular invasion, minimal extrathyroidal extension, or >5 metastatic lymph nodes (0.2–3 cm). High-risk patients have macroscopic extrathyroidal extension, incomplete tumor resection, distant metastases, or metastatic lymph nodes >3 cm. The NCCN guidelines for HCC (Version 1.2016) define low risk group as having minimal vascular invasion of ≤ 4 foci of invasion in an intrathyroidal HCC (Ahmadi and Stang 2016).

10.2.6.6 Genetic Counseling and Testing Strategies

Molecular marker analysis in thyroid cytology has been studied mostly in thyroid nodules with indeterminate cytology. The 3 reputable commercial tests include the Afirma Gene Expression Classifier (GEC, mRNA expression of 167 genes) (Alexander et al. 2012), a 7-gene panel of genetic mutation and rearrangement testing (Nikiforov et al. 2011), and a newer multiplexed next-generation sequencing (NGS) panel examining >400 known drivers of oncogenesis in thyroid cancer (ThyroSeq) (Haugen et al. 2016).

10.2.6.7 Management and Individualized Medicine

HCC is associated with more aggressive behavior than other differentiated thyroid cancers and has a higher rate of distant metastases. During initial follow-up of levothyroxine therapy, serum thyroglobulin/thyroglobulin antibody (Tg/Tg Ab) levels should be measured. In the ATA low-to intermediate-risk HCC patients, the measurement can be done every 12–24 months. However, for the ATA high risk HCC patients and those with indeterminate response to therapy the measurements will be every 6–12 months. Cervical ultrasound should be performed to assess the thyroid bed, central, and lateral cervical nodal compartments every 6–12 months in HCC patients after surgery. This should be continued periodically depending on each patient's category of risk and unique response to therapy (Ahmadi and Stang 2016).

10.2.7 Medullary Thyroid Carcinoma

10.2.7.1 Introduction

Medullary thyroid cancer (MTC) is a neuroendocrine tumor of the parafollicular or C cells of the thyroid gland. MTC is responsible for about 1–2% of thyroid cancers in the United States (Wells et al. 2015). Calcitonin production is a distinguishing feature of this tumor. Since the C cells originate from the embryonic neural crest, MTCs are similar in clinical and histologic features to other neuroendocrine tumors such as islet-cell tumors. About 25% of MTCs are familial as part of the multiple endocrine neoplasia type 2 (MEN2) syndrome (Michael Tuttle et al. 2017c), while the remaining are sporadic.

10.2.7.2 Pathophysiology and Classification

MTC is generally diagnosed as a solitary nodule of the neck with early spread to regional lymph nodes. Distant metastases can be seen in the liver, lung, bone, and brain. Sporadic MTC is usually unilateral. However, MTC associated with multiple endocrine neoplasia (MEN) syndromes is always bilateral and multi-centric. MTC is typically the first clinical manifestation of MEN 2 syndromes. MTC cells can produce several hormones including calcitonin, corticotropin, melanin, serotonin and prostaglandins (Anastasios 2017).

10.2.7.3 Genetics

Germline point mutation in the *RET* gene on chromosome 10q11.2 account for the hereditary MTC. The *RET* gene has 21 exons. It encodes a receptor tyrosine kinase containing four cadherin-related repeats and a cysteine-rich region in the extracellular domain. Most MEN2A (95%) and FMTC (85%) patients have mutations clustered in cysteine-rich extracellular domain (codons 609, 611, 618, and 620 in exon 10, and codons 630 and 634 in exon 11). Somatic *RET* point mutations have been found in about 50% of patients with sporadic MTC (Nose 2011).

10.2.7.4 Genotype-Phenotype Correlations

There have been several genotype-phenotype correlations. For example, patients with *RET* mutations in codon 634 are more likely to have pheochromocytoma and parathyroid hyperplasia or adenoma leading to a higher familial penetrance of the MEN2A phenotype (Machens and Dralle 2007).

10.2.7.5 Screening and Surveillance

All MTC patients and their family should be screened for related familial diseases. Genetic testing should be considered for all first-degree relatives of patients diagnosed with MTC compared to most other endocrine cancers (Dvorakova et al. 2008). Based on the American Thyroid Association (ATA), preoperative laboratory testing should be performed in patients with suspected MTC; in MEN2 patients, in case primary hyperparathyroidism and/or pheochromocytoma are identified, the surgical priorities would be altered. Mutation testing of *RET* can identify carrier members of the family allowing for early diagnosis, prophylactic thyroidectomy and treatment of affected individuals. Notably, prophylactic thyroidectomy is recommended for patients at risk for developing MTC. They include MTC patients with family history of MEN or MTC, elevated serum calcitonin level, or a *RET* mutation (Nose 2011). Finally, the extent of metastatic disease may be predicted based on preoperative laboratory testing which, in turn, determines the extent of preoperative imaging and may affect the surgical approach.

10.2.7.6 Genetic Counseling and Testing Strategies

Germline *RET* mutation testing is highly recommended in all patients with C cell hyperplasia and seemingly sporadic MTC. This testing should include sequencing of exons 10, 11, and 13 through 16 of the *RET* gene. Sequencing of the remaining exons in the *RET* gene should be carried out in patients with clinical features or family history of MTC but with no mutation in these hotspot exons (Moline and Eng 2011). Genetic counseling is highly recommended before and after genetic testing and informed consent requirements should be met (Wells et al. 2015). When a germline mutation is found in the proband, other family members should be offered genetic counselling and screening.

10.2.7.7 Management and Individualized Medicine

For patients with metastatic tumors smaller less than 1–2 cm in diameter and growing in diameter less than 20% per year, systemic therapy is not generally recommended. However, disease progression should be monitored and CT scan or MRI should be done every 6–12 months on known sites of metastatic disease. For patients with metastatic tumors with at least 1–2 cm in diameter but growing by at least 20% annually, or for patients with symptoms related to multiple metastatic foci, an oral tyrosine kinase inhibitor (TKI) should be administered. For initial TKI therapy, cabozantinib or vandetanib are suggested over sorafenib or sunitinib. Cytotoxic chemotherapy such as cyclophosphamide-vincristine-dacarbazine remains as an alternative option for patients who fail to tolerate TKIs (Sherman 2014).

10.2.8 Anaplastic Carcinoma and Other Thyroid Carcinomas

Anaplastic Thyroid Carcinoma

10.2.8.1 Introduction

Anaplastic thyroid cancers (ATC) are undifferentiated tumors of the thyroid follicular epithelium. As opposed to differentiated thyroid cancers, ATCs are highly aggressive, with about 100% of mortality. The very fast progression of disease and the poor treatment outcomes make necessary planning palliative care measures in the planning of disease management (Neff et al. 2008). Early disease diagnosis is essential for initiation of therapy.

10.2.8.2 Pathophysiology and Classification

ATC is widely believed to be originating from differentiated thyroid cancers of follicular cell origin. About 80% of ATC happens when long-term goiter is concurrent with an undiagnosed well-differentiated thyroid cancer. As a complex process, dedifferentiation mutational involves many events in the cell cycle and signal transduction pathway components. It is associated with gains and losses throughout the genome. Mutations of *BRAF* and *RAS* are common in ATC cases as well as in differentiated thyroid cancers. This suggests that the event could be an early step in tumorigenesis of the thyroid. *PIK3CA and PTEN* gene mutations also occur in both differentiated thyroid cancer and ATC (Keutgen et al. 2015).

10.2.8.3 Genetics

Genetic events in the C-*myc*, H-*ras*, and *Nm23*, *BRAF*, *RAS*, catenin (cadherin-associated protein) beta 1, *PIK3CA*, *TP53*, *AXIN1*, *PTEN*, and *APC* have been found in ATC. Chromosomal abnormalities are frequently observed (Smallridge et al. 2009).

10.2.8.4 Genotype-Phenotype Correlations

ATC is thought to develop from differentiated tumors through further mutational steps (Ricarte-Filho et al. 2009). Activating mutations in *BRAF* and RAS are found in both well-differentiated tumors and ATC cases. Therefore, they should be involved in early steps of cancer progression (Quiros et al. 2005). Later events

involve mutations in *TP53*, catenin (cadherin-associated protein) beta 1, and *PIK3CA* (Smallridge et al. 2009) suggesting that one or several of these mutations might be responsible for the highly aggressive behavior of ATC. In comparison, *RET* rearrangements are found in childhood and radiation-induced PTCs, and the *PAX8/PPARG* fusion gene is detected in follicular carcinomas, and are not observed in ATCs (Michael Tuttle 2017d).

10.2.8.5 Screening and Surveillance

Diagnosis of ATC is routinely confirmed through cytological examination (including routine light microscopy and immunohistochemistry) of cells taken by FNA biopsy or of tissue obtained by large needle (core) or surgical biopsies. The latter biopsies are normally done when the FNA shows necrotic or inflamed tissue but no diagnosis has been made (Michael Tuttle 2017d).

10.2.8.6 Genetic Counseling and Testing Strategies

Although the etiology of ATC is widely heterogeneous and includes different mutations of different genes as well as many chromosomal aberrations, NGS-based testing seems to be promising for the future testing. The following genes including C-*myc*, H-*ras*, and *Nm23*, *BRAF*, *RAS*, catenin (cadherin-associated protein) beta 1, *PIK3CA*, *TP53*, *AXIN1*, *PTEN*, and *APC* are among the panel of genes that could be tested in the ATC patients. ATC is a sporadic cancer disease and follows the general rules related to this sort of cancer.

10.2.8.7 Management and Individualized Medicine

An immediate assessment of the disease burden is the most important consideration in the management of ATC. Tumor could significantly increase in size in a matter of days, it can block the airway in patients and even make the tumor unresectable. In case, tracheal invasion is suspected, direct laryngoscopy and bronchoscopy should be considered. ATC patients who have undergone whole thyroid resection, should be subjected to intensive surveillance with cross-sectional imaging every 1–3 months in the first year, and repeated for every 4–6 months later. PET scan should be used to monitor recurrence or to evaluate the outcome of treatment Notably, thyroglobulin measurements and radioactive iodine scanning are not beneficial in the surveillance of ATC (Smallridge et al. 2012).

Many different targeted therapies have been used for ATC; some of these have shown efficiency in some thyroid malignancies (e.g., PTC and FTC) but not others that could be linked to the histotype and molecular profile (including both genetic and epigenetic alterations) (Guerra et al. 2013; Harris and Bible 2011).

- PLX4720 has also been verified to considerably reduce tumor size in both early and late stages of human ATC in vivo (Nucera et al. 2011).
- Another treatment option resembling PLX4720 is PLX4032 which is also known as vemurafenib. It has been suggested to work against ATC (Zhang et al. 2014).
- Targeting the Src family kinases with dasatinib has been put forth as a potential target therapy for ATC. It is already FDA approved for chronic myelogenous leukemia (CML) patients resistant to Imatinib (Chan et al. 2012). It can induce caspase 3/7 to activate apoptosis (Chan et al. 2012). It also inhibited ERK1/2 activation by reducing phosphorylation (Chan et al. 2012). Src inhibitors have been suggested to have a higher therapeutic efficacy in both PTC and ATC and can act at the same time as anti-tumor and anti-metastatic agent (Chan et al. 2012).

10.3 Prostate Cancer

10.3.1 Introduction

Prostate cancer is one of the most prevalent cancers in men worldwide, with an annual estimation of 1,600,000 cases and 366,000 deaths (Fitzmaurice et al. 2016). It has been estimated that in the United States alone, there will be 161,000 cases and 26,700 deaths in 2017 (Siegel et al. 2017). Prostate cancer clinical outcome can range from a microscopic, well-differentiated tumor with no clinical significance to a lethal high-grade cancer.

10.3.2 Pathophysiology and Classification

Prostate cancer (PC) is a multifactorial and multistep cancer with involvement of several genetic events. After the initial genetic event, mutation in such genes as *TP53 and RB1*, can lead to tumor progression and metastasis. About 95% of PCs are adenocarcinomas. About 4% are of transitional cell morphology possibly originating from the urothelial lining of the prostatic urethra. Rare PC cases have neuroendocrine morphology that might either arise from the neuroendocrine stem cells or from abnormal differentiation during cell transformation. Finally, less than 1% of PCs involves squamous cell carcinomas which may occur after exposure to radiation or hormone treatment. Most PCs are multifocal with involvement of clonal and non-clonal tumors (Gerald and Chodak 2017).

10.3.3 Genetics

Genetic studies have suggested that there is a strong familial predisposition in about 5–10% of PC cases. Men with a positive family history of PC are more likely to develop PC, 6–7 years earlier, compared to the general population.

- Different genes have been identified to be engaged in PC.
- Several variants in the 8q24 region have been associated with increased risk of PC.
- The *HPC1* (hereditary prostate cancer 1) gene and the *PCAP* (predisposing for cancer of the prostate) gene are both on chromosome 1.
- The human prostate cancer gene is on the X chromosome (*HPCX*).
- *BRCA2* mutations increase the risk of developing PC, which is of earlier onset and with a more aggressive behavior.
- *HOXB13* may be a risk factor for PC. The frequency of the rare G84E variant is significantly higher in men with early onset, familial PC than in those with late-onset, non-familial PC (Ewing et al. 2012).
- Men with Lynch syndrome have a two-fold increased risk of developing PC as compared to the general population (Raymond et al. 2013).

10.3.4 Genotype-Phenotype Correlations

Risk of developing PC is increased in men with a pathogenic variant in mismatch repair genes with earlier age of onset and a more aggressive phenotype (Haraldsdottir et al. 2014). Porkka et al. (2007) published the first miRNA expression study in PC. Benign and malignant cells were compared and it was found that many miRNAs were either up or down regulated. Since that hundreds of studies have investigated the role of miRNA PC and at least 26 unique miRNAs have been identified as possible markers (Wallis and Nam 2015).

10.3.5 Screening and Surveillance

While there is no consensus on the total prostate-specific antigen (PSA) levels that should be considered prior to prostate biopsy, a cutoff of 4.0 ng/mL is the widely acceptable standard compromising missing important cases at a curable stage with avoiding unnecessary prostate biopsies. Much effort is in progress to identify serum markers with greater diagnostic accuracy for PC.

At the current time, digital rectal examination (DRE) is not suggested anymore for PC screening. Most PC tumors (85%) are peripheral while DRE can detect tumors in the posterior and lateral positions of the prostate gland; Peripheral tumors

can be detected using digital examination. However, upon detection, they are normally at advanced stages since, by definition, stage T1 cancers are nonpalpable (Hoffman et al. 2017).

10.3.6 Genetic Counseling and Testing Strategies

Genetic testing for pathogenic variants in several genes associated with PC risk could be used to identify people at increased risk of developing PC. The susceptibility genes include: *BRCA1*, *BRCA2* mismatch repair (MMR) genes (such as *MLH1* and *MSH2)* and HOXB13 (Lange et al. 2003; Nastiuk et al. 1999; Pritchard et al. 2016). The OncogeneDx Prostate Cancer panel is a next generation sequencing (NGS)-based panel including analysis of the *BRCA1* and *BRCA2* genes as well as 10 other genes involving *ATM*, *CHEK2*, *EPCAM*, *HOXB13*, *MLH1*, *MSH2*, *MSH6*, *NBN*, *PMS2* and *TP53* (Gene 2017).

10.3.7 Management and Individualized Medicine

An individualized risk-adapted strategy based on PSA levels exists for early detection of PC. According to the PIVOT and the European Randomized Study of Screening for Prostate Cancer (ERSPC) trials, this strategy should be offered to men with at least 10–15 years of life expectancy. This includes testing every 2 years for at risk people but every 8 years for those with normal risk. Early PSA testing should be offered to men at increased risk of developing PC. Higher risk groups include men over 50 years old, or men over 45 years of age and with a positive family history of PC, or African-American men with a PSA level of >1 ng/mL at 40 years of age, men with a PSA level of >2 ng/mL at 60 years of age (Mottet et al. 2014).

Two different strategies exist to reduce overtreatment: active surveillance and watchful waiting.

Active surveillance is followed based on PSA data to determine the suitable timing for cure. Patients who are suitable for surgery and radiotherapy must be discussed about the options. Considering individual life expectancy (>10 years), patients will be subjected to prompt treatment when their data surpasses the predefined thresholds suggestive of potentially serious disease. The criteria for deciding >10 years of life expectancy needed for starting active surveillance include: initial stage of the cancer (cT1/2), PSA < 10 ng/mL, biopsy Gleason score <6, <2 positive biopsies, minimal biopsy core involvement (<50% cancer per biopsy). Follow-up should be based on PSA and repeat biopsies. Patients should be counselled on the need for further treatment in the future.

Watchful waiting (also known symptom-guided treatment) was routine in the pre-PSA screening era (before 1990) for which no standardized follow-up is

recommended. The latter strategy is applicable to patients not eligible for local curative treatment and also those with a short life expectancy. In locally advanced M0 patients, androgen-deprivation therapy (ADT), as monotherapy, can be administered in asymptomatic patients with a PSA DT >12 months and a PSA < 50 ng/mL and non-poorly differentiated tumor (Mottet et al. 2014). Androgen receptor gene status in plasma DNA has recently been correlated with response to chemotherapy (Conteduca et al. 2017).

10.4 Conclusion

Different aspects of endocrine cancers (MEN syndromes, thyroid and prostate cancers) were tried to be addressed in this chapter including pathology and classification, genetics, genotype-phenotype correlations, screening and surveillance, genetic counselling and testing strategies, and finally management and individualized medicine, based on our current understanding. While there is much information on some cancers such as MEN2 syndrome, there are still a lot of undetermined facts about many aspects of cancers such as Hürthle cell carcinoma and solitary tumors. As novel technologies such as next-generation sequencing are going all the way from bench to bedsides, our information on different aspects of cancers will be improved. Molecular-based profiling is predicted to further classify cancer histological subtypes into more categories for which distinct target therapy, an aspect of individualized medicine, would be developed. Novel technologies should be used in combination with clinical findings to help achieve outstanding results in the management of cancer cases.

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Chapter 11
A Comprehensive Look at Oromaxillofacial and Laryngopharyngeal Cancers

Samira Basir Shabestari, Iman Shirinbak and Roya Azadarmaki

Abstract Head and Neck cancer is the 9th most frequent cause of death from cancer globally. Genetic analysis and understanding of the majority of malignancies including Head and Neck cancer is of utmost importance, as this may allow for orchestration and implementation of targeted chemotherapy with the potential for higher success in treatment and overall survival. Successful treatment of the Head and Neck cancer patient involves a comprehensive approach with focus on removal of the tumor bulk, management of regional spread, treatment of the morbidities associated with the disease process, and psychological support and care of the patient. Quality of life is improved when a comprehensive approach is undertaken and the patient's morbidities from the cancer and its treatment are treated as seriously as the cancer itself. This involves psychological support of the patient and his/her caregivers and support team. Head and Neck cancer can be exceptionally tough for patients as a combination of airway protection, breathing, speech, ability to enjoy food, and cosmetic appearance can be impacted. This is in addition to the stress of the cancer diagnosis alone and its treatment journey. Prevention, early detection, and advances in therapy with a comprehensive approach to the Head and Neck cancer patient are ideal goals that can help improve outcomes in multiple dimensions. This chapter focuses on the brief classifications and key points of

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clinical and histopathological features of head and neck cancers, new advances in the field of diagnosis aids and treatment modalities and the interactions of practice aspects with psychosocial field.

Abbreviations

ACS	American Cancer Society
AJCC	American Joint Committee on Cancer
CBT	Cognitive Behavioral Therapy
CAM	Complementary and alternative medicine
CT	Computed tomography
CCRT	Concurrent chemo radiotherapy
CNB	Core needle biopsy
ENT	Ear, nose, and throat
EGFR	Epidermal growth factor receptor
EBV	Epstein-barr virus
FNA	Fine needle aspiration
FNAB	Fine-needle aspiration biopsy
FDG-PET	[18F]-fludeoxy glucose positron emission tomography
FDA	Food and Drug Administration
FOM	Floor of the mouth
FISH	Fluorescence in situ hybridization
HNC	Head and neck cancers
HNSCC	Head and neck squamous cell carcinoma
HPV	Human papilloma virus
HBO	Hyperbaric Oxygen
Image-guided radiotherapy [IGRT]	Image-guided IMRT
IHC	Immunohistochemistry
IMRT	Intensity-modulated radiotherapy
LP	Lichen planus
LLLT	Low level laser therapy
MRI	Magnetic resonance imaging
mTOR	Mammalian target of rapamycin
MMP	Matrix metalloproteinases
MORNJ	Medication-related osteoradionecrosis of the jaw
MTC	Medullary thyroid cancer
MEC	Mucoepidermoid carcinoma
MM	Multiple myeloma
NBI	Narrow band imaging
OC	Oral cancer
OR	Oral rehabilitation

OSCC	Oral squamous cell carcinoma
OPC	Oropharyngeal CAncer
OPSCC	Oropharyngeal squamous cell carcinomas
OS	Overall survival
PNS	Paraneoplastic syndromes
PEG	Percutaneous endoscopic gastrostomy
PTSD	Posttraumatic stress disorder
PMDs	Potentially malignant disorders
QOL	Quality of life
RT	Reverse transcription
SGC	Salivary gland cancer
SGT	Salivary gland tumors
SCC	Squamous cell carcinomas
TMJ	Temporomandibular Joint
TB	Toloiden blue
TORS	Trans oral robotic surgery
TNM	Tumor-nodes-metastasis

11.1 Introduction

Cancer is a result of an uncontrolled proliferation of cells due to accumulation of genetic mutations in a cell. Head and Neck cancer (HNC) is the 7th most common diagnosed malignancy and 9th most frequent cause of death from cancer globally. There is a worldwide incidence of over 600,000 new cases and 350,000 deaths each year (Ferlay et al. 2010). Unfortunately, the overall mortality rate for HNC is high at about 50% and is probably due to late diagnosis. So, it is one of the most challenging morbidities.

The most common type of OC is squamous cell carcinomas (SCC) (Eakin 2001). It represents the 11th and 16th most common cancer in males and females respectively. There is a report of 130,000 deaths each year as a result of oral cancer (OC), the 5th most prevalent cancer (Petti and Scully 2007). There is an increase in oral and pharyngeal cancer rate with age, especially among males. The incidence of OC shows a relative rise recently observed (Kerawala 1999).

11.1.1 Anatomy

Oral cavity extends from the wet-dry line of the lips to the oropharyngeal region posteriorly. It includes mucosal portion of the lips, labial and buccal mucosa, anterior two-thirds of the tongue, floor of the mouth (FOM), gingiva, retromolar

pad, and hard palate mucosa. Generally, OC is the sixth most common malignancy. Approximately 20% of anterior tongue and FOM cancers may have skipped metastasis to level IV lymph nodes (Deschler et al. 2014).

The pharynx consists of 3 subsites including the oropharynx, nasopharynx, and hypopharynx. The oropharynx starts from the soft palate and borders the oral cavity and extends inferiorly to the level of the hyoid. Its subsites include the soft palate, including uvula, tonsils, base of tongue, and lateral and posterior pharyngeal walls. The tonsillar area refers to the anterior and posterior pillars composed of the palatoglossus and palatopharyngeus muscles respectively and the lymphoid tonsillar tissue and its surrounding capsule. The base of tongue starts from the level of the circumvallate papillae to the vallecula and base of the epiglottis. The lymphatic drainage is primarily to level II jugulodigastric nodes followed by level III and IV respectively. Nodal drainage to the retropharyngeal and parapharyngeal space lymph nodes can occur, mostly from the lateral and posterior pharyngeal wall subsites.

The nasopharynx is the upper portion of the pharyngeal conduit and defines an area between the choana anteriorly, which is the posterior aspect of the nasal cavity, skull base superiorly, and soft palate inferiorly. Important subsites include the torus tubarius on the lateral wall, which refers to the Eustachian tube opening within the nasopharynx, a superiorly based recess above the torus tubarius within the lateral wall, referred to as the Fossa of Rosenmuller, and the adenoid pad, which is tonsillar and lymphoid tissue originating from the posterior wall. The fossa of rosenmuller is clinically significant as it the most common area of origin of nasopharyngeal carcinoma, and can be a hidden site that requires careful inspection. The primary site of lymphatic drainage are the retropharyngeal lymph nodes.

The hypopharynx is the posterior and caudal portion of the pharyngeal conduit that extends from the hyoid bone superiorly at the junction of the oropharynx to the cervical esophagus and the lower edge of the cricoid cartilage. Its subsites include the pyriform sinuses, posterior pharyngeal wall in the aforementioned territory, and the postcricoid region. The posterior pharyngeal wall is composed by the inferior constrictor muscles. The hypopharyngeal lymphatic drainage is to level II and III jugulodigastric nodes, and less commonly level IV. Retropharyngeal lymph node spread can also occur. Skipped metastasis is seen in these cancers.

The larynx extends from the inferior border of the oropharynx and lies anterior to the hypopharynx. It has three regions including the supraglottis, glottis, and subglottis. The supraglottis includes the epiglottis, arytenoids, aryepiglottic folds, false vocal cords, and ventricles. The glottis larynx includes the true vocal cords and anterior commissure. The subglottis starts several mm below the true vocal cords to the inferior border of the cricoid cartilage. The supraglottic larynx drains into level II–IV lymph nodes and can involve bilateral spread of disease. Glottic laryngeal cancers metastasize unilaterally however regional metastasis is less common than supraglottic cancers. The subglottic larynx drains primarily into level III, IV, and VI lymph nodes. Delphian lymph nodes are prelaryngeal lymph nodes located anteriorly between the thyroid and cricoid cartilages. Spread of laryngeal malignancy into these lymph nodes is associated with a high locoregional recurrence and poor prognosis.

11.1.2 Incidence, Epidemiology

Based on the SEER database, which is a United States based cancer surveillance and reporting database, approximately 48,330 new cases of oral cavity and pharyngeal cancers will be diagnosed in 2016 in the United States. The estimated number of deaths from oral cavity and pharyngeal cancers in 2016 is 9570. This is 2.9% of all newly diagnosed cancers and 1.6% of all cancer related deaths. The 5-year survival is reported at 64% (Cheng and Wright 2011). Approximately 300,682 individuals were living with oral cavity and pharyngeal cancer in the United States in 2013. Based on the SEER database, the estimated number of new individuals diagnosed with laryngeal cancer in 2016 will be 13,430 with the estimated number of deaths at 3620. This is 0.8% of all new cancers diagnosed with 0.6% of all cancer related deaths. The 5-year survival rate is reported as 60.7%. The number of people living with laryngeal cancer in 2013 in the United States was an estimated 89,081.

Oropharyngeal Cancer: The incidence of oropharyngeal cancers (OPC) is rising despite the drop in overall incidence of Head and Neck squamous cell carcinomas (HNSCC). This trend is seen mostly in the developed world and is most likely related to the Human papilloma virus (HPV) association of this cancer. Based on some reports, as many as 90% of oropharyngeal cancers are reported as HPV positive (Torrente et al. 2011). It is estimated that more than 3100 case of HPV related oropharyngeal squamous cell carcinomas (OPSCC) are diagnosed in woman and more than 12,600 in men each year in the United States alone (Viens et al. 2016).

The prevalence of OPSCC is more common in men and the age of presentation is mostly between 50–70 years of age. Men are affected 3–5 more times than women. HPV positive related cancers are seen in individuals 40–59 years of age, white men, nonsmokers and nondrinkers, with a higher number of oral sexual partners. Despite this trend, alcohol and tobacco still pose as a risk factor for OPC and cancers of other head and neck subsites. A diet poor in fruits and vegetables, mate' consumption (stimulant beverage), and chewing of betel squid are other associated factors.

More than 90% of oropharyngeal cancers are squamous cell carcinoma. Lymphoma, melanoma, minor salivary gland tumors, sarcoma, and palsmacytoma are other forms of malignant lesions that can be seen in the oropharynx (Crawford et al. 1979).

Hypopharynx Cancer: Cancer of the hypopharynx accounted for 5.2% of head and neck cancers from 2000–2008. Approximately 1800 new cases are diagnosed each year in the United States (American Cancer Society 2013). As in all head and neck cancers, it is more common in men than woman. The stage of presentation is typically more advanced and the survival rate is slightly worse than head and neck cancers of other sites. Tobacco is a strong risk factor and alcohol potentiates this effect. There is a 100-fold increase risk of hypopharyngeal cancer in individuals who consume both alcohol and tobacco. Moderate to heavy alcohol use seems to

increase the risk of hypopharyngeal cancer even in non-smokers. Hard liquor seems to increase the risk more than light liquor and the causative role of alcohol is more evident in hypopharyngeal cancers compared to laryngeal cancers. 20% of hypopharyngeal cancers have been reported as HPV positive. Hypopharyngeal cancers are known for the tendency for submucosal spread and skipped lymph node metastasis (Deschler et al. 2014).

Laryngeal Cancer: Laryngeal cancer is one of the more common head and neck cancers. The incidence is 3–5 times more common in men than women. The majority of laryngeal cancers remain localized. There is a strong correlation with tobacco use. The role of alcohol in laryngeal cancer is not clear. Heavy marijuana use is linked to laryngeal cancer in the younger age groups. Of the 3 subsites, the rate of glottic cancers is higher than supraglottic malignancies. Subglottic cancers are less frequent than the other subsites. Approximately 25% of laryngeal cancers are HPV positive.

Nasopharyngeal cancer: Nasopharyngeal carcinoma is distinct from other head and neck cancers clinically, pathologically, and epidemiologically (Ali and al-Sarraf 2000). The incidence of nasopharyngeal cancer has an interesting distinct and geographically based distribution worldwide. Global incidence has been reported as 84,400 new cases and 51,600 deaths annually (Louis et al. 2014). In Southern China that incidence is as high as 5–15 per 100,000. In Europe and the US the incidence is 0.5–2 per 100,000 (Ali and al-Sarraf 2000). In the United States, the estimated number of people diagnosed with this cancer in 2016 is 3200 (Cancer. Net 2016). This is a rare disease entity in the United States but there are high to intermediate rates reported in Southern China, Southeast Asia, Northern Africa, Kuwait, and among the Inuit populations of Alaska, Greenland, and Northern Canada; and in migrants of Chinese and Filipino descent. It is now believed that there may be a bimodal distribution in presentation with the first peak in the late teenage years and the second peak later in life between 65 and 79 years of age. Five-year survival in Europe is 50% with better survival in younger and earlier stages of disease (Cancer Research UK 2015).

The 5 year survival rate in the United States ranges from 72% for stage I nasopharyngeal carcinoma to 38% for stage IV (American Cancer Society 2016). Like other forms of head and neck cancer, this cancer is seen more commonly in men than women with a male to female ratio of 33.5:1 (Ali and al-Sarraf 2000).

Unlike squamous cell carcinoma of other subsites of the head and neck, alcohol and tobacco do not have a great role in nasopharyngeal cancer development. The etiology appears to be a combination of genetic, viral, and environmental factors. Nitrosamines, poor hygiene, poor ventilation, smoking, and nasal balms have also been implicated. The role of EBV in nasopharyngeal carcinoma is well known. This is a B-lymphocyte herpes virus that is found in precancerous lesions and undifferentiated nasopharyngeal carcinoma seen in endemic areas but not within normal epithelium of the nasopharynx (Louis et al. 2014).

According to the World Health Organization classification for head and neck cancer, nasopharyngeal carcinoma is classified into type I, II, and III. Type I refers to squamous cell carcinoma, type II refers to non-keratinized carcinoma, and type

III refers to undifferentiated carcinoma. Epstein-Barr virus (EBV) is mostly associated with type II/III nasopharyngeal carcinomas and HPV has been seen in nasopharyngeal carcinomas with oropharyngeal extension. There is argument that in the latter group, the tumor may be originating from the oropharynx and extending into the nasopharynx (Louis et al. 2014).

11.1.3 Survival and Mortality Rate

The survival rate varies, depending on Several factors, including age, sex, ethnic group, stage at the time of diagnosis and anatomic location (Chen et al. 2004) (Table 11.1). The overall 5-year survival rate in the modern era remains suboptimal at 50% (Jemal et al. 2009). The increase in survival rate in the past several years is not substantial despite advances in diagnostic and treatment modalities (Howlader et al. 2015). Locoregional recurrence is seen in 30–40% and distant metastasis in 20–30% of cases (Murar and Forastiere 2008). The overall mortality rate for OC is high at about 50% and is probably due to the diagnosis (Table 11.2).

11.2 Etiology and Risk Factors

11.2.1 Tobacco and Alcohol

Tobacco and Alcohol are associated with up to 75% of all head and neck cancers (Hashibe et al. 2009). Recently, new molecular studies have demonstrated that tobacco-related cancers could be more aggressive than cancers in non-smokers patients (Cao et al. 2016). HNC patients who associated with tobacco and alcohol

Table 11.1 Overall 5-year survival based on subsite

Subsite %	5-year survival rate
Oral cavity and pharynx	64
Lip	89
Tongue	65
FOM	52
Gum and other oral cavity	59
Tonsil	73
Oropharynx	42
Hypopharynx	33
Larynx	61
Nasopharynx	61
Salivary gland	72

Howlader et al. (2015), American Cancer Society (2016) last revised: 8 August 2016

Table 11.2 Estimated incidence, mortality and 5-year prevalence: both sexes

Cancer	Incidence		Mortality	
	(%)	ASR (W)	(%)	ASR (W)
Lip, oral cavity both sex	2.1	4.0	1.8	1.9
Nasopharynx both sex	0.6	1.2	0.6	0.7
Other pharynx both sex	1.0	1.9	1.2	1.3

Incidence and mortality data for all ages. 5 year prevalence for adult population only. Number of new cancer cases (thousands)
Age standardized rate = ASR (W). proportions per 100,000
Source GLOBOCAN (2012)

consumption often exhibit mutated *pRb, TP53* overexpression, and decreased *p16* expression. So, lifestyle modifications have a pivotal role in prevention and control programs of OC patients.

11.2.2 Human Papilloma Virus

The role of HPV in OPC has been established, but its etiologic role in OC is unclear (Lingen et al. 2008). Large scale gene sequencing efforts in HNSCC has been reported in several studies. 80% of HPV-negative HNSCC display chromosomal instability (Leemans et al. 2011; Pickering et al. 2013; Zack et al. 2013). In HPV-negative tumors the consistent findings were that a large number of tumor suppressor genes were mutated. The tumor suppressor gene, *p53*, has been found to be commonly mutated in all the sequencing studies. 46–73% of HNSCCs are shown to have a mutation the *p53* gene. Mutations in the *CDKN2A* tumor suppressor gene is seen in many HNSCCS. Homozygous deletion of *CDKN2A* is seen in up to 30% of HNSCCs and a form of mutation of this gene is seen in another 10–20% of samples (Riaz et al. 2014). The novel finding in these sequencing efforts was the discovery of the mutation of the NOTCH1 transmembrane receptor, which has a role in cell differentiation and embryonic development. Depending on the cell context, *NOTCH* can serve as a tumor suppressor or oncogene. It has been demonstrated that *NOTCH1* serves as a tumor suppressor gene in HNSCC with reduced expression in these tumors (Golub and Vogelstein 2011). Additional genetic alterations have been seen in *CASP8, FAT1*, and *HRAS* genes in addition to genes involved in the bodies management of oxidative stress and chromatin structure.

HPV contain 2 oncogenes, *E6* and *E7*, which inactivate *p53* and *Rb* respectively. These 2 proteins are believed to be important mediators for producing a malignant phenotype. Mutations of the *p53* gene are almost never seen in HPV-positive tumors as *p53* is inhibited by *E6*. HPV-positive tumors are found to have *PIK3CA*

mutations more commonly compared to HPV-negative tumors. *PIK3CA* is the second most common gene mutated in all forms of cancer. Alterations in this pathway is thought to play a role in cancer cell growth, metabolism, and survival (Kandoth et al. 2013; Engelman 2009).

11.2.3 Nutritional Factors

Various micro-nutrients, including vitamin A, C and E, fibers, and flavonoids reduces the risk of OC and OPC (Gillison 2007). Some studies suggest that total calories, saturated oil, starchy foods and processed meat have emerged increasing OC risk. Whether or not vitamin D on cancer development is controversial. Some studies have showed that the anti-cancer effects of vitamin D is due to inhibition of proliferation (Buttigliero et al. 2011). Vitamin D3 can impact HNSCC cancer progression and development by regulating the immune system (Rita et al. 2013).

11.2.4 Genetic Predisposition to Head and Neck Cancer

Although the majority of HNSCC is linked to exposure to carcinogens and viruses, there are several familial predispositions identified. There is an increased risk of HNSCC in patients with Fanconi Anemia, which is an autosomal recessive genomic instability syndrome (Moldovan and D'Andrea 2009). In addition, rare clusters of HNSCC has been identified in families with germ-line mutations in *CDKN2A* and *ATR* (Schneider-Stock et al. 2003; Tanaka et al. 2012).

Pooled analysis reveals an increased risk of developing HNC in first degree relatives of patients with HNSCC with an odds ratio of 1.7. There is earlier data suggesting a 3.5-fold increase risk. Hypothetically genetic differences in cell cycle regulation, DNA repair, and carcinogen metabolism can increase the risk of carcinogenesis associated with tobacco and alcohol use. Phenotypic differences have been demonstrated in lymphocytes of patients with HNSCC (Cheng et al. 1998).

11.3 Pathogenesis

The molecular aspects of carcinogenesis include a cumulative effect on cell differentiation and division which are resulted from epigenetic changes or mutations in two extended group of genes: Tumor suppressor genes and Proto-oncogenes (Harari and Huang 2000).

11.3.1 Extracellular Enzymes

Matrix Metalloproteinases (MMP) are involved in dissolution of the exracellular matrix. They play an important role in cell behaviors such as proliferation, migration, differentiation, and apoptosis (Van and Libert 2007). Dissolution of extracellular matrix is also required for local invasion and distant metastasis of tumors.

Head and neck cancers are linked to upregulation of these enzymes. Overexpression of MMP-1 and MMP-9 mRNA is associated with progression of oral dysplasia to oral SCC. MMP-2, MMP-7, and *MMP-9* overexpression is seen in supraglottic SCC and *MMP-2, MMP-9,* and *MMP-20* overexpression is seen in laryngeal SCC (Specenier and Anja 2015).

Several growth factors are implicated in HNSCC. Polymorphism of the *vascular endothelial growth factor (VEGF)* gene may impact the initiation or progression of OC. Many studies have linked increased *VEGF* expression with worse HNSCC outcome, including a tendency for lymph node metastasis (Ghias 2014). There is a suggested role for connective tissue growth factor in the invasive and migratory abilities of Oral squamous cell carcinoma (OSCC) cell lines (Yang et al. 2012).

Elevated *epidermal growth factor receptor (EGFR)* expression detected by immunohistochemistry (IHC) is present in over 90% of HNSCC specimens (Mehra et al. 2008). Elevated *EGFR* expression levels in HNSCC tumor has been correlated with decreased survival (Grandis et al. 1998; Ang et al. 2002). Increased *EGFR* expression in HNSCC is the result of gene amplification and transcriptional activation (Chung et al. 2006; Grandis et al. 1996). In addition to HNSCC, *EGFR* overexpression has also been noted in premalignant dysplastic lesions (Rubin Grandis et al. 1998). Cetuximab, which is used in management of HNSCC, is a monoclonal antibody which binds to *EGFR* and competitively inhibits *EGF* and other ligands.

11.3.2 Immunosuppression

The immune system has an outstanding role in the development and progression of HNC (Ferris 2015). Treatment strategies can be designed based off of the endogenous immune responses generated against these malignancies and the expression of immunologic markers (Pardoll 2012). Immunosuppressive states induced by cancer in patients may explain the unsatisfactory results of clinical vaccine trials (Sakakura and Chikamatsu 2013).

11.4 Paraneoplastic Syndromes

Paraneoplastic syndromes (PNS) accompany the malignant tumor but are not directly related to the cancerous mass, its invasion, or metastasis. An estimated 1–7.4% of all malignancies are associated with a paraneoplastic syndrome (Baijens

and Manni 2006). The rate of presentation of PNS in HNC patients is unknown. The majority of such occurrences are reported as case presentations or reports. PNS related to HNC can be categorized as: endocrine, cutaneous or dermatologic, hematologic, neurologic, osteoarticular or rheumatologic, ocular syndromes (Ferlito et al. 2007). In some cases, PNS can be more serious than the consequences of the primary tumor and other times it can become misleading by taking attention away from the primary malignancy (Glick and William 2015).

Bazex syndrome is a frequently reported paraneoplastic dermatological syndrome. The cutaneous paraneoplastic syndromes associated with OC include Sweet syndrome, Bazex syndrome, and paraneoplastic pemphigus (Toro et al. 2010). Review of articles showed that the development of malignant cutaneous neoplasms (SCC, Malignant Melanoma) within the hyperkeratotic lesions of the syndrome (Papillon-Lefèvre syndrome) is very rare (Al-Benna et al. 2009; Schackert et al. 2014). In the literature laryngeal cancers have seldom been associated with cerebellar degeneration and Eaton-Lambert myasthenia syndrome, which are the most reported neurologic PNS (Baijens and Manni 2006).

11.4.1 Premalignant Lesions

Premalignant lesions are morphologically altered mucosa with a higher probability of malignant transformation compared to their surrounding mucosa. These lesions can occur in all areas of the oral cavity and laryngopharynx. The main premalignant disorders of the oral cavity are erythroplakia, leukoplakia, oral lichen planus (LP), actinic keratosis, discoid lupus erythematosus, oral submucous fibrosis, epidermolisys bullosa and dyskeratosis congenital (Napier and Speight 2008). As most oral premalignant lesions are asymptomatic, they are incidentally diagnosed. The premalignant lesions of the larynx are mostly found at the level of the glottis and along the mucosal lining of the true vocal cords. Premalignant lesions in the supraglottis and subglottis are not common. Of all oral cavity subsite malignancies, FOM lesions are most likely to arise from an area of erythroplakia or leukoplakia. In 5–25% of oral leukoplakias we can expect malignant transformation. However, histopathologically carcinoma in situ is seen in 90% of erythroplakic lesions (Neville et al. 2016). Based on some studies, the rate of malignant transformation in Oral LP is judged to be quite low (between 0.07 and 2%) (Lodi et al. 2005) (Fig. 11.1). It is shown that malignancy transformation rate in submucous fibrosis between 2 and 7% (Hazarey et al. 2007).

Current treatment modalities for treatment of premalignant lesions of the oral cavity and laryngopharynx include observation and serial biopsies in lesions with a lower likelihood of progressing to cancer versus cryotherapy, laser ablation or other modes of surgical resection in the more aggressive forms.

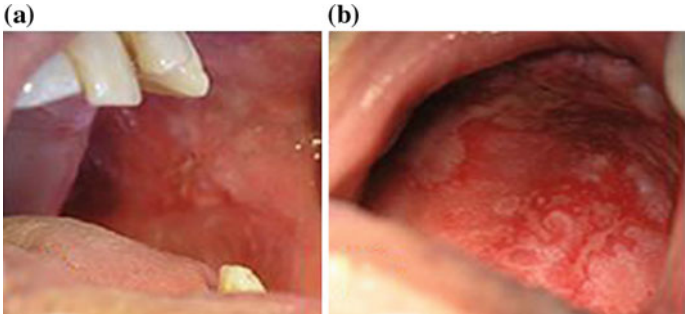

Fig. 11.1 Intra-oral view: atrophic oral lichen planus with malignant transformation. **a** Buccal and, **b** palatal mucosa (Courtesy of Dr. Basir shabestari)

11.5 Clinical Presentation

Patients with oral cavity or laryngopharyngeal cancer can have a wide range of presentations from an asymptomatic patient with incidental findings on physical examination, dental work, or radiographic imaging to a patient with a wide variety of symptoms. These symptoms include but are not limited to localized pain, dysphagia, odynophagia, sore throat, hoarseness, globus sensation, otalgia, unexpected weight loss, oral mucosal ulceration, oral bleeding, upper airway bleeding, difficulty with speech articulation or mastication, trismus, localized paresthesias or paresthesias along a sensory nerves distribution or anesthesia in the absence of a history of trauma, or persistent mass in the mouth or neck (Stewart and Kleihues 2003) (Fig. 11.2).

Historically, the oral exam has served as the 1st step in oral cancer screening and diagnosis in patients who present with signs and symptoms of OC and potentially malignant disorders (PMDs) (Lingen et al. 2008). Loosened teeth (Basir Shabestari et al. 2012) or poorly fitting dentures should raise a red flag and warrant close inspection of the alveolar ridge for any masses or lesions (Fig. 11.3b). Possible tissue changes in oral cavity or larynopharyngeal cancers may include mucosal surface discolorations such as a red and or white lesion; a change in the surface

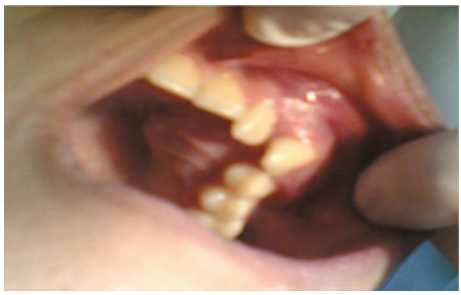

Fig. 11.2 A persistent mass in the mouth accompanied by loosened and displaced teeth may be an indicator for malignancy. Basir Shabestari et al. (2012), with permission

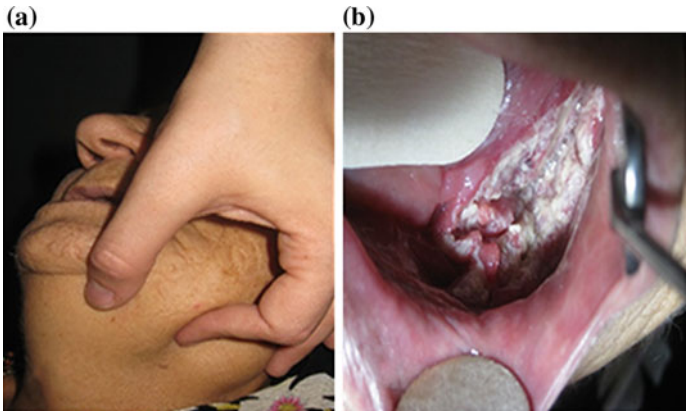

Fig. 11.3 **a** Extra-oral view: enlarged lymph nodes, firm and fixed in palpation, **b** intra-oral view: SCC of the edentulous alveolar ridge presented as indurated ulcer with irregular border (Courtesy of Dr. Basir shabestari)

texture producing a smooth, rough, granular, crusted, or exophytic lesion; versus an indurated ulcer. With locoregional spread and lymphatic metastasis, lymph nodes can become enlarged, firm to hard in texture, and fixed in the presence of extra-capsular spread (Bsoul et al. 2005) (Fig. 11.3a).

11.6 Diagnostic Evaluation and Histopathologic Features

A complete oral cavity, oropharynx, laryngopharynx, and neck exam is the first step in diagnosing a patient with head and neck cancer. In addition to a thorough inspection of the oral cavity and oropharynx, base of tongue digital palpation and fiberoptic nasopharyngolaryngoscopy are important office-based exam tools for thorough evaluation of patients.

Diagnosis is mostly rely on histopathological evaluation as a gold standard (Ogawa et al. 2004). For easily accessible lesions that are not a significant bleeding risk, office based biopsy will allow for tissue sampling. Intraoperative direct laryngoscopy or nasal endoscopy with biopsy is a necessity in the majority of cases for tissue diagnosis. Fine needle aspiration (FNA) of a cervical lymph node with or without ultrasound guidance is another method of tissue sampling that can establish the diagnosis of HNC in cases of regional lymph node involvement. In cases of lymphoma, lymph node FNA is usually not adequate and an excisional biopsy is required.

Squamous cell carcinoma, which is the most common form of HNC, arises from dysplastic surface epithelium. Histopathologically it consists of invasive islands and cords of malignant squamous cells. Keratin pearls may be seen within these lesions. Basal lamina degradation and connective tissue modulation is seen.

(Neville et al. 2016). Invasive carcinomas involve basement membrane and connective tissue invasion and can demonstrate penetration into the vascular system (Regezi et al. 2012).

11.6.1 Immunohistochemistry

In cases with questionable or in determinant diagnosis based on microscopic evaluation alone, a secondary confirmatory test such as IHC is required (Hayat 2005). The IHC panel staining has efficacy in understanding and differentiating the PMDs and their malignant progression, defining metastatic tumors, and in the diagnosis and differential diagnosis of benign and malignant neoplasms (Halliday 2010; Wick 2008). It should be mentioned that the number of tumor-specific markers is very few. IHC reactivity alone is often not diagnostic but in combination with clinical history and histopathology can support arriving at the correct diagnosis (Hunt 2011).

11.6.2 Cancer Screening and Early Detection

Early diagnosis of head and neck cancers can positively affect outcomes and allow for more conservative medical therapy with reduced morbidity and mortality (Masthan et al. 2012). Although oral cancer typically arises in accessible areas, with an incomplete exam, delayed diagnosis is common. As symptoms are uncommon and subtle in earlier stages of the disease, the diagnosis is typically made in more advanced stages (Bsoul et al. 2005). A combination of public awareness of early symptoms as well as proper education of health care providers, particularly primary care physicians, should lead to early detection, increase cure rates, and decrease treatment-related morbidity (Gomez et al. 2010).

11.6.3 Diagnostic Aids

11.6.3.1 Toluidine Blue and Lugol Staining

Toluidine Blue (TB) is an acidophilic metachromatic dye, which has an affinity to bind with DNA (Satoskar and Dinakar 2006). It helps in better visualization of high risk areas involved with rapid cell proliferation as seen in PMD and OSCC. To date, TB is cleared by the US Food and Drug Administration (FDA) as an adjunct marking aid in combination with a chemiluminescence light device (Rethman et al. 2010). Lugol staining may have a role for early detection of oral cavity, pharynx,

and laryngeal lesions. Staining with Lugol or TB allows for analysis of the surface epithelium and by definition does not play a role in early detection of submucosal lesions (Vergez et al. 2013).

11.6.3.2 Endoscopy

Endoscopy under general anesthesia, associated with palpation, adequate tissue sampling and biopsies, proper and detailed reporting, in addition to dated drawings or pictures and video clips are recommended for diagnosis and staging purposes. Panendoscopy should be performed for a more thorough evaluation to rule out a synchronous lesion that can occur in up to 8.5% of cases (Dhooge et al. 1998).

11.6.3.3 Novel Endoscopy Techniques

The dynamics of tissue composition and physical properties of light has introduced a new era in evaluating the head and neck for precancerous lesions and in the early detection of cancer. Tissue contains endogenous fluorophores that vary between healthy and pathological tissue (Vergez et al. 2013). Excitation of tissue induces autofluorescence. New bioendoscopy techniques use this property to detect tissue pathology. Narrow band imaging (NBI) endoscopy uses emission wavebands in the range of hemoglobin absorption spectra and as such can improve visualization of microvascularization, which is seen in cancer and precancerous lesions (Muto et al. 2010). This technology has been proposed for early detection in the oral cavity, pharynx, and larynx. Confocal endomicroscopy can potentially demonstrate in vivo cell level tissue images (Vergez et al. 2013). This technique is being investigated in Ear, Nose, and Throat (ENT) oncology.

Optical technology and bioendoscopy is as its infancy. Although the efficacy of these techniques have yet to be demonstrated, the promise of such technology warrants time and financial investment in research protocols.

11.6.3.4 Visual Adjunctive Tools

Vizilite and the VEL scope are two optical based devises used in visualizing the oral cavity. Vizilite uses a non-toxic chemiluminescent light. Vizilite Plus technology is used in identifying soft tissue abnormalities and has been used in screening of the oral cavity. The FDA licensed VEL scope for direct auto fluorescence visualization in the oral cavity. The VEL scope has been recently introduced into the market as a diagnostic adjunct for OC detection (Cheng and Wright 2011). There is no general consensus on the specificity and sensitivity of these devices, and the ability for early disease detection (Wilder-Smith et al. 2010).

11.6.3.5 Brush Cytology

Oral brush biopsy with computer-assisted analysis is commercially available, which has high sensitivity for detecting OC (Mehrotra et al. 2011). Cytology of the oral mucosa is useful in the assessment of cellular morphology although final diagnosis is made by an oral and maxillofacial pathologist based on standard histopathologic evaluation (Maurer et al. 2013).

11.6.3.6 Vocal Cord Motion

For glottic and laryngeal tumors proper evaluation and documentation of the true vocal cord motion is key and mandatory for staging purposes.

11.6.3.7 Fine-Needle Aspiration Biopsy

Fine-Needle Aspiration Biopsy (FNAB) or core needle biopsy (CNB) is a relatively inexpensive, safe and rapid procedure in the evaluation of suspicious masses in other areas of the head and neck, or in cases that conventional biopsy is contraindicated (Glick and William 2015). Despite the potential drawbacks of FNAB, it still remains an accurate diagnostic aid, comparable to frozen section, for diagnosis of salivary gland tumors (Stewart et al. 2000). Ultrasound guided FNAB has a major role in tissue sampling of deep jugulodigastric nodes. In fact, open biopsy is frowned upon in cases of suspected HNSCC as this can disrupt the natural and relatively predictable pattern of lymphatic drainage. Such disruption can impact the role of selective neck dissection, which is needed for accurate staging and further treatment planning.

11.6.4 Salivary Diagnostics

11.6.4.1 Salivary Gland Tumor Diagnostic Markers

Molecular biomarkers based on blood or saliva samples can be used for screening, enabling the early detection of OC long before conventional microscopic or imaging techniques (Seoane Leston and Diz Dios 2010; Arellano-Garcia et al. 2010). Compared with to blood biomarkers, salivary biomarkers have advantages including ease and non-invasive sampling (Tiziani 2009). Salivary metabolomics is arising as a diagnostic and screening tool for leukoplakia and OC (Wang et al. 2014). *Thioredoxin, ODZ, SAT, IL-8*, and *IL-1b* are examples of salivary biomarkers that have been used successfully in the detection of OC (Eisbruch et al. 2003). Some studies show that use of these markers as screening tools to detect OC is controversial (Ishikawa et al. 2016).

11.6.5 Imaging Modalities

Computed tomography (CT) of the neck and chest should be performed in oral cavity, larynx, and pharyngeal cancers for evaluation of extent of local disease and as part of initial staging. Magnetic resonance imaging (MRI) is an effective tool in assessing local extent of tissue and soft tissue involvement in oral cavity and oropharyngeal cancers. Extent of mandibular invasion can be seen on both CT and MRI. Dynamic CT imaging with contrast can be useful in detecting the extent of laryngeal and hypopharyngeal cancers. CT imaging of the neck and chest is the first line for evaluation of lymph node involvement from the level of the skull base to superior mediastinum.

[18F]-Fludeoxyglucose Positron emission tomography(FDG-PET) has increased sensitivity in detecting small primary tumors and non-palpable nodal disease. Improvement in preoperative staging accuracy and treatment planning can be attributed to this diagnostic tool (Gupta et al. 2011). In HNSCC of unknown primary, CT neck and chest and FDG-PET/CT should be a part of the diagnostic workup. Imaging should proceed endoscopy and biopsy.

11.7 Prognosis

Tumor site, size, thickness and depth of invasion, involvement of adjacent structures, presence of extracapsular spread and perivascular and perineural invasion are some important prognostic factors in HNSCC. New studies showed that delayed therapy results in a worse prognosis among HNC patients (Polesel et al. 2017). The most important recognized prognostic indicator affecting the overall and disease-free survival is the presence of regional cervical lymph node metastases, which if present reduces the survival rate by 50% (Leemans et al. 1994). Locoregional lymph node metastasis is seen in 10–50% of head and neck cancers (Ebrahimi et al. 2011).

HNC Prognosis may be influenced by some patient-related factors such as ethnicity, socioeconomic status and comorbidity (Prieto et al. 2005).

The predominance of HPV-positive tumors in the oropharynx and the difference in the nature of these tumors relative to the tobacco and alcohol HNSCC, adds another layer of prognostic factors in tumors of this subsite. Studies have shown that patients with HPV-positive OPC have 2× better 5 year and 10-year overall survival (OS) compared with HPV-negative tumors (Chaturvedi et al. 2011).

11.7.1 Staging

Staging is the foundation for of diagnosis, treatment planning and application, patient surveillance post treatment, and clinical research. The American Joint Committee on Cancer (AJCC) has developed Tumor-Nodes-Metastasis

(TNM) staging system of cancer, which is an reliable and important predictor of survival rate (Edge et al. 2010) (Table 11.3). The Tumor-Nodes-Metastasis staging system differs from one type of cancer to another type (Table 11.4). Compared to microscopic grading, clinical staging correlates much better with prognosis (Neville et al. 2016). The staging system for HNSCC classify lesions as stages I through IV with stages I and II representing early disease and stages III and IV being advanced disease (Edge et al. 2010) (Tables 11.5, 11.6, 11.7 and 11.8).

11.8 Treatment

Cancer treatment aims to cure disease, and improve the quantity and quality of life (QOL) with minimal side effects (Boyle 2001). The choice of treatment depends on cell type and degree of differentiation, the presence of bone invasion, TNM stage of tumor at the time of diagnosis,the ability to achieve adequate surgical margins, patient's general health status and the absence or presence of metastases (Kowalski et al. 2005; Ribeiro et al. 2000). The time from diagnosis to treatment initiating should ideally be 2 weeks or less, and no longer than 4 weeks (Vergez et al. 2013). The rate of tumor control with radiation may be decreased by 1% for each week in delay of treatment (Wyatt et al. 2003). Team approach is the best option in treating HNC patients. In this method, each involved specialist have an important role for an optimal treatment outcome. In patients with HNC restoration of function, aesthetics, and prevention of infection should be major factors before cancer therapy.

The primary modality of treatment of OC is surgical as tumors of this area are more readily accessible for surgical excision. In addition, radiation has a higher chance of inducing osteoradionecrosis secondary to the tumor's vicinity to the mandible or maxilla. OC are also less sensitive to chemoradiation relative to OPC and laryngeal cancers. Advanced stage disease may require post excision radiation. Chemoradiation is administered when there are positive surgical margins, extensive lymph node disease and extracapsular spread, or distant metastasis (Shanthi and Forastiere 2016).

Advances in access to the oropharynx with transoral robotic and laser-assisted surgery has been a shifting paradigm in management of oropharyngeal malignancy. In the past radiation was used for early stage disease and chemoradiation for more advanced tumors. Currently trans oral robotic surgery (TORS) and trans oral laser-assisted surgery has opened a new chapter in management of oropharyngeal tumors with more of a role for surgical resection.

Hypopharyngeal cancers have traditionally been treated with surgical resection. There is more success with radiation and chemoradiation with organ preservative in the past years.

Both radiation alone and surgical excision, mostly laser assisted, have good control rates for early stage tumors. There is more of a debate on management of more advanced staged tumors. Chemoradiation has shown to achieve better control rates in the recent years leaving room for T3 and early T4 disease to allow for organ

Table 11.3 Tumor-node-metastasis staging system

T—Primary tumor oral cavity[a]	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ
T1	Tumor 2 cm or less in greatest dimension
T2	Tumor more than 2 cm but not more than 4 cm in greatest dimension
T3	Tumor more than 4 cm in greatest dimension
T4 (lip)	Tumor invades through cortical bone, inferior alveolar nerve, floor of mouth, skin of face (chin, nose)
T4a (oral cavity)	Tumor invades adjacent structures (e.g., through cortical bone, [mandible or maxilla] into deep [extrinsic] muscle of tongue [genioglossus, hyoglossus, palatoglossus, and styloglossus], maxillary sinus, skin of face)
T4b (Lip and oral cavity)	Tumor invades masticator space, lateral pterygoid muscle, pterygoid plates, or skull base, or encases the internal carotid artery
T—Primary tumor oropharynx	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ
T1	Tumor 2 cm or less in greatest dimension
T2	Tumor more than 2 cm but not more than 4 cm in greatest dimension
T3	T3 Tumor more than 4 cm in greatest dimension or extension to lingual surface of epiglottis
T4a	Moderately advanced local disease. Tumor invades the larynx, deep/extrinsic muscle of the tongue, medial pterygoid, hard palate, or mandible
T4b	Very advanced local disease. Tumor invades the lateral pterygoid muscle, pterygoid plates, lateral nasopharynx, or skull base, or encases the carotid artery
T—Primary tumor supraglottis	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ
T1	Tumor limited to one subsite of the supraglottis with normal vocal fold mobility
T2	Tumor invades mucosa of more than one adjacent subsite of the supraglottis or glottis or region outside the supraglottis (e.g., mucosa of base of tongue, vallecula, medial wall of pyriform sinus) without fixation of the larynx
T3	Tumor limited to the larynx with vocal fold fixation and/or invades any of the following: postcricoid area, pre-epiglottic tissues, paraglottic space, and/or inner cortex of thyroid cartilage

(continued)

Table 11.3 (continued)

T4a	Moderately advanced local disease. Tumor invades through the thyroid cartilage and/or invades tissues beyond the larynx (e.g., trachea, soft tissues of neck including deep extrinsic muscle of the tongue, strap muscles, thyroid, or esophagus)
T4b	Very advanced local disease. Tumor invades prevertebral space, encases carotid artery, or invades mediastinal structures
T—Primary tumor glottis	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ
T1	Tumor limited to the vocal fold(s) (may involve anterior or posterior commissure) with normal mobility
T1a	Tumor limited to one vocal fold
T1b	Tumor involves both vocal folds
T2	Tumor extends to the supraglottis and/or subglottis, and/or with impaired vocal fold mobility
T3	Tumor limited to the larynx with vocal fold fixation and/or invasion of paraglottic space, and/or inner cortex of the thyroid cartilage
T4a	Moderately advanced local disease. Tumor invades the outer cortex of the thyroid cartilage and/or invades tissues beyond the larynx (e.g., trachea, soft tissues of the neck, including deep extrinsic muscle of the tongue, strap muscles, thyroid, or esophagus)
T4b	Very advanced local disease. Tumor invades prevertebral space, encases carotid artery, or invades mediastinal structures
T—Primary tumor subglottis	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ
T1	Tumor limited to the subglottis
T2	Tumor extends to the vocal cord(s) with normal or impaired mobility.
T3	Tumor limited to the larynx with vocal fold fixation.
T4a	Moderately advanced local disease. Tumor invades cricoid or thyroid cartilage and/or invades tissues beyond the larynx (e.g., trachea, soft tissues of the neck including deep extrinsic muscles of the tongue, strap muscles, thyroid, or esophagus)
T4b	Very advanced local disease. Tumor invades prevertebral space, encases carotid artery, or invades mediastinal structures
T—Primary tumor hypopharynx	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ
T1	Tumor limited to one subsite of the hypopharynx and is 2 cm or less in greatest dimension

(continued)

Table 11.3 (continued)

T2	Tumor invades more than one subsite of the hypopharynx or an adjacent site, or measures more than 2 cm but not more than 4 cm in greatest dimension without fixation of the hemilarynx or extension to the esophagus
T3	Tumor more than 4 cm in greatest dimension or with fixation of the hemilarynx or extension to the esophagus
T4a	T4a Moderately advanced local disease. Tumor invades thyroid/cricoid cartilage, hyoid bone, thyroid gland, esophagus, or central compartment soft tissue
T4b	Very advanced local disease. Tumor invades prevertebral fascia, encases carotid artery, or involves mediastinal structures
N—Regional lymph nodes Involvement[b]	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis are involved
N1	Metastasis in a single ipsilateral lymph node, 3 cm or less in greatest dimension
N2	Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6 cm in greatest dimension; or in multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension; or bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension
N2a	Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6 cm in greatest dimension
N2b	Metastasis in multiple ipsilateral lymph nodes, not more than 6 cm in greatest dimension
N2c	Metastasis in bilateral or contralateral lymph nodes, not more than 6 cm in greatest dimension
N3	Metastasis in a lymph node more than 6 cm in greatest dimension
M—Distant metastasis[c]	
MX	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis

[a]Superficial erosion alone of bone/ tooth socket by gingival primary is not sufficient to classify a tumor as T4

[b]The regional lymph nodes are the cervical lymph nodes. Midline nodes are considered ipsilateral nodes

[c]*Note* Metastases at level VII are considered regional lymph node metastases

From lip and oral cavity. In: Edge et al. AJCC Cancer Staging Manual (2010a, b)

preservation. Advanced T4 disease would benefit from surgical resection and concomitant chemoradiation.

Early stage nasopharyngeal carcinoma is treated with radiation and advanced staged tumors are treated with chemoradiation. Surgery is rarely used and has more of a salvage role in addressing neck disease or tumor of the primary site.

Adjuvant radiation and chemotherapy should start 4–6 weeks postoperatively. This may be delayed if postoperative complications arise.

Table 11.4 Staging of salivary gland tumors

TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ
T1	Tumor 2 cm or less in greatest dimension without extra parenchymal extension[a]
T2	Tumor more than 2 cm but not more than 4 cm in greatest dimension without extra parenchymal extension
T3	Tumor more than 4 cm in greatest dimension and/or tumor having extra parenchymal extension
T4a	Tumor invades skin, mandible, ear canal, and/or facial nerve
T4b	Tumor invades skull base and/or pterygoid plates and/or encases carotid artery
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in a single ipsilateral lymph node, 3 cm or less in greatest dimension
N2	Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6 cm in greatest dimension In multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension In bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension
N2a	Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6 cm in greatest dimension
N2b	Metastasis in multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension
N2c	Metastasis in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension
N3	Metastasis in a lymph node more than 6 cm in greatest dimension
M0	No distant metastasis
M1	Distant metastasis

[a]Extraparenchymal extension is clinical or macroscopic evidence of invasion of soft tissues. Microscopic evidence alone does not constitute extraparenchymal extension for classification purposes

From lip and oral cavity. In: Edge et al. AJCC Cancer Staging Manual (2010a)

11.8.1 Surgery

Many surgical approaches are available to remove a tumor and to restore function and appearance. Minimally invasive and endoscopic resection of HNC is in sync with the natural evolution of surgical disciplines and applied in the multidisciplinary treatment paradigm of HNC (Herron and Marohn 2008). Laser is commonly used in surgical treatment of HNC patients (Jerjes et al. 2011).

Transoral robotic surgery (TORS) has become a common practice in management of HNC with the potential advantage of application in less accessible sites (Richmon et al. 2014). TORS appears to be a safer and more generalizable surgical innovation for HNC treatment, which has demonstrated maintaining excellent oncologic and improved functional outcomes in swallowing and speech (Shah et al. 2014). TORS is a minimally invasive approach associated with a decreased length

Table 11.5 Staging of nasopharyngeal tumors

T—Primary nasopharynx	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ
T1	Tumor confined to the nasopharynx or tumor extends to the oropharynx and/or nasal cavity without parapharyngeal extension
T2	Tumor with parapharygeal extension
T3	Tumor involves bony structures of skull base and/or paranasal sinuses
T4	Tumor with intracranial extension and/or involvement of cranial nerves, hypopharynx, orbit, or with extension to the infratemporal fossa/ masticator space
N—Regional lymph node involvement	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis are involved
N1	Unilateral metastasis in cervical lymph node(s), 6 cm or less in greatest dimension, above the supraclavicular fossa, and/or unilateral or bilateral retropharyngeal lymph nodes, 6 cm or less in greatest dimension[a]
N2	Bilateral metastasis in cervical lymph node(s), 6 cm or less in greatest dimension, above the supraclavicular fossa[a]
N3	Metastasis in lymph node)[a] >6 cm and/or to supraclavicular fossa[a]
N3a	Greater than 6 cm in dimension
N3b	Extension to the supraclavicular fossa[b]
M—Distant metastasis[a]	
MX	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis

[a]*Note* Midline nodes are considered ipsilateral nodes

[b]*Note* Supraclavicular zones or fossa is relevant to the staging of nasopharyngeal carcinoma and is the triangular region originally described by Ho. It is defined by three points: (1) the superior margin of the sternal end of the clavicle, (2) the superior margin of the lateral end of the clavicle, (3) the point where the neck meets the shoulder. Note that this would include caudal portions of Levels IV and VB. All cases with lymph nodes (whole or part) in the fossa are considered N3b Deschler et al. (2014)

of hospitalization, cost, and morbidity and mortality compared to other surgical techniques (Weinstein et al. 2010). In recently published article, there was a decline in the rate of TORS related major complications over the time from 2010 to 2015. The probability of complication occurrence following TORS, were 3 times greater in HNC patients over 60 years old (Hay et al. 2017). Although HNC patients who undergo surgery have better survival rates, morbidities associated with altered body image and poor QOL, speech and swallowing impairment, and airway challenge, nonetheless leave their mark.

Table 11.6 Tumor-node-metastasis (TNM) clinical staging categories for oral squamous cell carcinoma with corresponding survival rates

Stage	TNM classification	Five-year relative survival rate		
		Oral cavity[a] (%)	Tongue (%)	Lip[b] (%)
0	Tis N0 M0			
I	T1 N0 M0	72	71	96
II	T2 N0 M0	58	59	83
III	T3 N0 M0 and T1, T2 or T3 N1 M0	45	47	57
IV	Any T4, N2, N3 or M1	32	37	48
IVA:	T4a N0M0 and T4aN1M0 and T1, T2 or T3N2M0 and T4aN2M0			
IVB:	Any TN3 M0 and T4b Any N M0			
IVC:	Any T Any N M1			

[a]From lip and oral cavity. In Edge et al. AJCC Cancer Staging Manual (2010a)
[b]Based on SEER data for patients treated from 1988 to 2001. *Source* American Cancer Society (2013)

Table 11.7 TNM staging for the larynx, oropharynx, hypopharynx, and salivary glands

Stage grouping	
Stage 0	Tis N0 M0
Stage I	T1 N0 M0
Stage II	T2 N0 M0
Stage III	T3 N0 M0
	T1 N1 M0
	T2 N1 M0
	T3 N1 M0
Stage IVA	T4a N0 M0
	T4a N1 M0
	T1 N2 M0
	T2 N2 M0
	T3 N2 M0
	T4a N2 M0
Stage IVB	Any T N3 M
	T4b Any N M0
Stage IVC	Any T Any N M1

Deschler et al. (2014)

11.8.2 Radiation Therapy

Radiotherapy uses high-energy x-rays or particles to eradicate cells by producing charged molecules interacting with cellular biochemical processes, and subsequently leading to direct damage to DNA. In head and neck cancer, radiation can be

Table 11.8 Stage grouping for the nasopharynx

Stage grouping	
Stage 0	Tis N0 M0
Stage I	T1 N0 M0
Stage II	T2 N1 M0
	T2 N0 M0
	T2 N1 M0
Stage III	T1 N2 M0
	T2 N2 M0
	T3 N0 M0
	T3 N1 M0
	T3 N2 M0
Stage IVA	T4 N0 M0
	T4 N1 M0
	T4 N2 M0
Stage IVB	Any T N3 M0
Stage IVC	Any T Any N M1

Deschler et al.(2014)

used as a single treatment modality in early staged disease or as an adjuvant alone post-surgical excision. In advanced cases it can be used concomitantly with chemotherapy or as an adjuvant with chemotherapy post-surgical excision.

Radiation may be administered using implant techniques (brachytherapy) or by using external beam radiation. The size of the high-dose field of irradiation is decreased and the exposure of adjacent vital structures is limited with technical advances such as intensity-modulated radiotherapy (IMRT), newer faster forms of image-guided IMRT [image-guided radiotherapy (IGRT)], and proton beam therapy. Proton are better able to concentrate within the targeted tissue with minimized radiation concentration in the surrounding structures due to their inherent physical characteristics (Hall 2006).

11.8.3 Chemotherapy

Traditionally chemotherapy aim to initiate disruption of the cell cycle. Concurrent chemoradiotherapy (CCRT) protocols are the standard of care for stage III and IV as primary therapy and for disease with poor prognostic findings following surgery (Epstein et al. 2012).

In head and neck cancers, chemotherapy is administered in one of 3 ways. Concomitant adjuvant, (administered with radiation simultaneously postoperatively), adjuvant (administered after radiation or surgery), or palliative (Deschler et al. 2014). The most common method of administration is concomitant chemotherapy, which is believed to potentiate the effects of radiation. Concomitant

chemotherapy improves locoregional control and in some cases, overall survival. The downside is that it also increases the local toxic effects of radiation.

Platinum based agents have an established role in head and neck cancer. The common first line chemotherapeutic agents for HNC include cisplatin, carboplatin, and 5-fluorouracil. For incurable disease, cisplatin, carboplatin, 5-FU, and cetuximab are used. The goal of palliative therapy is to improve survival and quality of life. The most widely studied compound in HNC is the *EGFR* monocloncal antibody, cetuximab (Deschler et al. 2014).

Chemoprevention is an investigational approach with the goal of reversing the premalignant process in field cancerization, thereby returning the affected tissue to a state of normal function and maturation (National Cancer Institute 2013).

11.8.4 Gene Therapy

Gene therapy is defined as the transfer of genetic material for treatment purpose, which involve replacing defective genes with enhancing the expression of certain key genes, functional variants, or suppressing genes that contribute to disease (Waleed et al. 2015).

Gene therapy encompasses 3 major categories: gene addition therapy, gene disruption therapy, or epigenetic modification therapy (Gong et al. 2016). Gene addition therapy targeting *p53* has been well established and has been tested in HNSCC by using an adenovirus vector expressing a wildtype *p53* gene (Chung et al. 2004; Chen et al. 2014). RNA interfere technique has been used to disable a group of genes that play a role in HNSCC including *VEGR, EGFR*, and *Cyclin D1* (Martinez et al. 2015; Parsel et al. 2016; Nozawa et al. 2006). Difference in expression of *miRNA*s have been detected in normal and cancer cells in various tumors including HNSCC. Epigenetic modification therapy addressing miRNA have demonstrated anticancer potential (Negrini et al. 2009). Establishing successful genetic therapy in HNC is yet to occur, but will most likely be a major research focus in the years to come.

11.8.5 Targeted Molecular Therapy

Many of the traditional chemotherapeutic agents act on rapidly proliferating cells, normal and cancerous alike. Targeted therapy which selects cancer cells over normal cells is ideally what is desired. Epidermal growth factor receptors are upregulated in up to 95% of head and neck tumors and is one of the most important targets in HNSCC (Kalyankrishna and Grandis 2006). Cetuximab, an *EGFR* monoclonal antibody, as mentioned previously, is one of the most studied

compounds in HNC with proven efficacy in cancer control. It is the only biologic agent approved by the FDA for use in HNSCC (Stewart et al. 2009).

Other cell surface receptors that are potential targets for molecular therapy in HNSCC include Human epidermal growth factor receptor-2 (HER2) and the *Vascular endothelial growth factor receptor* (*VEGFR*) (Gong et al. 2016). Molecular therapy acting at the level of these receptors are being studied at the level of clinical trials and investigations in HNSCC.

Cellular signaling pathways altered in HNSCC include the *PI3K/Akt/mTOR, RAS/RAF/MEK/ERK*, and the newly discovered *JAK/STAT* pathways (Gong et al. 2016). The first *mammalian target of rapamycin* (*mTOR*) inhibitor rapamycin, is an FDA approved macrolide antibiotic with discovered immunosuppressive and antineoplastic properties (Boers-Doets 2013; Liu et al. 2011). Enhanced efficacy was noted when targeted therapy for the *PI3K/Akt/mTOR* pathway was used in combination with other drugs in patients with HNSCC (Polivka and Janku 2014). Molecular targeted therapies involving all aforementioned pathways are being studied and have the potential of becoming a part of the future of established head and neck treatment protocols.

11.8.6 Immunotherapy

Unfortunately, HNSCC cells have the ability to manipulate the immune system through diverse mechanisms, promoting tumor growth and spread (Economopoulou et al. 2016). Immunotherapy is often used to reduce tumor recurrence, treat the presence of minimal disease, and to reduce toxicity (Heimdal et al. 2000). Cancer vaccine is anticipated as a novel modality to improve the poor survival rate of SCCHN. Two HPV vaccines has approved by The FDA (Neville et al. 2016). Vaccines for several types of solid tumors have developed by inducing high immune response rates with the use of tumor-associated peptides. Melanoma antigen proteins *(MAGED4B)* are overexpressed in OSCC. *MAGED4B* peptides can be considered as vaccine agents for OSCC as they induce anti-tumor immune responses (Montoro et al. 2012).

11.9 Palliative Care

Palliative care improves the QOL of cancer patients with advanced illness and their families, regardless of the possibilities of cure. These cares are simple and available with low cost (Parikh et al. 2013). An interdisciplinary approach is essential in palliative medicine. Dentists should be a part of this interdisciplinary team as dental care is often overlooked (Saini et al. 2011).

11.9.1 Complementary Alternative Medicine

Complementary Alternative Medicine (CAM) is a kind of medical practices that are not part of routine medical care. The most frequently administered interventions for all conditions include Dietary supplements, Herbal medicine, nutrition, massage therapy, meditation, yoga, Tai Chi or Qi Gong, traditional Chinese medicine, and pharmaceuticals. The most proven useful modalities included management of chronic pain, stress and depression (Horrigan et al. 2012). With the aid of CAM we can expect great improvement in patients psychosocial status in contrast to nonusers (Gilbar et al. 2001).

The anticancerogenic property of green tea is attributed to *polyphenol-epigallocatevhin-3-gallate* (*EGCG*), which modulates multiple signaling pathways including inhibition of activation of *EGFR, VEGF, NF-Kappa-B* and activation of the *p53* and *p73*. Apoptosis, cell cycle arrest, growth inhibition, anti-angiogenesis, and inhibition of metastasis can result from the combined effects of *EGCG* (Kim et al. 2010). Turmeric is a polyphenol derived from the plant *Curcuma longa*. Its pharmacological properties include anti-tumor, anti-inflammatory, and anti-oxidant effects and it also believed to be a radiosensitizer (Arora et al. 2005). Some in vitro studies demonstrated that curcumin stops cell growth and proliferation of human Oral SCC by inhibiting *NFkB* with abrogation of *IkBα kinase,COX-2* expression, and activation of *caspases* (Aggarwal et al. 2004; Sharma et al. 2006).

11.9.2 Acupuncture

Acupuncture is a part of alternative medicine. That may be helpful in cancer patients to control nausea and vomiting, neuropathy, xerostomia, chemotherapy-related neutropenia, pain and shoulder dysfunction after radical neck dissection, dysphagia related to chemoradiation therapy, cancer fatigue, and psychologic stress, depression, and anxiety (Lu et al. 2009; Pfister et al. 2010; Weidong et al. 2010; Vickers et al. 2004; Kober et al. 2003). But review of articles demonstrated that, there is controversy regarding application of acupuncture in cancer patients.

11.10 Prevention

The ultimate goal of cancer prevention is to decrease the mortality and incidence of cancer and to improve the QOL of patients. Primary prevention has been aimed at life style modifications such as tobacco cessation (Ganz et al. 2006). Secondary cancer prevention considered as the treatment of pre neoplastic lesions to stop their progression to invasive cancer (Stewart et al. 2003).

11.11 OroMaxillofacial Cancers

11.11.1 Malignant Tumors of the Salivary Glands

Malignant salivary gland tumors (SGT) are not as prevalent as HNSCC. They usually present in the 6th decade of life. The palate and the parotid gland are the most common site for minor and major SGT, respectively. Mucoepidermoid carcinoma (MEC) is the most common malignant neoplasm of the salivary glands. Adenoid cystic carcinoma is the most common minor salivary gland malignant tumor. Adenocarcinoma, ex-pleomorphic adenoma, acinic cell carcinoma, squamous cell carcinoma, and epidermoid carcinoma are other types of malignant neoplasms of the salivary glands (Neville et al. 2016).

Minor salivary glands are widely dispersed in the upper airway to include areas of the palate, lip, laryngopharynx including the nasopharynx, and parapharyngeal space. They are most densely populated in the hard and soft palate. As such, the most common site of minor salivary gland malignant tumors is in the palate area. Less commonly minor salivary gland malignancies may arise in other areas of the upper airway.

The pathogenesis of salivary gland cancer (SGC) remains obscure, but ionizing radiation exposure may be one risk factor (Ettl et al. 2012). Many genetic changes are proposed in the pathogenesis of SGC including variable expression and mutations related to *EGFR, MUC1, BIM, c-kit, Ki-67, BAX, Bcl 2,* and *her-2* to name a few, but these findings are far from universal.Viral sources such as CMV have been reported as a possible etiology (Melnick et al. 2011). Malignant SGT most commonly presents as a painless mass however oral ulcerated lesions can also be found. Trismus, mass fixation, overlying skin changes, and neuropathy can occur from tumor invasion of adjacent structures(Bjørndal et al. 2011). For masses in the major salivary glands, FNA or CNB is the preferred method of biopsy (Schmidt et al. 2011). Regardless of type, treatment continues to consist of surgical resection and radiotherapy, followed by chemotherapy as a palliative method in metastatic cases. SGC has a dismal prognosis; however, surgical resection and aggressive radiotherapy may provide benefit. Inoperable, unresectable, and recurrent tumors have been shown to respond to fast neutron-beam radiation therapy or accelerated hyperfractionated photon-beam schedules (Pommier et al. 2006).

11.11.2 Malignant Tumors of the Jaw

11.11.2.1 Osteosarcoma

After multiple myeloma, osteosarcoma (Osteogenic Sarcoma) is the most common primary malignancy of bone (Zarbo and Carlson 2003). Osteogenic Sarcoma of the jaws are more frequently seen in the fourth decade. of life with unknown etiology.

Risk factors include hormonal factors, ionizing radiation and chromic oxide exposure, alkylating agents, Paget disease of bone, Li-Fraumeni syndrome, Bloom syndrome, Werner syndrome, Diamond-Black fan anemia, Rothmund-Thompson syndrome and, hereditary retinoblastoma (Neville et al. 2016). Some of the genetic changes that seems to have a role in the development of osteosarcoma include *p53*, *RB1*, *MDM2*, *SAS* and *CDK4* (Lopes et al. 2001). Jaw osteosarcoma is slightly more common in the mandible than in the maxilla. But maxillary lesions of osteosarcoma often are more difficult to resect and are associated with a worse prognosis (Neville et al. 2016). These lesions often present with localized pain and swelling (Paparella et al. 2013). Additional symptoms consist of mobility and or pain of teeth, paresthesia, and nasal obstruction. Distinct features include older age at presentation, longer median survival, difficult to control rare local recurrences typically leading to death of the osteosarcoma patients. The essential microscopic criterion is direct production of fibrous connective tissue, osteoid, and chondroid by tumor cells (Neville et al. 2016). The "sun ray" appearance is the classic radiographic appearance. Another ominous signs are widening of the mandibular canal. Osteosarcoma of the jaw metastasizes less than extragnathic lesions. The most common sites of metastasis are the lungs and brain. Death occurs more often from uncontrolled local disease than from distant metastases. The treatment of choice is radical surgical resection. But, the additional use of radiotherapy and/or chemotherapy, (neo)adjuvant chemotherapy is controversial (Granowski-LeCornu et al. 2011).

11.11.2.2 Chondrosarcoma

Chondrosarcoma is a rare primary malignant neoplasm composed of cartilage showing varying degrees of cellularity and maturation. It has a male predilection. Gnathic chondrosarcomas have a tendency to occur along the anterior maxilla and the posterior mandible. Patients with Maffucci syndrome and Ollier disease have an increased risk for head and neck chondrosarcoma. Presenting signs include painless swelling, nasal obstruction and epistaxis, loosening of teeth, congestion, photophobia (Neville et al. 2016). Typically chondrosarcomas of the head and neck are locally aggressive with low metastatic potential. Death can occur by direct extension and destruction of vital organs. The histological grade of tumors guides treatment and management. Wide surgical resection is the treatment of choice for chondrosarcoma, because it is traditionally radioresistant. Radiotherapy is often advised for high-grade lesions of chondrosarcoma, and chemotherapy has a palliative role (Glick and William 2015).

11.11.2.3 Ewing Sarcoma

Ewing sarcoma is a rare malignant neoplasm which composed of small and undifferentiated round cells with controversial origin. Children and adolescents are

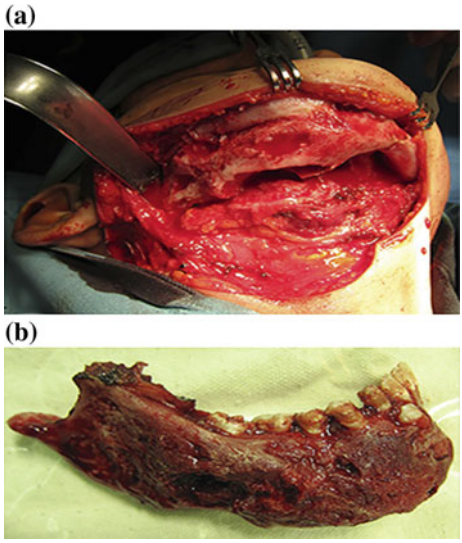

Fig. 11.4 Ewing's sarcoma of the right posterior mandible in a 9-year-old boy. **a** Intraoperative view of the lesion. This massive tumor had been presented for many months before the patient sought treatment. **b** The surgically resected specimen (Courtesy of Dr. Shirinbak)

affected mostly. It less commonly occurs in the head and neck. Pain and swelling are the most common clinical findings (Fig. 11.4). Paresthesia and tooth mobility may result from jaw involvement. This is more common in the mandible. Making the correct diagnosis can be difficult and care must be taken to not mistake this tumor for other pediatric small round cell tumors. Cytoplasmic PAS-positive glycogen granules and membranous immunoreactivity for *CD99* (*MIC2*) is exhibited in Ewing's sarcoma. These findings are nonspecific. *Fli1* expression has also been reported. The diagnosis can be confirmed with the identification of characteristic chromosomal translocations by reverse transcription (RT)-PCR or fluorescence in situ hybridization (FISH). To date, it has been understood that high level of *CD133* expression are characteristic of chemo resistant variant of Ewing sarcoma (Brazao-Silva et al. 2010). *Ki-67* is another prognostic IHC marker to identify a best treatment for each patient (Wexler et al. 2003). So, some researchers suggest that IHC evaluation of *Ki-67* and *CD133* must be added in routine histopathologic analysis of biopsy samples in this malignancy. This may be useful to suggest a beneficial treatment protocol (Karimi et al. 2011). Treatment can include multidrug chemotherapy with surgery (Fig. 11.4) and/or radiation therapy (Neville et al. 2016).

11.11.2.4 Multiple Myeloma

Multiple Myeloma (MM) is the most common primary malignant tumor of bone. It is a plasma cell malignancy arising within bone marrow. It is a monoclonal proliferation tumor in which the tumor cells are all identical to the malignant cell of origin. Large quantities of identical nonfunctional immunoglobulins are produced

by the malignant plasma cells. The etiology of this malignancy is unknown (Neville et al. 2016). Multiple genetic abnormalities play a key role in the pathogenesis of myeloma (Palumbo and Anderson 2011). MM usually affect adults with a slight male predilection. Initial signs and symptoms of oral and maxillofacial MM may involve pain, swelling, tooth mobility, paresthesia, radiolucency, petechial hemorrhages of the oral mucosa, and diffused enlargement of the tongue. Radiographic changes in patients with MM vary from typical punched-out radiolucencies to ragged radiolucent lesions. Diffuse, monotonous sheets of neoplastic, variably differentiated, plasmacytoid cells are seen histologically, in which invade and replace normal tissue. Treatment goals include controlling the cancer and prolonging survival in addition to making the patient comfortable and improving QOL (Neville et al. 2016). Palliation of painful bony lesions can be achieved through localized radiation. Bone marrow transplantation is reserved for patients who fail chemotherapy. Skeletal complications among patients with MM can be reduced with the use of bisphosphonates. As such, bisphosphanates are now a drug of choice for MM therapy.

11.11.3 Sarcomas of the Soft Tissues

Sarcoma is a malignant neoplasm arising from mesenchymal tissue. Sarcomas consist of 1–2% of all head and neck malignancies. Of all sarcomas, 4–10% arise in the head and neck. Soft tissue sarcomas of the oral cavity are rare. 5-year survival rate is reported at 60% (Peng et al. 2014). Metastasis at the time of presentation is seen in 10% of cases (Farhood et al. 1990).

In children, the most common head and neck sarcoma is rhabdomyosarcoma followed by osteosarcoma. Most common adult head and neck sarcomas include malignant fibrous histiocytoma (MFH), Kaposi sarcoma, and hemangiosarcoma (Peng et al. 2014). Slow- or rapidly-growing swelling of the mucosa involving any subsite of the oral cavity can be seen with soft tissue oral cavity sarcoma. Treatment usually consists of surgery, with adjuvant radiotherapy reserved for cases with high-grade tumors and/or positive margins after surgery (De Bree et al. 2006).

11.11.4 Mucosal Melanoma

Mucosal melanoma of the head and neck is a rare and highly aggressive malignant disease entity. Of the subsites in the head and neck region, the sinonasal cavity and oral cavity are the most common areas of presentation with other areas of origin including the pharynx and larynx. There is a predilection among women of child-bearing age; in contrast, a man predilection is seen among older patients (Neville et al. 2016). The prevalence is higher among black-skinned and Japanese people. There is no distinct clinical appearance to oral melanoma. There is a wide

variety to the coloration and surface architecture of oral melanomas (Wu et al. 2014). Approximately a third of oral melanomas are amelanotic, which is particularly difficult to diagnose and it may be misdiagnosed as a SCC or benign tumor (Tanaka et al. 2004). IHC positivity for *S-100 protein, MART-1, HMB-45,* and *Mitf* may aid in diagnosis. Enlarged atypical melanocytes with varying degrees of nuclear pleomorphism and hyperchromatism is seen in mucosal melanoma.

Unlike cutaneous melanomas, the etiology does not involve sun exposure (Lourenco et al. 2014). The etiology of mucosal melanoma is not clearly understood but a sequential genetic and molecular alteration in melanocytes is believed to occur in these tumors. *KIT*, *BRAF*, *N-RAS*, and *GNAQ*, and molecular pathways like *PI3K-Akt-mTOR* are a number of frequently altered genes identified in mucosal melanomas (Lopez et al. 2016). Of the rare cases of laryngeal mucosal melanomas reported, a smoking history was present in the majority of patients, suggesting a possible role for smoking as seen in laryngeal SCC. The 5-year survival is dismal at under 25%, reflecting the aggressiveness of this malignancy and its tendency for aggressive locoregional spread, recurrence, and distant metastasis (Lourenco et al. 2014).

Management includes surgical resection with adjuvant radiotherapy. Unfortunately, although adjuvant radiation may offer better locoregional control, it has not shown to increase overall survival (Konuthula et al. 2017). Chemotherapy is considered in cases with distant metastasis or in unrespectable cases. The FDA has approved two novel treatments for metastatic mucosal melanoma: ipilimumab and vemurafenib (Neville et al. 2016).

11.12 Metastases to the Head and Neck

Metastasis to the head and neck is not common. In 20–35% of cases it may be the first clinical sign of an occult malignancy (Barnes 2009; Basir Shabestari et al. 2012). Most methods of spread are hemotagenous other than the metastasis to the parotid gland, which is enriched in lymph nodes. Clinical and radiographic work-up in combination with histopathologic examination and in certain cases, immunohistochemical techniques, is often required to determine the primary site of malignancy (D'Silva et al. 2006) (Figs. 11.5, 11.6 and 11.7). *MMP 2 & 9*, *VEGF* tumor markers are mostly used to identify metastasis and neoangiogenesis (Raica et al. 2009). Management of the metastatic lesion in the majority of cases is palliative and should be coordinated with the overall treatment plan (Neville et al. 2016).

The most common sites for oral soft tissue metastases are the tongue and the gingiva. Metastasis to soft tissue of oral cavity is frequently seen in male. The lesion usually appears as a exophytic nodular mass that resembles a hyperplastic or reactive growth or as a surface ulceration. Adjacent teeth may become loose secondary to destruction of the alveolar bone (Neville et al. 2016). Metastasis to the oral cavity can occur from any malignancy from anybody site. Oral cavity soft

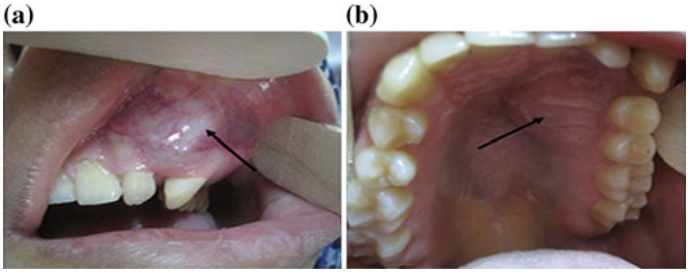

Fig. 11.5 Intraoral view: **a** buccal vestibular swelling with displacement of teeth number 10 and 11, **b** palatal mucosa swelling with displacement of teeth. Basir Shabestari et al. (2012), with permission

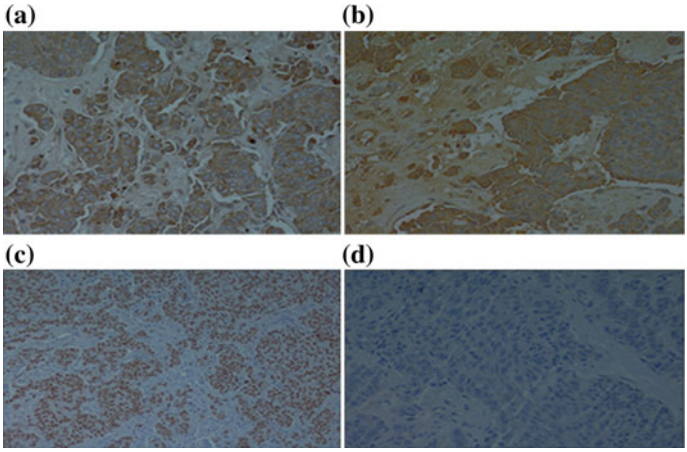

Fig. 11.6 Immunohistochemistry analysis: ×400. **a** pan-cytokeratin^{+}, **b** calcitonin^{+}, **c** TTF-1 $^{+}$, **d** thyroglobulin. Basir Shabestari et al. (2012), with permission

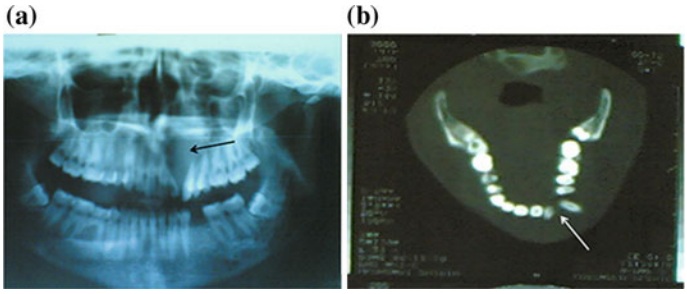

Fig. 11.7 Radiographic view. **a** Panoramic view: a relatively well-defined mixed radiolucency was seen. **b** CT scan view: left maxillary teeth were displaced by destructive mass. Basir Shabestari et al. (2012), with permission

tissue metastasis has been reported from the lung, kidney, skin (melanoma) and liver, breast, uterus, cervix, and ovaries. Metastasis to the oral cavity is a poor prognostic indicator (Hirshberg et al. 2008).

Metastasis to the oropharynx can be to the base of tongue and tonsil. Base of tongue metastasis has been reported from the lung, kidney, and skin (melanoma). Skin (melanoma), lung, breast and kidney are the most frequent primary tumors to involve the tonsil.

Metastasis to the larynx is rare, perhaps secondary to the larynx being a terminal organ in the lymphatic-vascular channel. The majority of tumors that metastasize to the larynx are either melanomas or renal, gastrointestinal, lung, breast, or prostate carcinomas. Bone and soft tissue sarcoma metastasis has also been reported. Nasopharyngeal metastasis from cutaneous melanoma, kidney, lung, breast, colon, and cervix has been reported (Barnes 2009).

Metastasis to the parotid gland is common as they are enriched with lymph nodes. The most common metastasis is from cutaneous skin cancers, squamous cell carcinoma and malignant melanoma, of the head and neck. Aggressive patterns of metastasis are seen in immunocompromised organ transplant patients with cutaneous SCC. Spread of cancer from head and neck primaries other than the skin can also occur.

11.12.1 Metastatic Tumors to the Jaws

Cancers of the breast, lung, thyroid, prostate, and kidney are the most common primary sites that metastasize to bone (Hirshberg et al. 2008). Swelling, paresthesia, pain, tooth mobility, trismus and gingival enlargement are the most common signs and symptoms associated with jaw metastasis (Fig. 11.5a, b). The posterior aspect of the mandible is more commonly predisposed to metastasis. Treatment of a solitary metastatic focus may involve surgery, radiation, or both (Seoane et al. 2009).

11.12.2 Distant Metastasis in Head and Neck Cancer

Locoregional extension of disease in HNC with regional lymph node involvement is more commonly seen than distant metastasis. In fact, the incidence of distant metastasis is small in HNSCC. Head and neck subsites more commonly associated with distant metastasis include the hypopharynx, oropharynx, and oral cavity.

Distant metastasis is more commonly seen in cases with advanced nodal involvement, especially in the setting of extensive soft tissue disease and jugular vein invasion. The lung is the most common site of distant metastasis followed by metastasis to the bone and liver respectively. The lung accounts for 66% of distant metastasis (Ferlito et al. 2001). Skin, mediastinum, and bone marrow metastasis may also occur.

11.13 Complications of HNC Treatments

Both HNC itself and its related treatments cause dysfunction and disfigurement due to the role of the head and neck in vital functions and overall appearance (Rogers et al. 2007). HNC therapy is associated with morbidities that may affect the quantity and quality of life. The side effects of the various treatments that HNC patients undergo are enormous, including intense pain, disfigurement, mucositis, salivary gland hypofunction, osteoradionecrosis, swallowing disorders, airway compromise, and psychological problems (Parliament et al. 2004; Hassanein et al. 2001).

11.13.1 Surgical Complications

Surgical complications of head and neck resection include hemorrhage, infection, flap loss, aspiration, wound breakdown, and fistula. Surgery of the oral tongue and/or the FOM often adversely affects the oral phase of deglutition. Detection of food particles in the oral sulcus is impaired with the lack of tongue sensation due to lingual nerve damage. Speech and swallowing dysfunction are common problems in glossectomy OC patients (Godoy et al. 1991). Oral competence is reduced with associated drooling and oral motor problems with the loss of teeth and lip function. Skin grafting may help prevent ankylosis of the residual tongue. Laryngopharyngeal resections are associated with airway and swallowing dysfunction. Surgical reconstruction with the goal of enhancement of esthetics and functional results for QOL purposes is critical in many surgical defect sites (Ship 2002).

11.13.1.1 Percutaneous Endoscopic Gastrostomy Tube and Tracheostomy

In individuals in which significant airway compromise from the tumor itself or secondary to radiation induced edema is seen or anticipated, a tracheostomy is a necessity for airway protection. Considerations for the level of tracheal entry and tube placement should be made in patients with an anticipated laryngectomy, who will also ultimately rely on a laryngeal stoma for breathing.

A PEG tube should be discussed and recommended in individuals who have already suffered from significant malnutrition or who have extensive dysphagia, as malnutrition can significantly impact cancer treatment and wound healing. If significant dysphagia and trouble with mastication and swallowing is anticipated based on the size and location of the tumor and its outlined treatment protocol, a PEG tube should be placed.

11.13.1.2 Rehabilitation in HNC Patients

Aggressive post treatment speech and swallow rehabilitation is a must in patients for restoration of swallowing and speech function. The extent of therapy needed depends on the extent of resection and its subsequent deficits and the impact of radiation.

The oral rehabilitation (OR) in HNC patients is a challenge for the physician. The main goal of OR is to restore the patient's oral functions following surgery. Multidisciplinary team must make the effort to give this patient the best OR according to the clinical condition (Falcao et al. 2015).

11.13.2 Complications of Radiation

Mucositis and subsequent fungal superinfection with dysphagia and odynophagia are immediate complications that can occur from radiation therapy. Early complications include changes to soft tissue including mucosal changes and alteration in saliva (Glick and William 2015). Xerostomia impacts QOL with resulting dry mucosal linings, soft tissue fibrosis, dental caries and deterioration, damage to bone, tongue mobility, and oral excursion. Eating, oral hygiene, and articulation are adversely affected (Chambers et al. 2005). Radiotherapy also negatively impacts diet and HNC patient weight (Gellrich et al. 2015).

11.13.3 Toxicity in Both Chemotherapy and Radiation Patients

11.13.3.1 Mucositis

Mucositis is a common and painful side effect of radiation therapy. It occurs in about 80% of HNC patients who receive chemotherapy or radiotherapy (Kakoei et al. 2013). Mucositis typically starts 2 weeks after initiation of radiotherapy, peaks at 1–2 weeks after completion of therapy, and can last up to 4–5 weeks after the last dose. The recommendations for management of mucositis includes oral pain control, oral hygiene and nutritional support (McGuire et al. 2013). There is currently no known intervention proven to completely and successfully treat mucositis. Various therapeutic measures have been suggested, with various response rates. Low level laser therapy (LLLT) is a noninvasive modality used for management and prevention with demonstrated analgesic effect and inflammation reduction (Fekrazad and Chiniforush 2014). Prophylactic LLLT declines severe oral mucositis and its associated pain (Oberoi et al. 2014). Granulocyte macrophage colony stimulating factor, topical corticosteroids, the prostaglandin-E2 and the amifostine with radio-protector properties are other agents trialed for mucositis. In

field of traditional medicine for reduced the side effect of cancer treatment, some studies showed that curcumin (diferuloylmethane) has radioprotective property (Arora et al. 2005). It is not only lessens the grade of oral mucositis, but also reduces the size of oral cavity lesions that may occur secondary to radiotherapy (Aggarwal et al. 2003).

Honey has antimicrobial qualities (Mavric et al. 2008). Regular oral administration of honey during or after radiotherapy and chemotherapy may prevent moderate to severe oral mucositis (Cho et al. 2015). Positive effect on cell epithelialization and regrowth are suggested mechanisms of actions. A number of studies have also demonstrated that honey can promote wound healing effects when applied as a dressing (Molan 2006).

11.13.3.2 Pain

Patients with HNC usually experience pain (Hall and Boswell 2009). In patients undergoing chemotherapy, patient-controlled analgesia with transdermal fentanyl or morphine is suggested for pain control (Saunders et al. 2013).

Topical anesthetics for relief of mouth and throat pain, especially in radiation patients, should be considered. Based on the area of concern, swishing and spitting or gargling and swallowing of a solution composed of equal parts of 2% viscous lidocaine (Xylocaine), diphenhydramine (Benadryl), and aluminum hydroxide–magnesium hydroxide–simethicone (such as Mylanta or Maalox) can be helpful in relieving generalized mouth and throat pain. This can be used before meals and bedtime for comfort. Topical anesthetic ointments for painful oral cavity ulcers or discrete areas of inflammation can be used. Overuse of the topical anesthetics should be avoided secondary to the possible side effects (Louis et al. 2014).

11.13.4 Oral Toxicity in Head and Neck Radiation Patients

11.13.4.1 Oral Mucositis

Erythema and epithelial sloughing presenting by the end of the first week of a conventional 2 Gy/day, five times a week radiation regimen, are the first signs of oral mucositis. This usually resolves during the one month following cancer treatment cessation (Al-Dasooqi et al. 2013). Therapies that induces mucosal cell propagation and DNA replication inhibit mucositis development (Davies and Finlay 2005).

11.13.4.2 Salivary Gland Dysfunction

The primary cause of xerostomia is damage to minor and major salivary glands by radiation (Chambers et al. 2005). Dryness of the mouth, mouth burning, with

speech, mastication, and swallowing compromise are a list of subjective complaints. Palliative measures for xerostomia include aggressive hydration, use of artificial saliva, and sialagogue therapy (pilocarpine hydrochloride). BioXtra mouth wash and spray were similarly effective in relieving the symptoms of radiotherapy induced xerostomia (Bakhshi et al. 2014). Another method that may decrease xerostomia is the application of implant techniques (brachytherapy) in combination with external beam radiation therapy. Major salivary gland function can be spared with highly conformal radiation delivery utilizing IMRT (Chambers et al. 2005). The role of stem cell replacement therapy in the treatment of radiation-induced hyposalivation is being investigated. Specific growth factors can induce differentiation of cells into functional units (Pringle et al. 2013).

11.13.4.3 Oral Infections

Bacterial, viral, and fungal oral mucosal infection results from xerostomia, and/or damage to the mucosa from radiotherapy or chemotherapy. Oropharyngeal candidiasis is frequently associated with a metallic taste changes and burning mucosal sensation (Lalla et al. 2010; Terai and Shimahara 2007). Following chemotherapy bacterial infections can cause localized mucosal lesions, sialoadenitis, pericoronitis, or periodontal abscesses. There is a high risk of rampant dental caries that may initiate within 12 weeks of completing radiation therapy if changes in either the amount or quality of saliva persist. HNC patients undergoing radiation therapy should be recommended on the lifelong use of daily supplemental high-dose fluoride gel. Brushing with a soft toothbrush is recommended. Antiseptic mouthwashes (chlorhexidine) may also be administered (McGuireet al. 2013).

11.13.4.4 Trismus

Radiation therapy can induce fibrosis and loss of elasticity of masticatory muscles of the temporomandibular joint (TMJ) which restricts the normal ability to open the mouth. This can be further aggravated by growth of tumor into the TMJ/masticatory muscles and surgical procedures. So, this may impact speech, food intake, swallow, and compromise oral hygiene. Patients should be advised to open and close the mouth as far as possible to prevent and treat jaw stiffness (Glick and William 2015).

11.13.4.5 Medication-Related Osteoradionecrosis of the Jaw

Patients with skeletal metastatic disease who use either injectable or oral bone-stabilizing agents (Antiangiogenics, Bisphosphonates, Denosumab), in which are designed to minimize the risk of fracture, can develop Medication-related Osteoradionecrosis of the jaw (MORNJ) (Allen and Ruggiero 2014). This disease entity has been classically characterized as the presence of exposed oral bone along

the gingiva and alveolar ridge for more than 2 months in the absence of a history of head and neck radiation. A significant number of these cases have been associated with dentoalveolar surgeries. When possible dental infections should be managed nonsurgically (Ruggiero et al. 2006). So, oral and dental checkup before starting treatment has been recommended. The doctor should stop the bisphosphonate treatment if MORNJ occurs.

Osteoradionecrosis can be a devastating complication of antiangiogenic therapies and or radiation therapy. Clinical manifestations include sensory disturbances, severe pain, fistula, infection, pathologic bone fracture, and full-thickness involvement of jaw bone (Peterson et al. 2010). The standard of care for advanced MORN patients is surgical resection with free osteocutaneous flap reconstruction (Jacobson et al. 2010). Surgical dentoalveolar procedures in patients with recent radiotherapy is contraindicated due to risk of osteonecrosis (Scully and Felix 2006). If an invasive dental and or oral surgical procedure is required, use of antibiotics and/or pre-treatment with hyperbaric oxygen (HBO) therapy before and after surgery should be considered, but clinical efficacy is inconclusive (Peterson et al. 2010). This approach is in need of thorough presurgical examination and multidisciplinary consultation.

11.14 Oral Care Protocols for Oncology Patients

The oral cavity is the most frequently documented source of sepsis in the immunosuppressed cancer patient. Oral and dental health should be assessed before initiation of cancer treatment. The protocols should be focused on oral health improvement, pain management, promotion of salivary function, fabrication of dental prostheses, and application of osseointegrated implants if indicated (McGuire et al. 2013). Palliative care dentists must demonstrate empathy and kindness. Dentists can play an important role in evaluation and promotion of oral health and also reducing the physical and psychological burden created by the reality of dying in HNC patients during their follow-ups (Wiseman 2000).

11.15 Factors Affecting Quality of Life in HNC Patients

Cancer and its treatment have impact on patient QOL factors such as physical, social, psychological, and spiritual. The decision of which treatment modality to consider has a lot to do with QOL considerations (Hassan and Weymuller 1993). Depressive symptoms have a significant role in the loss of QOL (Handschel et al. 2013). Psychosocial variables impact patient's QOL more than medical facts (Holloway et al. 2005).

In patients recently diagnosed with cancer, social support is identified as the most important contributor to overall QOL. Particular attention should be paid to

assessing and controlling physical function, fatigue, psychological distress and pain from the time of diagnosis. Health-care professional involvement with specific expertise in the management of HNC-related symptoms, psychological distress and loss of social and physical functions, integrated with volunteer support, should be regarded as the new standard of HNC patients care with decreased QOL (Pesut et al. 2012; Lee et al. 2005).

11.15.1 Social Issues in HNC Patients

HNC patients often encounter complicated social issues after treatment. Successful rehabilitation and optimal swallowing and speech outcomes rely on a multiphase and continuous team approach and ongoing communication among clinicians. HNC patients express similar concerns and functional problems (Radford et al. 2004). Patients with severe weight loss avoid appearing in public more frequently because of eating and appearance impairments. Diet have a great impact on mucosal problems in patients receiving cancer treatment (Chambers et al. 1995). Diet should consist of soft and nontraumatizing foods to avoid puncture of the vulnerable mucosal epithelium (Fleming et al. 1995). Patients should be advised to avoid sweet, acidic or spicy foods, and to avoid usage of toothpicks, alcohol and tobacco. The perception of greater social support can have a positive impact on the psychological health of caregivers (Longacre et al. 2012).

11.15.2 Psychological Concerns in HNC Patients

HNC in addition to its somatic impact, can lead to psychologic challenges associated with accepting death, body image changes, facial disfigurement, depression and anxiety, which obviate the need for a comprehensive approach to treatment (Tomar et al. 2011). HNC patients are often plagued by worry about recurrence, and are at increased risk of developing posttraumatic stress disorder (PTSD), particularly during the 6-month interval after cancer diagnosis (Longacre et al. 2012). In addition to adversely affecting QOL, this may interfere with treatment and rehabilitation. In the rehabilitation process many patients are faced with long hospitalizations that require vocational therapy, speech therapy, physical and occupational therapy, psychotherapy, dietary management, and family therapy (Cogwell Anderson and Anderson Franke 2001). Patients with HNC face difficulties with swallowing, speech, mastication, and breathing as well as changes in appearance. Some studies suggest that psychological therapy based on the principles of Cognitive—Behavioral Therapy (CBT) has benefits in cancer care by reducing psychologic morbidity, especially in patients with facial disfigurement (Newell and Clarke 2000).

In guidelines intervention is recommended because of the overwhelming evidence of psychological distress in HNC patients (Verdonck-de Leeuw et al. 2009). Alternative forms of delivery such as self-help, group treatments and brief therapies, and a stepped care approach may be useful (Carlson and Bultz 2004). Symptoms of distress in most case of HNC can be resolved with psychological support alone. Highly anticholinergic drugs should be avoided in HNC patients with xerostomia when psychotropic drug therapy is indicated. Spiritual and psychosocial support to patients and their families from the time of diagnosis and throughout the treatment course of the disease is just as important as pain control in anesthetic relief (Walter-Ginzburg et al. 2001). Benefits of social support in numerous cancer populations has been demonstrated in studies (Falagas et al. 2007). Each specific HNC problems need different kinds of supportive measures during therapy and recovery period. Difficulties in speech production and facial disfigurement may lead to depression and social isolation (DiMatteo et al. 2000). Physical activities after diagnose of cancer are associated with an enhancement in QOL, and a decreased risk of mortality (Sammut et al. 2014). A wider range of demographics and survival outcomes is being observed by specialized oncologists. Rehabilitation programs, social interventions, relaxation exercises and stress management, and ongoing monitoring for support and guidance should be offered to HNC patients (Gold 2012).

11.16 Public Awareness Regarding HNC

Many studies show lack of knowledge regarding HNC in population (West et al. 2006; Pakfetrat et al. 2010). These findings emphasize on need for increasing public awareness and educational programs about possible risk factors for HNC with the aid of mass media. Health promotion strategies, life style modifications and rapid diagnosis of PMD lesions must be discussed with at risk population.

11.16.1 Discussion

At the end of this chapter, there is a brief discussion on three interesting HNC cases.

11.16.2 Case 1

A healthy, 1-month postpartum 21-year-old woman with chief complaint of massive, non-tender swelling in the anterior left maxilla, with 2 months' period was referred to us. Her past medical history revealed a thyroidectomy operation 7 years ago. she thought that as a goiter treatment. Biopsy revealed a metastatic Medullary

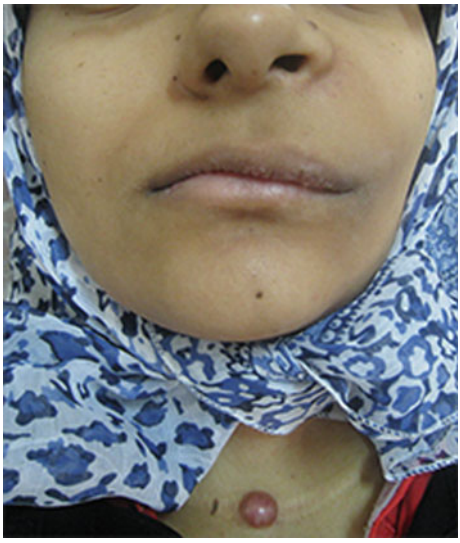

Fig. 11.8 Extra oral view of patient: the nasolabial fold was flattened, and slight deviation of left nostril was seen. The neck showed a scar from prior thyroid surgery. Basir Shabestari et al. (2012), with permission

thyroid cancer (MTC) to left maxilla. Further investigations regarding her past surgery informed us that she has been diagnosed with MTC (Fig. 11.8).

11.16.2.1 Case Analysis and Discussion

This case reflects a cultural hazard that aims at hiding a medical diagnosis from a patient. This culture is seen in some parts of the world with the potential for grave consequences. The problem of lack of patient understanding of their disease process is not solely related to hiding medical information. Based on patient education and socioeconomic status, even when a diagnosis is clearly delivered, some patient's may not grasp the significance and extent of their ailment and this can also lead to lack of appropriate intervention and post intervention surveillance.

The diagnosis of cancer ideally should be delivered to the patient and their family and social support. At times the medical team may be able to predict when a challenge arises in the patient's ability to understand the significance of the disease process. Here is where the presence of a case management team is imperative in assuring follow up with these patients and repetitive reminders of the disease process and its definition to assure that the patient and their support understands what needs to be done. Psychologist can be imperative in counseling patients and their families, however culturally this may be rejected by the same class of patients who opt to hide diagnoses and ignore follow up.

The bottom line is that management of patients requires a comprehensive approach and does not rely on diagnosing the disease and treating it alone. Beyond biopsy, surgical resection, chemotherapy, and radiation, understanding patient culture and expectations and assigning a support team to engrave the reality of the

situation and follow through is imperative. Despite this, situations like this case will continue to occur and resources available will not be able to prevent such occurrences.

> Talk to the patient at their level and help them climb the steps of reality until they reach a place that allows for the marriage of modern medical care and patient compliance.

11.16.3 Case 2

A 9-year-old boy was referred for surgical management of the previously misdiagnosed mandibular dental abscess. The swelling first appeared 18 months ago as an intraoral mandibular enlargement. His dentist diagnosed him as a dental abscess and initiated antibiotic therapy. Although the lesion decreased in size but never disappeared. After long time of antibiotic therapy his dentist referred him to us. His biopsy unfortunately revealed mandibular Ewing sarcoma.

11.16.3.1 Case Analysis and Discussion

Initial misdiagnosis unfortunately occurs in many medical circumstances when a malignant lesion has a subtle presentation. Several factors should raise concern and earlier suspicion for a cancerous process. Factors raising the possibility of malignancy have been presented in the chapter. Lack of response to antibiotic therapy after a reasonable several day course, in retrospect, should have been a trigger for earlier imaging and biopsy. The time to biopsy should be minimal and the time from obtaining a diagnosis to referral to a head and neck cancer team is just as imperative.

Earlier diagnosis allows for management of cancer at an earlier stage, with the possible need for less aggressive treatment measures. This may allow for a smaller surgical defect post resection, leaving the patient with less of a deformity and its associated functional, aesthetic, and emotional sequel and a higher likelihood of reducing the financial burden of disease control on the patient and society.

11.16.4 Case 3

A 77-year-old non-smoker and non-drinker with a heart transplant underwent an emergent tracheotomy after presenting to an outside hospital in respiratory distress with critical narrowing of his subglottic airway.

Seven years after his transplantation patient developed progressive hoarseness. Intraoperative evaluation and biopsy revealed a diagnosis of respiratory papillomatosis. A biopsy 1.5 years later of the laryngeal mass revealed evidence of severe

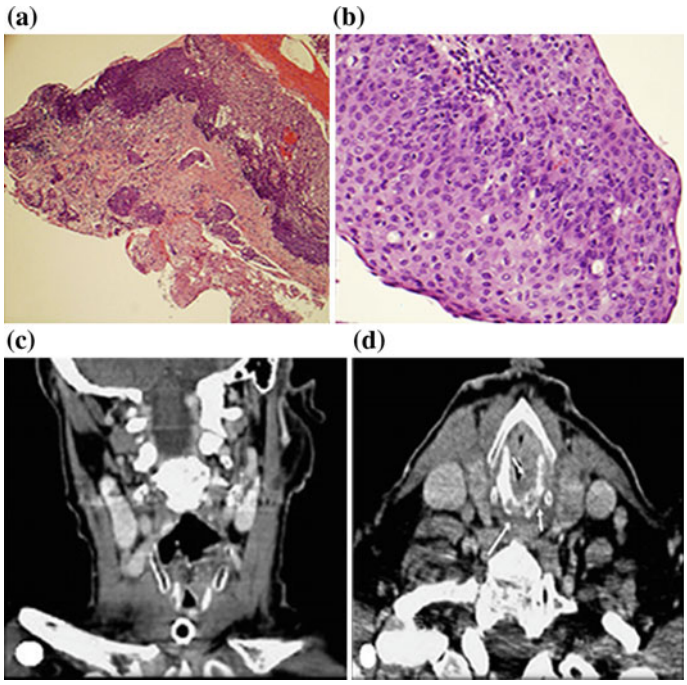

Fig. 11.9 **a**, **b** Histopathologic view. **a** SCC of subglottic larynx, **b** dysplasia of tracheal wall. **c**, **d** Radiographic view. **c** CT scan Coronal cut with view of larynx and occupying soft tissue lesion, **d** axial cut with view of narrowed subglottic larynx and invasive soft tissue lesion (*arrows*). Azadarmaki and Lango (2013), with permission

dysplasia (Fig. 11.9 a ,b). Several months later the patient developed respiratory distress with airway compromise and underwent an emergent tracheotomy as previously mentioned. A diagnosis of malignant conversion of the respiratory papillomatosis was made at this time (Fig. 11.9).

Intraoperative evaluation at our institution revealed an obstructing subglottic mass and mild papillomatous changes along the left true vocal fold. Malignant transformation of the respiratory papilloma to moderately differentiated SCC had occurred primarily at the level of the subglottis (Fig. 11.9a). Papillomatous changes had also extended inferiorly along the anterior tracheal. Severe dysplasia was seen at the level of the anterior tracheal wall (Fig. 11.9c, d).

11.16.4.1 Case Analysis and Discussion

Over 20% of cancers can be attributed to an infectious cause with more than 15% associated with a viral etiology. Virally mediated malignancies have a disproportionately higher incidence in immunosuppressed patients.

It is well known that cutaneous HPV related SCC has a more aggressive behavior and presentation in solid organ transplant patients. Respiratory papillomatosis, although a benign HPV related lesion with uncommon malignant conversion rates, should be surveillanced much more aggressively in immunocompromised patients with serial biopsies. Serial imaging can also pick up malignant properties such as cartilage and bony invasion that is not demonstrated in a benign disease process.

11.17 Conclusion

Head and Neck cancer is the 7th most common cancer worldwide with a strong association with tobacco and heavy alcohol use. The most common form of head and neck cancer remains squamous cell carcinoma. The rising awareness and noted association of oropharyngeal cancers with HPV and the better prognosis of HPV positive cancers has opened a whole new chapter in head and neck cancer prevention strategies and areas of research. Despite all the advances and research, survival rates remain suboptimal.

Individual and practitioner education and awareness of risk factors and signs and symptoms of head and neck cancer remain key components in prevention and early detection of head and neck cancer, which can ultimately lead to improved survival rates and quality of life. Healthcare access remains a key limiting factor in poverty stricken areas or areas underserved. This matter and concern is not only limited to developing countries.

Ideally a multidisciplinary team focusing on medical management, speech therapy, psychotherapy, dental care, and social support is desired in treating a head and neck cancer patient. Multiple factors including type of malignancy, stage of disease, location of primary, presence of metastasis, and patient's medical comorbidities are taken into consideration in deciding between surgical resection, radiation, chemotherapy, and or a combination of the aforementioned treatment modalities. Targeted genetic therapy is a highly researched area in head and neck cancer with the potential of revolutionizing cancer therapy. The ultimate goal in head and neck cancer therapy is improving survival rates and patient quality of life.

11.18 Summery

Head and Neck cancer (HNC) is the 7th most common diagnosed malignancy and 9th most frequent cause of death from cancer globally. There is a worldwide incidence of over 600,000 new cases and 350,000 deaths each year. There is a strong association with tobacco and heavy alcohol use. The most common form of HNC remains squamous cell carcinoma. The rising awareness and noted association of oropharygneal cancers with HPV and the better prognosis of HPV positive

cancers has opened a whole new chapter in HNC prevention strategies and areas of research. Despite all the advances and research, survival rates remain suboptimal.

Individual and practitioner education and awareness of risk factors and signs and symptoms of HNC remain key components in prevention and early detection of HNC, which can ultimately lead to improved survival rates and quality of life. Healthcare access remains a key limiting factor in poverty stricken areas or areas underserved. This matter and concern is not only limited to developing countries.

Successful treatment of the HNC patient involves a comprehensive approach with focus on removal of the tumor bulk, management of regional spread and distant metastasis, treatment of the morbidities associated with the disease process, and psychological support and care of the patient. Ideally a multidisciplinary team focusing on medical management, speech therapy, psychotherapy, dental care, and social support is desired. Multiple factors including type of malignancy, stage of disease, location of primary, presence of metastasis, and patient's medical comorbidities are taken into consideration in deciding between surgical resection, radiation, chemotherapy, and or a combination of the aforementioned treatment modalities. Quality of life is improved when a comprehensive approach is undertaken and the patient's morbidities from the cancer and its treatment are treated as seriously as the cancer itself. This stresses the importance of psychological support of the patient and his/her caregivers and support team.

Targeted genetic therapy is a highly researched area in HNC with the potential of revolutionizing cancer therapy. The ultimate goal in HNC therapy is improving survival rates and patient quality of life.

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Chapter 12
Gastrointestinal Cancers

Mohammad Amin Tabatabaiefar and Abbas Moridnia

Abstract Gastrointestinal cancers (GI) are an important category of cancers. This chapter reviews the updates on different aspects of GI cancers including pathophysiology, classification, genetics, genotype-phenotype correlations, screening, surveillance, genetic counseling, testing strategies, management and individualized medicine of esophageal cancers (squamous cell carcinoma and adenocarcinoma), gastric cancer (intestinal and diffuse subtypes), and colorectal cancers (CRCs) (with focus on Lynch syndrome and adenomatous polyposis colon cancer which are two common hereditary form of CRCs). Our current understanding of different aspects of GI cancers and future directions especially in the issues of genetic profiling and targeted therapies in GI cancers are tried to be address in this chapter.

Keywords Gastrointestinal cancer · Esophageal cancer · Gastric cancer · Colorectal cancer · Lynch syndrome · Hereditary non-polyposis · HNPCC · MSI analysis · Familial adenomatous polyposis

Abbreviations

AAPC	Attenuated adenomatous polyposis coli
ACG	American College of Gastroenterology
ACP	American College of Physicians
ACS	American Cancer Society
AGA	American Gastroenterological Association
ASCO	American Society of Clinical Oncology
ASGE	American Society for Gastrointestinal Endoscopy
AMPK	AMP-activated protein kinase
APC	Adenomatous polyposis coli

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cfDNA	Cell-free nucleic acid
CHRP	Congenital hypertrophy of retinal pigment epithelium
CK	Cytokeratin
CNV	Copy number variations
CRC	Colorectal cancer
CT	Computed tomography
CTC	Circulating tumor cell
DGC	Diffuse gastric cancer
EBV	Epstein–Barr virus positive
EC	Esophageal cancer
EpCAM	Epithelial cell adhesion
ESCC	Esophageal squamous cell carcinoma
FAP	Familial adenomatous polyposis
FDA	Food and Drug Administration
GC	Gastric cancer
GERD	Gastroesophageal reflux disease
GI	Gastrointestinal cancers
HDGC	Hereditary diffuse gastric cancer
HPV	Human papillomavirus
IGC	Intestinal gastric cancer
IHC	Immunohistochemistry
IPAA	Proctocolectomy with ileal pouch–anal anastomosis
IRA	Ileorectal anastomosis
MMR	Mismatch repair
MRI	Magnetic resonance imaging
MSI	Microsatellite instability
MSS	Microsatellite stable
NCI	National Cancer Institute
NCCN	National Comprehensive Cancer Network
NGS	Next-generation sequencing
PCR	Polymerase chain reaction
piRNA	Piwi-interacting RNA
RFA	Radiofrequency ablation
RFLP	Restriction fragment length polymorphism
SCC	Squamous cell carcinoma
TERT	Telomerase reverse transcriptase

12.1 Esophageal Cancer

12.1.1 Introduction

Esophageal cancer (EC) is one of the most lethal and aggressive cancers. This section outlines pathophysiology and classification, genetics, screening and surveillance,

genetic counseling and testing strategies. The esophagus is a muscular tube extending from the 7th cervical vertebra to the 11th thoracic vertebra. Anatomically, the esophagus is divided into the cervical, thoracic and abdominal parts (Beasley 1997). Esophageal cancer (EC) is one of the most lethal and aggressive cancers. Esophageal cancer is the seventh most frequent cause of death from cancer in males. The male-to-female ratio is 3–4:1 (Siegel et al. 2016). The incidence of EC is about 3–6 cases per 100,000 individuals (Siegel et al. 2016). The main histologic types of EC include squamous cell carcinoma (SCC) and adenocarcinoma. SCC may involve any part of the esophagus, most often in the upper half of the esophagus. In comparison, adenocarcinoma classically develops from specialized intestinal metaplasia known as Barrett metaplasia which, in turn, develops as a result of gastroesophageal reflux disease (GERD). Therefore, adenocarcinoma would normally originate in the lower half of the distal esophagus (Kelsen 2007).

The etiology of EC could be partly due to exposure of the esophageal mucosa to harmful or toxic stimuli (ex. certain foodstuffs, hot liquids, certain fungi, molds, or yeasts etc.) leading to a sequence of dysplasia conversion to carcinoma in situ and finally to carcinoma (Kubo and Corley 2006; Lagergren et al. 1999). High body mass index (BMI), GERD and the resultant Barrett esophagus are among major risk factors for developing EC (Lagergren et al. 1999). Certain nutritional deficiencies such as deficiencies in vitamins (e.g., riboflavin) or micronutrients may play a contributory role in parts of China and Iran (Kamangar et al. 2009).

Based on the Netherlands Cohort Study, a prospective study on 120,852 subjects, the combined effects of smoking and alcohol consumption increased the risk of SCC of the esophagus (Steevens et al. 2010). However, no association was found between alcohol consumption and esophageal adenocarcinoma. Human papillomavirus (HPV) infection has been associated with EC (Sitas et al. 2012).

Survival in EC patients is correlated with the stage of the disease. SCC and adenocarcinoma appear to show stage-dependent survival rates. Lymph node and solid organ metastases are associated with poor prognosis (Siegel et al. 2016). Stage IV cancers have a 5-year survival rate of less than 5%.

12.1.2 Pathophysiology and Classification

The histologic types of EC include squamous cell carcinoma (SCC), adenocarcinoma, undifferentiated cancers, and rare cancers including melanoma, lymphoma and sarcomas. SCC may involve any part of the esophagus, most often in the upper half of the esophagus. In comparison, adenocarcinoma classically develops from specialized intestinal metaplasia known as Barrett metaplasia which, in turn, develops as a result of GERD. Therefore, adenocarcinoma would normally originate in the lower half of the distal esophagus. They are the most common cancers of the esophagus (Kelsen 2007). In undifferentiated cancers, type of cells from which cancer has developed is unclear. GERD is the most common risk factor for developing esophageal adenocarcinoma. The reflux of acid and bile can cause

lesions which advances through to the intestinal Barrett metaplasia. In the next step, they can become low-grade dysplasia which, in turn, could turn into high-grade dysplasia, and finally to adenocarcinoma (Kelsen 2007).

The evolution of Barrett metaplasia to adenocarcinoma involves alterations in gene expression, and protein structure (Koppert et al. 2005). Barrett metaplasia increases the risk of developing EC between 30–60 times that of the general population (Graham et al. 2016).

12.1.3 Genetics

EC follows a complex inheritance involvement of multiple genetic loci and gene-environment interactions. For example, in a genome-wide association study, seven susceptibility loci in esophageal SCC were identified on chromosomes 5q11, 6p21, 10q23, 12q24, and 21q22 (Wu et al. 2011). Numerous genetic and epigenetic alterations in different category of genes such as tumor suppressor genes, DNA repair genes, oncogenes, cell adhesion molecules, cell cycle regulatory genes are involved in the development and progression of EC.

For esophageal SCC (ESCC), mutations in *RHBDF2*, which causes tylosis, has been identified. By sequencing 30 ESCC archival tissue samples using exome sequencing, Donner et al. prioritized shared, deleterious and rare variants in ESCC samples compared to Finnish and population subset specific controls. A nonsense mutation in *DNAH9* (p.Tyr1573Ter) was found in four unrelated patients. Also, missense variants in *GKAP1, BAG1, NFX1, FUK*, and *DDOST* and *EP300* were identified. These variant should be confirmed in an independent ESCC sample set (Donner et al. 2017).

Genetic factors related to EC are summarized as follows:

Tumor suppressor genes: The genes are involved in the cell cycle control, DNA repair, and cell death:

- P53: Over 100 ESCC mutations have been identified for *TP53*. About 50% of ESCC samples have *TP53* mutations. Evidence suggests that the mutations in the gene might be an early event in carcinogenesis of the esophagus (Gholipour et al. 2016; Horvath et al. 2016). The prognosis of ESCC patients with p53 mutations was significantly poorer than those with intact p53 (Kihara et al. 2000).
- P16INK4a: Abnormally higher expression of p16 and *TP53*, not *MDM2*, have been identified in the tumor tissue of ESCC patients (Taghavi et al. 2010).
- BRCA2: *BRCA2* is a nuclear protein tumor suppressor gene. Deleterious *BRCA2* variants have been identified in 7.6% of patients with ESCC (Akbari et al. 2008).

Some of the oncogenes with a role in EC include:

- Epidermal growth factor receptor (EGFR): EGFR gene family act as receptors with intracellular tyrosine kinase activity. In a meta-analysis, EGFR over-expression

was identified to be positive in 722 of 1150 patients (63%) and was associated with a poor prognosis (Wang et al. 2014a).

- Human epidermal growth factor 2 (HER-2): HER-2 can have either protein overexpression or *HER2* gene amplification in EC which are independently associated with poor prognosis (Prins et al. 2013).
- Cyclin D1: the proto-oncogene is associated with increased lymph node metastasis, distant metastasis, high tumor grade, poor response to chemotherapy, and poor prognosis (Saemi et al. 2016).
- Telomerase reverse transcriptase (TERT): TERT is the reverse transcriptase catalytic subunit of telomerase, which promotes tumor invasion and metastasis in esophageal cancer as well as several other gastrointestinal cancers such as gastric and liver cancers (Wu et al. 2017).
- ZNF208: Genetic variants in the *ZNF208,* possibly acting as a tumor suppressor, are associated with EC in a Chinese Han population (Wang et al. 2016). This gene can also predicts the response to imatinib mesylate treatment in patients with gastrointestinal stromal tumor.

Genetic polymorphisms in enzymes have also been studied in EC including:

Genetic polymorphisms affect the activity of enzymes metabolizing carcinogens, thereby modifying the individual response to carcinogenic exposure. Some important genes include: Cytochrome P450 (CyPs), Glutathione S-transferases (GSTs), alcohol dehydrogenases (AdHs)/aldehyde dehydrogenases (ALds), implicated in the pathogenesis of SCC (Malik et al. 2010).

NGS-based methods have recently been applied to clarify the etiology of EC:

Researchers have investigated 164 esophageal carcinomas using integrated clustering of somatic copy number variations (CNVs), DNA methylation, mRNA and microRNA expression data. Their results showed that adenocarcinoma of the esophagus had increased E-cadherin (*CDH1*) signaling and upregulation of ARF6 and FOXA pathways, which regulate E-cadherin (Carneiro et al. 2012). In contrast, SCCs showed upregulation of Wnt, syndecan and p63 pathways (Carneiro et al. 2012). Thus, distinct molecular profiles occur in these two subtypes.

Concordant with the theory, SCC mostly show recurrent mutations in *TP53*, *NFE2L2*, *MLL2*, *ZNF750*, *NOTCH1* and *TGFBR2* (Cancer Genome Atlas Research et al. 2017; Cheng et al. 2016; Qin et al. 2016). While adenocarcinoma exhibited mutations in *TP53*, *CDKN2A*, *ARID1A*, *SMAD4* and *ERBB2* (Cancer Genome Atlas Research et al. 2017; Dulak et al. 2013). Interestingly, *CDKN2A* and *TP53* mutations are prevalent among dysplastic Barrett esophagus which is an adenocarcinoma precursor.

CNVs that were frequent in adenocarcinoma of the esophagus but totally absent in SCC involved amplifications of *VEGFA* (6p21.1), *ERBB2* (17p12), *GATA6* (18q11.2) and *CCNE1* (19q12), and deletion of *SMAD4* (18q21.2). On the other hand, frequent CNVs in SCC included amplifications of *SOX2* (3q26.33), *TERT*

(5p15.33), *FGFR1* (8p11.23), *MDM2* (12q14.3), *NKX2-1* (14q13.2) and deletion of *RB1* (13q14.2) and *VGLL4/ATG7* (3p25.2) (*Cancer Genome Atlas Research* et al. 2017).

12.1.4 Genotype-Phenotype Correlations

EC is a multifactorial disease. Molecular profiling may help in the future to correlate each genetic variant profile with a phenotype. Also, different molecular profiles would determine a distinctive entity. Some recent findings were discussed in the previous section (Sect. 12.1.3).

12.1.5 Screening and Surveillance

Direct visualization and biopsies of the tumor has been facilitated using Esophagogastroduodenoscopy. The depth of tumor penetration and metastases to the peripheral lymph nodes has been made possible through endoscopic ultrasonography. In patients who appear to have localized esophageal cancer, positron emission tomography (PET) scanning can be useful as part of the baseline staging. Other imaging studies may assist in selected patients (You et al. 2013).

Recommendations for screening and surveillance of patients with GERD and/or Barrett esophagus have been put forth by several associations including: American Society for Gastrointestinal Endoscopy (ASGE), American College of Gastroenterology (ACG), American Gastroenterological Association (AGA), and American College of Physicians (ACP).

As a general rule, chronic GERD, a male gender, age over 50 years of old and obesity make necessary upper gastrointestinal endoscopy in order to screen for Barrett esophagus or esophageal adenocarcinoma (Shaheen et al. 2016). However, whether it truly prevents cancer or prolongs survival is yet to be established (Shaukat et al. 2015).

Based on the ACP best practice, the following recommendations have been offered for upper endoscopy for GERD (Shaheen et al. 2012):

- Screening endoscopy is not recommended for women at any age or men younger than 50 years old.
- Screening is highly recommended for everyone with GERD symptoms even under medical treatment.
- In case upper endoscopy shows no Barret esophagus, there is no need for further surveillance.
- If there is a Barrett esophagus with no dysplasia, the surveillance should be done every 3–5 years; however, if the patient has Barrett esophagus and dysplasia, shorter intervals should be considered.

- For patients with low-grade dysplasia, endoscopy should be repeated every 6 months to confirm the diagnosis.
- For patients with high-grade dysplasia, endoscopic resection should be done; radiofrequency ablation (RFA) may be considered for flat lesions; Patients who do not choose surgery or ablation should be under surveillance.
- In comparison, the AGA 2011 position statement has many similarities with the above mentioned guideline but includes some differences in the following items:
- In case there is no dysplasia, upper endoscopy should be followed every 3–5 years.
- For low-grade dysplasia, the interval should be 6–12 months.
- In high-grade dysplasia with no eradication therapy, the interval period is reduced to every 3 months.

Based upon the ACG guidelines endoscopic ablative therapies is the procedure of choice for patients with confirmed high or low dysplasia. The ACG recommends against using antireflux surgery as an antineoplastic measure (Shaheen et al. 2016).

12.1.5.1 Genetic Counseling and Testing Strategies

EC follows a complex inheritance. It is a multi-step cancer with co-occurrence of many genetic alterations, some important of which were discussed in the previous section. Therefore, EC follows the general rule of cancer genetic counseling.

As soon as the genetic profiling of EC subtypes including adenocarcinoma and SCC are determined, NGS-based genetic testing will develop (Mikhail et al. 2015).

12.1.6 Management and Individualized Medicine

Surgery has conventionally been the treatment of choice for EC, with the first successful resection done in 1913 by Torek (1913). Non-operative therapy remains as alternative choice for patients with certain clinical conditions or advanced disease.

Recently, studies have been conducted to evaluate the genome of esophageal cancers and to identify genetic alterations that may be used to for targeted therapy. Despite early promising results, they generally have led to little achievements. For example, erlotinib induced response to therapy in 15% of SCC patients (Ilson et al. 2011). Many other chemotherapeutic agents have been investigated but little therapeutic responses have been achieved.

12.2 Gastric Cancer

12.2.1 Introduction

Gastric cancer (GC) is the fourth common cancer worldwide and ranks second in the mortality rate among cancers (Jemal et al. 2011; Mehrabani et al. 2013). GC has been estimated to be the eleventh cause (1.8%) of all deaths until 2030 (Mehrabani et al. 2013). Approximately 22,220 patients are diagnosed annually in the United States, of whom 10,990 are expected to die (Rugge et al. 2015). Most of the GC cases are sporadic. Familial GC constitutes up to 10% of the GC cases (Ekström et al. 2000) and a minority of GC cases are hereditary (1–3%) (Henson et al. 2004; Palli et al. 1994). According to Lauren histological classification, GC is classified as intestinal (well-differentiated, moderately- and poorly-differentiated) and diffuse (with or without signet ring cells) types (Power et al. 2009), with different morphological, epidemiological and pathogenicity features, and distinct genetic profiles.

Intestinal GC (IGC) is more common than the diffuse type (Crew and Neugut 2006). It is more frequent among men than women, increases considerably with age and shows differences in geographic distribution, with the highest rates found in Eastern Asia, Eastern Europe, and South America while the lowest rates are observed in North America, Northern and Western Europe, Northern and Western Africa, and southeast Asia; (Ajani et al. 2010); The diffuse gastric cancer (DGC), in comparison, shows an equal sex ratio, a younger age distribution, and little changes with geographical migration (Ajani et al. 2010; Woods 2007).

The most important environmental risk factor for GC is chronic gastritis secondary to *Helicobacter pylori* (*H. pylori*) infection (Suerbaum and Michetti 2002). The interplay between the bacterium and the host immune reaction might be complex (Azadegan-Dehkordi et al. 2015). While about two third of the world population (mostly living in developing countries) is infected with *H. pylori*, only a small proportion develop GC. This could be due to several reasons:

1. Genetic characteristics of the bacterial strain. Young individuals infected with certain *H. pylori* genotypes have an increased risk of developing cancer (Tiwari et al. 2008). Certain strains of *H. pylori* can lead to higher plasma malondialdehyde levels and nitric oxide levels (Tiwari et al. 2010).
2. Genetic features of the host. Certain sequence variants in the *TNF* gene (encoding tumor necrosis α) and *IFNGR1* (encoding interferon gamma receptor 1) considerably increase the risk for GC, especially in people infected with virulent strains of the *H. pylori* (Canedo et al. 2008). Thus, both bacterial and the host genetic features can affect the progression of gastritis to chronic atrophic gastritis and intestinal metaplasia and finally leading to GC (Correa et al. 2000).

Persistent administration of vitamin C and β-carotene, and anti- *H. pylori* therapy can help revert gastric precancerous lesions, atrophy, and intestinal metaplasia in the stomach (Correa et al. 2000). The improved public health in developed countries has been an important factor in declining the *H.pylori*-mediated GC

(Roosendaal et al. 1997). Other environmental factors such as smoking, salt, pickled foods and low fruit- and vegetable diet can increase the risk for GC (Gonzalez and Lopez-Carrillo 2010).

Although for DGC, no precursor is known, *H. pylori* infection would also be a player in its etiology (Kamangar et al. 2006). Epigenetic effects of *H. pylori* on promoter hypermethylation of *CDH1* have been shown which could be reversed using antibiotic treatment (Perri et al. 2007).

12.2.2 Pathophysiology and Classification

Lauren's histological classification of GC includes intestinal (well-differentiated, moderately- and poorly-differentiated) and diffuse (with or without signet ring cells) types (Power et al. 2009). Each categories has different morphological, epidemiological and pathogenicity features, and distinct genetic profiles.

Most GC cases are sporadic. About 10% of cases are familial, a minority of which are hereditary (HGC) (Henson et al. 2004).

International Gastric Cancer Linkage Consortium has defined the criteria of HDGC which include:

1. A positive history of GC in two or more members of the first or second degree relatives with at least 1 established DGC diagnosis before 50 years of age.
2. Three or more confirmed DGC cases among the first or second degree relatives, independent of age.
3. A DGC patient younger than 40 years old with no positive family history.
4. A personal or family history of DGC and lobular breast cancer with one or more members diagnosed under 50 years of age (Pinheiro et al. 2014).

Mutations in the *CDH1* gene are the most frequent cause of HDGC and sporadic DGC (Moridnia et al. 2017; Worthley et al. 2012). The rate of *CDH1* gene mutations shows an inverse relationship with the incidence of GC. In low incidence countries, such as North America, the mutation rate in the *CDH1* gene is about 51.6%, while is reduced to 25% in the medium incidence countries and to 22.2% in high incidence countries such as Italy (Pinheiro et al. 2014).

It is estimated that between 15 and 50% of HDGC families have germline mutations in the *CDH1* gene (van der Post et al. 2015). HDGC follows an autosomal dominant mode of inheritance. It is an undifferentiated, infiltrating, and highly invasive tumor with a poor prognosis. The infiltrating pattern leads to thickening of the gastric wall (Linitis plastica). Signet ring cell carcinoma (SRCC) and isolated cell type carcinoma are the two histopathologic subcategories of diffuse GC (DGC) (Pagon et al.). E-cadherin encoded by *CDH1* is downregulated in the DGCs, while it remains intact in IGC. IGC originates from the precursor intestinal metaplasia. It is more prevalent than DGC (Crew and Neugut 2006). It involves tubular or glandular formations typical of adenocarcinomas of the intestinal tract.

The tumor cells could be of different differentiation stages (Lynch et al. 2005). It forms a large projected, ulcerated, or infiltrative lesion in the stomach.

E-cadherin is a transmembrane protein with three domains including extracellular, transmembrane, and cytoplasmic domain (Guilford et al. 1999). E-cadherin is tumor suppressor protein involved in the maintenance of the epithelial tissue architecture (Moran et al. 2005). It prevents nuclear signaling function of the proto-oncogene b-catenin (Gottardi et al. 2001). The age-related penetrance (until 80 years of age) of *CDH1* mutations is 80% in both sexes, while women have also a 60% risk of developing lobular breast cancer (Davis and Sano 2001).

The NIH Cancer Genome Atlas project has classified gastric cancer into four subtypes based on molecular profiles:

(1) Epstein–Barr virus positive (EBV) which is associated with extreme DNA hypermethylation
(2) Microsatellite instability (MSI)-positive tumors associated with high mutation rates. These tumors are, however, genomically stable. They normally have diffuse histology;
(3) Chromosomally instable tumors which have intestinal histology (Lee et al. 2010).

Emerging biomarkers including microRNAs, lncRNA etc. may be useful for screening, prognosis and management of GC in the future (Mocellin and Pasquali 2015).

12.2.3 Genetics

Mutations in the *CDH1* gene are the most frequent etiology of HDGC and sporadic DGC (Worthley et al. 2012). The *CDH1* gene encoding E-cadherin is located on 16q22.1 and is composed of 16 exons (Guilford et al. 1999). Germline mutation (a 2 bp germline deletion in exon 2) in *CTNNA1* (encoding the alpha-E-catenin) has also been reported from a large HDGC kindred (Majewski et al. 2013). Other families with DGC have recently been reported with *BRCA2* and *PALB2* mutations (Hansford et al. 2015); It is likely that other HDGC-associated genes will be discovered through next-generation sequencing (NGS). For example, using a combination of NGS and genetic linkage study, mutations in *MAP3K6* have recently been described (Gaston et al. 2014).

According to the catalogue of somatic mutations in cancer (COSMIC), the top genes frequently mutated include *TP53, APC, CDH1, TRRAP, PIK3CA, MLL3, RNF213, KMT2D, MLL, CTNNB1, CREBBP, AKAP9, CACNA1D, MYH9, ZNF521, SETBP1, KRAS,CDH11* and *ATM*. In one study on GC, whole exome sequencing was performed and (Wang et al. 2011) previously known drivers genes such as *TP53, PTEN* and *CTNNB1* were reported. *ARID1A*, as a key member of the SWI-SNF complex, was also found to be frequently mutated in GC cases. *ARID1A* mutations are clearly related to better prognosis of GC. *FAT4*, a member of the E cadherin family,

was another gene found for GC (Zang et al. 2012). In a more recent study, previously known genes such as *TP53, ARID1A,* and *CDH1* were found to be mutated in GC tumors and new genes such as *MUC6, CTNNA2, GLI3*, and *RNF43* were discovered to be mutated in GC. *RHOA* mutations were identified in about 15% of DGC but not among IGC tumors (Wang et al. 2014b). Different populations might have different profile of gene variants in GC (Saffari-Chaleshtori et al. 2017).

12.2.4 Genotype-Phenotype Correlations

The lack of E-cadherin immunoreactivity detected in hereditary DGC cases who are carriers of *CDH1* germline mutations confirms the Knudson's two-hit hypothesis that the second allele has been inactivated (Barber et al. 2008a). Germline *CDH1* mutations are distributed in the entire coding sequence, and involve frameshifts (37.5%), splice-site (23.1%), nonsense (17.3%), and missense mutations (17.3%), and large rearrangements (8.7%) (Fitzgerald et al. 2010). Over 75% of the mutations are truncating while the remaining include splice site and missense mutations (Pedrazzani et al. 2007). Germline deletions of *CDH1* have been observed in 4% of DGCs (Oliveira et al. 2009a). Generally, missense mutations pose lower risk for DGC. For clarifying the pathogenicity status of the missense variants several analyses such as determining variant frequencies in healthy control population, co-segregation of the variant in the pedigree, recurrence of the variant in independent families, in silico predictions, and in vitro functional studies are recommended (Figueiredo et al. 2013).

– In over half of the sporadic DGCs with somatic *CDH1* mutation, promoter methylation (34.2%) of the second allele has been reported. Loss of heterozygosity (LOH) (7.4%) at the *CDH1* locus, and a second *CDH1* mutation (5.6%) have been also reported (Barber et al. 2008a; Machado et al. 2001; Oliveira et al. 2013, 2009a, b; Pinheiro et al. 2014). In lymph node metastases, LOH has been found as the most prevalent 2nd allele inactivation mechanism (41.7%), followed by a combination of LOH and promoter hypermethylation (Oliveira et al. 2009b). The frequencies of *CDH1* somatic mutations in sporadic DGCs can vary from 3% to greater than 50%. In countries with high incidence of sporadic GC (such as East Asia), the frequency of germline mutations in familial GC cases is low. However, in low-incidence countries, the inverse trend is observed (Carneiro et al. 2008; Oliveira et al. 2013).

Nearly half of the patients suspected of HDGC do not carry *CDH1* mutations. The etiology in these cases has been investigated:

– In one single *CTNNA1*-harbouring HDGC family reported so far, several genes have been known to be *somatically* mutated (e.g. *LMTK3, MCTP2, PIK3CA, ARID1A, RHOA*) (Majewski et al. 2013). *PIK3CA, ARID1A* and *RHOA* have also reported from sporadic DGC tumors (Pinheiro et al. 2014).

- *PIK3CA* and *TP53* have been the most frequently somatically mutated genes in both DGC and IGC. *PIK3Ca* mutations are more frequently observed in DGC than IGC.
- A SNP in the *PSCA* gene (rs2976329) has been reported to be associated with increased risk of DGC in Korean and Japanese populations (Sakamoto et al. 2008).
- Mutations in *ARID1A* are frequently detected in MSI- and in EBV-positive GCs (Wang et al. 2011; Zang et al. 2012).
- Genomic gains and losses have been reported in DGCs. Losses in chromosomes 16, 17, 19, 20, 21, and 22, and gains of chromosomes 3, 7, 8, and 13 have been reported. Tumor suppressor genes including *CDH1, PLA2G2A, RUNX3, SMAD2,* and *TP53* are located in these genomic losses. Proto-oncogenes such as *MYC, MET* (Machado et al. 2001), *MOS* and *ZHX2* have been shown to be in the genomic gains of DGC samples (Wang et al. 2011). Somatic mutations have been identified to be clustered in exons 7–10 of *CDH1* in sporadic GC tumors (Machado et al. 2001).
- Blood group A has been associated with GC especially the DGC. Part of this could be due to its association with pernicious anemia, which in turn, pose higher risk for the development of GC (Woods 2007).

IGC can be part of the spectrum of cancers clustered with Lynch syndrome. *PIK3CA (*PIK pathway) mutations have been shown to be highly mutated in the MSI positive IGC tumors and are associated with good prognosis (Cristescu et al. 2015). Alterations in the mTOR pathway and receptor tyrosine kinase (such as *KRAS*) have been more significantly identified in good prognosis groups. *TP53* mutations have been associated with intermediate prognosis (Cristescu et al. 2015).

12.2.5 Screening and Surveillance

Routine surveillance in individuals with a *CDH1* germline mutation would not be much useful. Because, DGC tends to spread in the submucosa and therefore is not normally detected until it is in an advanced stage (Norton et al. 2007; van der Post et al. 2015).

Germline *CDH1* mutation carriers are generally recommended to undergo total prophylactic gastrectomy. Otherwise, they should be screened through detailed 30 min endoscopic examination of the gastric mucosa every 6–12 months by upper endoscopy with random biopsies (Pinheiro et al. 2014; van der Post et al. 2015). Although it has been used to detect cancer and determine the stage of cancer in gastrointestinal (GI) tumors, its effectiveness is under question in detecting precursor lesions (Fitzgerald and Caldas 2004; van der Post et al. 2015). Several other tools have been examined in DGC including positron emission tomography (PET) scan (Pinheiro et al. 2014), endoscopic ultrasound, stool-based molecular

screening, abdominal CT, and multiple random stomach biopsies (Barber et al. 2008b; van der Post et al. 2015).

For women with a germline *CDH1* mutation, lobular breast cancer (LBC) risk management is necessary which resembles that for women with a *BRCA1* germline mutation: monthly breast self-examinations and every 6 month period of clinical breast examination. They should be introduced to a high risk breast cancer screening program. However, instead of mammography, MRI should be considered, which is more sensitive in detecting such tumors (Pinheiro et al. 2014). The screening should begin by 35 years of age or 5–10 years before the onset of cancer in the related family (Fitzgerald et al. 2010).

Data on monitoring other potential sites are controversial. However, it has been recommended that colonoscopy (for detection of colon cancer) should be done every 3–5 years starting at age 40 years or 10 years before the onset of colon cancer in the family members (Fitzgerald et al. 2010).

The potential utility of blood-based biomarkers such as circulating tumor cells (CTCs) and cell-free nucleic acids (cfNAs) have been realized especially in recent years (Alix-Panabières and Pantel 2013). These novel markers have great potential to enable early detection of cancer, predication of prognosis, monitoring of tumor dynamics, response to therapy and development of novel targeted therapies. Detecting CTCs and cfNAs from the blood has been called "liquid biopsy" meaning that it is much more non-invasive than the conventional biopsies using surgical or endoscopic biopsy methods (Tsujiura et al. 2014).

Liquid biopsy generally involves a positive selection with antibodies against tumor-associated antigens, such as epithelial cell adhesion molecule (EpCAM) and cytokeratins (CKs), and negative selection with antibodies against the common leukocyte antigen such as CD45. Of interest, EpCAM has been used for GC (van der Gun et al. 2010). Among several technologies based on antibody-based isolation, the CellSearch system (Veridex) has been mostly used.

After enrichment of CTCs, downstream genetic, proteomic and genomic methods can be followed ranging from cytometric/protein-based approaches to polymerase chain reaction (PCR)-based approaches and to NGS (Heitzer et al. 2013). Wu et al. developed a sensitive assay using a high-throughput colorimetric membrane array, in which several markers such as human TERT, cytokeratin 19 (CK19), carcinoembryonic antigen (CEA) and MUC1, were measured altogether and served as a prognostic indicator for overall survival and postoperative recurrence/metastasis in GC (Wu et al. 2006). Non-coding RNAs such as miRNAs and Piwi-interacting RNAs (piRNAs), have been proven to alter their expression in carcinogenesis and tumor progression and have the potential to be also used in liquid biopsies (Mei et al. 2013). Very recently, miR-21-5p has been offered as a potential non-invasive biomarker for GC (Kao et al. 2017). In summary, recent technological advances have provided considerable progress and interest in the detection of CTCs in various cancers, including GC.

12.2.6 Genetic Counseling and Testing Strategies

The risk of developing GC in the first-degree relatives of the proband has turned out to be much more in DGC than in IGC (seven times vs. 1.5 times higher than the general population) (Woods 2007). In most DGC cases, the pathogenic variant in the *CDH1* gene has been inherited from one parent. Due to reduced penetrance, the parent may have remained healthy although he or she is carrying the variant. Alternatively, HDGC may develop cancer as the result of either a de novo pathogenic variant in the offspring or germline mosaicism in a parent (Shah et al. 2012). To ensure about the latter possibilities, molecular genetic testing of parents should be considered. If a parent is affected and/or has a pathogenic variant in *CDH1*, the chance of inheriting the pathogenic variant by the sibs is 50%. The recurrence risk would be indeterminate in case of germline mosaicism and very low in the situation of de novo mutation. When neither parent of a proband with an autosomal dominant condition has the pathogenic variant or clinical evidence of the disorder, it is likely that the proband has a de novo mutation. However, possible non-medical explanations such as non-paternity, alternate paternity or maternity (e.g., with assisted reproduction), or undisclosed adoption should also be sought.

Predictive testing in asymptomatic at risk adults (younger than 18) in the pedigree is possible, provided that *CDH1* mutation has been identified. Notably, in 50–70% of HDGC families, genetic etiology remains unknown after *CDH1*genetic testing. Cancer susceptibility is caused by unknown genetic factors; NGS offers new possibilities to identify novel variants and genes involved in genetic diseases including cancer syndromes (Gaston et al. 2014; Tabatabaiefar et al. 2017; Schrauwen et al. 2012). For example, studying a large Canadian HDGC family, Gaston et al. were able to identify a germline coding variant (p.P946L) in mitogen-activated protein kinase kinase kinase 6 (*MAP3K6*) (Gaston et al. 2014).

Genetic testing in asymptomatic at risk individuals younger than 18 years of age is a debatable issue. It has been suggested that testing of this group may be beneficial (Fitzgerald et al. 2010; Guilford et al. 1998). To maximize the benefits to the child while minimizing the risks, it has been suggested that genetic testing should be permitted about the age of onset of cancer in the related family (Fitzgerald et al. 2010; Kodish 1999).

In case molecular genetic testing of *CDH1* is performed in at risk relatives, in carriers of mutations early diagnosis and treatment could be offered (Pinheiro et al. 2014).

Families with the HDGC criteria should be offered genetic testing for *CDH1*. In case there is a familial gastric cancer but without *CDH1* mutations or there is certain familial syndrome involving gastric cancer, *H. pylori* infection should be first addressed. Endoscopies may be performed annually. These families could be subjected to NGS for possible discovery of their genetic etiology.

12.2.7 Management and Individualized Medicine

Several serum markers such as CEA, the glycoprotein CA 125 antigen (CA 125) and cancer antigen 19-9 (CA 19-9) have been reported to be raised in gastric cancer patients (Lai et al. 2001). However, due to their low sensitivity and specificity they have not been included in diagnostics of GC. Their serum elevations have sometimes be used independently to implicate poor prognosis preoperatively (Mihmanli et al. 2003). The National Comprehensive Cancer Network (NCCN) guideline does not include any tumor marker for preoperative evaluation of GC (Ajani et al. 2013). GCs producing alpha-fetoprotein (AFP) (Liu et al. 2010) tend to be aggressive and of a poor prognosis.

In a recent study, Tu et al. used five stomach-specific circulating serum biomarkers including pepsinogen I (PGI), PGII, PGI/II ratio, anti-Helicobacter pylori (*H. pylori*) antibody, and gastrin-17 (G-17) to identify high-risk individuals for further diagnostic gastroscopy and to predict risk of developing GC. They concluded this could improve prediction beyond traditional risk factors and would be applied to targeted screening and precision prevention in the future (Tu et al. 2017).

KRAS amplification in advanced GC ha been associated with lack of response to EGFR targeted therapies. Similarly, *PIK3CA* mutations reduced responsiveness to EGF-targeted therapies (Markman et al. 2010). *PIK3CA* mutations have been associated with bone metastasis recurrence. *CDH1* or *ARID1A* mutations and *EGFR* amplification is associated with peritoneal recurrence (Kuboki et al. 2015).

So far, 2 targeted molecular therapeutic agents, trastuzumab and ramucirumab, have been approved by the Food and Drug Administration (FDA). Several clinical trials are already targeting STAT3, c-MET, mTOR, and PD-1/PD-L1(Al-Batran et al. 2016; Muro et al. 2016; Shitara and Ohtsu 2016). Combination therapy with vorinostat (a histone deacetylase inhibitor) and radiotherapy is being investigated for GC (ClinicalTrials.gov identifier: NCT01045538) (Abdelfatah et al. 2016). *MET* amplification is observed in about 5% of GC patients for which crizotinib is in a clinical trial phase (ClinicalTrials. gov identifier: NCT02435108) (Lennerz et al. 2011; Network 2014). For advanced GCs, several targeted molecular agents have been assessed in clinical trials (Bang 2013). For example, the monoclonal antibody trastuzumab has been shown to be effective against human epidermal growth factor receptor (HER) 2-positive advanced GC (Farran et al. 2017). However, some studies have concluded otherwise (Xu et al. 2017). Immune therapies are also promising. For example, pembrolizumab may provide a way to tackle chemotherapeutically resistant GC tumors (Lianos et al. 2015). Angiogenesis is an important process in cancer development. Thus, VEGFs and receptors are potential targets for treatment (Claerhout et al. 2011).

To identify the molecular foundation of GC, an NGS-based RNA-sequencing approach (RNA Seq) was applied to quantitatively characterize the entire transcriptome of GC. The central metabolic regulator AMP-activated protein kinase (AMPK) was found to be a potential therapeutic target for early-stage gastric cancer

in Asian patients (Kim et al. 2012). microRNAs are useful biomarker for progression and prognosis of gastric cancer (Cho 2013).

Thanks to the combined application of NGS and novel preclinical model strategies, genome-based and phenotype-based strategies are being developed to be used in precision medicine of GC (Liu and Meltzer 2017).

12.3 Colorectal Cancer

12.3.1 Introduction

Colorectal cancer (CRC) is the fourth most common cancer in the United States and the second leading cause of cancer-related death (Al-Hajeili et al. 2017). Over 65% of CRC cases are found in developing countries. It is more common in men than in women (McGuire 2016). Important signs and symptoms may include blood in the stool, a change in bowel movements, weight loss, and feeling tired all the time (Harriss et al. 2009).

Surgery, radiation therapy, chemotherapy and targeted therapy are among most available treatments for CRC. As a general paradigm, cancers limited within their capsules are mostly curable with surgery while cancers that have metastasized to distant regions are usually not curable. In these cases, their management is mostly focused on improving the quality of life (Mathis and Pemberton 2015). Major risk factors include old age, genetic predisposition, lifestyle factors such as diet, smoking, obesity, and sedentary life, and such disorders as inflammatory bowel disease (including Crohn's disease and ulcerative colitis) (Mundade et al. 2014; Tezcan et al. 2016).

12.3.2 Pathophysiology and Classification

CRCs originate from dysplastic adenomatous polyps in most of cases. Tumorigenesis is a multi-step process in which a variety of tumor suppressor and DNA repair genes are inactivated while a series of proto-oncogenes become activated. Therefore, as genetic alterations are accumulated, a selective growth advantage is endowed to the epithelial cells of the colon and the normal epithelium transforms to adenomatous polyp, and finally to invasive CRC (Grady and Markowitz 2015). Germline mutations are inherited in hereditary colon cancer syndromes. Sporadic cancers, however, are caused by a step-wise buildup of somatic genetic mutations. A single germline mutation in the adenomatous polyposis coli (APC) tumor suppressor gene accounts for the familial adenomatous polyposis (FAP). The second allele is inactivated by a mutation, deletion and epigenetic mechanism, also known as the second hit (Van Cutsem et al. 2011).

Hereditary non-polyposis colon cancer (HNPCC) is the second type of inherited CRC. HNPCC is the most prevalent type of hereditary CRC, the incidence of which in the United States is 2–5%, or 7500 new cases annually (Winawer et al. 2003).

In HNPCC patients, there is increased risk of other cancers such as endometrial, ovary, small intestine, stomach, hepatobiliary tract, pancreas, prostrate and brain It is a dominantly inherited syndrome. HNPCC is clinically subcategorized into Lynch syndrome I (familial colon cancer) and Lynch syndrome II (HNPCC associated with other cancers of the gastrointestinal (GI) or reproductive system (Frei 1992).

12.3.3 Lynch Syndrome (Hereditary Non-Polyposis Colorectal Cancer, HNPCC)

12.3.3.1 Genetics

HNPCC can be caused by defects in mismatch repair (MMR) genes. HNPCC is responsible for 2–5% of all CRCs. More than 90% of HNPCC patients show a high microsatellite instability (MSI-H), meaning that 2 or more genes have been mutated in HNPCC tumors (FC et al. 2016).

MMR genes encode proteins engaged in the repair of mismatches during DNA replication.

Thus far, the 7 identified distinct MMR genes include:

- *hMLH1* (*3p22)*
- *hMSH2* and *hMSH6* (2p16)
- *hPMS1* (3p32) and *hPMS2* (7q22)
- *hMSH3* (5q14.1)
- *EXO1* (1q43)

Mutations of *hMLH1* and *hMSH2* represent about *70% of MMR mutations in HNPCC;* hMSH6 mutations constitute 10% of the HNPCC causes (FC et al. 2016; Rau et al. 2017). The remaining genes as well as novel ones account for the remaining etiology.

12.3.3.2 Genotype-Phenotype Correlations

Approximately 35% of families in which the diagnosis of HNPCC is based on the Amsterdam criteria do not appear to harbor mutations in the DNA-mismatch repair genes (Scott et al. 2001).

The group had a distinct pattern of cancer incidence compared to those harboring MMS mutations: a modest increase in the incidence of CRC (standardized incidence ratio of 2.3, compared to 6.1 in families) and no increase in the risk of other

malignancies. Lindor et al. (2005) suggested the designation 'familial colorectal cancer type X′ for this type of familial aggregation of CRC (Lindor et al. 2005).

Van der Post et al. (2010) concluded that patients with Lynch syndrome, particularly those carrying MSH2, *hMSH2* mutations, have an increased risk of urinary tract cancer, which may warrant surveillance (Skeldon et al. 2013; van der Post et al. 2010).

Both alleles of an HNPCC gene must be inactivated before the MSI phenotype appears. Truncating mutations account for most inactivating mutations of *hMLH1* and *hMSH2*. The result is the inability to repair replication errors and leads to aggregation of mutations (FC et al. 2016).

When an individual with an HNPCC mutation is not subjected to a partial or total colectomy, after the initial malignancy, he would have a risk of 30–40% for developing a metachronous tumor within 10 years and a 50% risk within 15 years. While in the general population, the risk is 3 and 5% within 10 and 15 years, respectively (Brychtova et al. 2017).

Loss of MSH6 expression is the associated with early-onset CRC. Endometrial cancer has also been associated with MSH6 mutation (Giráldez et al. 2010).

12.3.3.3 Screening and Surveillance

The Amsterdam I criteria focused on the number and ages of family members with CRC that were published in 1990. However, they were later found out to be of a low sensitivity to include Lynch syndrome patients (Bellizzi and Frankel 2009; Wijnen et al. 1997).

The Amsterdam I criteria include at least three relatives with CRC while all of the following criteria should be met:

(1) One should be the first-degree relative of the other two patients
(2) At least two successive generations must be affected
(3) At least one of the relatives affected with CRC must be diagnosed before 50 years old
(4) FAP should be excluded
(5) Tumors should be confirmed by pathologic examination

The Amsterdam II criteria were proposed in 1999 (Vasen et al. 1999). To meet the Amsterdam II criteria, at least three relatives must have a cancer associated with Lynch syndrome including CRC. They could also have related cancers: small bowel, transitional cell carcinoma of the upper urinary tract, stomach, ovarian, brain (Turcot syndrome) and sebaceous gland adenomas or keratoacanthomas (Muir-Torre syndrome). In addition, all of the following criteria should be present:

(1) One must be a first-degree relative of the other two
(2) At least two successive generations must be affected

(3) At least one relative with cancer associated with Lynch syndrome should be diagnosed before age 50
(4) FAP should be excluded
(5) Tumors should be confirmed by pathological examination.

The Amsterdam criteria are useful but do not identify up to 30% of potential Lynch syndrome carriers. The Bethesda criteria were developed and later revised by the National Cancer Institute (Umar et al. 2004). Based on the revised Bethesda guidelines, tumors from individuals should be subjected to MSI testing in the following situations:

(1) CRC is diagnosed in a patient younger than 50 years old.
(2) Synchronous or metachronous CRC or other related tumors occur regardless of age.
(3) CRC with MSI-high histology is diagnosed in a patient younger than 60 years of age.
(4) CRC is diagnosed in a patient with one or more first-degree relatives with a related cancer, one patient should be diagnosed younger than 50 years of age.
(5) CRC is diagnosed in a patient with two or more first- or second-degree relatives with related cancer regardless of age.

The Bethesda criteria may be more sensitive than Amsterdam criteria I and II in identifying families with HNPCC, but they are not diagnostic of HNPCC. Since MSI can also be observed in 15% of sporadic tumors, DNA testing should be followed for confirmation.

Screening the individuals fulfilling the clinical criteria includes the following:

Colonoscopy: Colonoscopy is the preferred and cost-effective screening test for HNPCC patients. Based on some data, patients, who have undergone colonoscopic adenoma removal, are more likely to survive. According to the current American Cancer Society (ACS) guidelines, colonoscopy should be offered to patients with a family history of colorectal cancer since age 20–25 years or 5–10 years earlier than the onset of CRC in the family. It should be repeated every 2–3 years. After 40 years of age, it should be performed every 1–2 years. Female patients carrying *MSH6* mutations have a lower risk of CRC. Therefore, Colonoscopy is recommended to start at age 30 years instead of the age 20–25 years (Bonadona et al. 2011).

Virtual colonoscopy. In virtual colonoscopy, computed tomography (CT) scanning is applied to generate a 3-dimensional (3-D) image of the air-extended, prepared colon. While virtual colonoscopy lacks the risk of sedation or perforation observed in the conventional colonoscopy, removal of a polyp, if detected, would have to be performed using colonoscopy. This test is not presently recommended as a screening test for patients carrying an MMR gene mutation (Levine and Yee 2014).

12.3.3.4 Genetic Counseling and Testing Strategies

Germline mutations are often inherited but may also arise spontaneously or de novo in an individual and in the next generation. The latter situations are mostly identified when patients develop CRC early in life. The mode of inheritance of the Lynch syndrome is autosomal dominant. Therefore, 50% of the offspring of an affected individual inherit a mutant allele (FC et al. 2016; Rau et al. 2017). If an individual inherits the mutant allele, there is a 70–80% lifetime risk of developing CRC. Using genetic predictive testing, individuals harboring the deleterious allele can be identified. If one declines genetic testing, endoscopic surveillance should be carried out.

Genetic diagnosis of Lynch syndrome includes the process of genetic counseling in which cancer history is reviewed and pedigree is drawn. Usually, tumor testing should be performed using either immunohistochemistry (IHC) or MSI testing or both followed by genetic testing of the individual to identify the underlying germline genetic variant.

The following history findings may imply HNPCC:

- Several CRC patients or many adenomatous polyps diagnosed in different generations
- Patients diagnosed younger than 50 years old
- The combination of syndrome-related tumors in other organs
- Synchronous or metachronous tumors present in one person.

Significant suspicion for HNPCC makes necessary further evaluation of the patient and his or her family (Munoz et al. 2017).

The American College of Gastroenterology (ACG) has released the following recommendations for the management of patients with hereditary GI cancer syndromes—especially Lynch syndrome, (Syngal et al. 2015):

- Collection of a family history of cancers and premalignant GI conditions is the preliminary necessary step.
- Age at diagnosis and lineage (maternal and/or paternal) should be noted for all diagnoses, especially in the first- and second-degree relatives.
- When indicated, genetic testing for a germline mutation should be offered on patient ascertained through the family history evaluation and/or tumor analysis. This is an important step to confirm a diagnosis and would later allow for predictive testing of pedigree members.
- Pre- and post-test genetic counseling should be performed to ensure the patient's informed decision making prior to genetic testing.
- Patients meeting the clinical criteria for Lynch syndrome and people with identified pathogenic germline mutations should receive appropriate surveillance measures to decrease their risk of developing the cancer.

When a family meets the Amsterdam or Bethesda Criteria, testing of the tumor tissue is indicated. Tests include IHC testing, MSI testing and DNA analysis.

IHC: For Lynch syndrome, monoclonal antibodies are used to show which MMR proteins are present in a tissue sample. MLH1, MSH2, MSH6, and PMS2 can be assessed. If the protein of interest is not stained, we can conclude that there must be a mutation in the encoding gene.

- An IHC pattern with absent staining for MLH1 and PMS2 but positive staining for MSH2 and MSH6 suggests a mutation in *MLH1* (*Levin* et al. 2008).
- An IHC pattern with no staining for MSH2 and MSH6 but positive for MLH1 and PMS2 indicates a mutation in *MSH2*. In IHC pattern compatible with MSH2, there is a 5% chance of finding a germline mutation in MSH6. Thus, in these cases if no mutation was identified for *MSH2* DNA analysis of *MSH6* should be considered. *MSH6* mutations are associated with early-onset CRC and also with the related endometrial cancer in the family (Giráldez et al. 2010).
- An IHC pattern with absent staining for MSH6 but positive for the remaining MMR proteins suggests *hMLH6* DNA analysis. However, if no mutation is found in the gene, the genetic testing of *MSH2* may be considered.

Compared to MSI analysis, IHC can determine the MMR gene to be subjected to DNA analysis. IHC is specifically sensitive for truncating variants such as frameshift, splice site mutations, large genomic rearrangements. In substitution mutations, the protein may become non-functional but it is still detected using IHC.

MSI analysis. MSI is a molecular phenotype of defective MMR genes. It is found in CRC tumor but not in the normal tissues of individuals with MMR mutations. MSI is characterized by change in the number of short repeated DNA sequences (known as short tandem repeats or STRs). Over 90% of HNPCC tumors and 15% of sporadic tumors show MSI. The presence of instability throughout genome in STRs indicates a defective MMR gene.

Different marker panels have been used to assess the MSI status (Park et al. 2017; Ziadi et al. 2014). In 1997, the National Cancer Institute (NCI) Workshop on Hereditary Nonpolyposis Colorectal Cancer Syndrome suggested 5 markers for evaluation of MSI. The frequently used panel, 5–6 markers include D2S123, D17S250, D5S346, BAT25, has been applied. MSI can be categorized as MSI-high (MSH-H), when at least 30% of markers show instability, or MSI-low (MSH-L), if less than 30% of the markers show instability. Tumors with no positive markers are known as microsatellite stable (MSS) (Umar et al. 2004).

About 90% of *hMLH1* and *hMSH2* mutations result in the MSH-H phenotype, whereas nearly 10% of *hMLH6* mutations may result in the MSI-L or MSS phenotype. *hMLH6* mutation carriers particularly related to endometrial carcinomas usually present MSS phenotype. Thus, in case MSS phenotype is identified using MSI testing, IHC of MSH6 should be considered (Levin et al. 2008).

MSI analysis has a high sensitivity (about 93%) for detecting MMR defects. However, this test cannot be used to indicate which of the MMR genes should be chosen for later DNA analysis.

Genetic testing of MMR genes

Prior to genetic tests, pre-genetic counseling of the patient and his or her family should be done. A blood sample is taken to identify mutations of the MMR genes for sequence, deletion/duplication or rearrangement analyses.

Genetic testing should be offered when HNPCC is strongly suspected. Patients fulfilling the Amsterdam or Bethesda criteria can undergo genetic testing. According to the new guidelines, before moving to genetic testing, tumor testing of the patient should be done using MSI or IHC analyses, followed by germline mutation testing. *hMLH1* and *hMSH2* mutation testing will be considered for those with MSI-H tumors or tumors negative for expression of one of the MMR gene products upon IHC. *hMSH6* germline mutation testing should be performed when the tumor tests MSS.

Alternatively, high-risk individuals have an option to be tested using NGS-based testing. In the following conditions genetic testing will be the only options: if tissue testing is not feasible (e.g., the tumor sample is unavailable), or if HNPCC is suspected but MSI/IHC testing reveals MSI-L or MSS/normal *hMLH1* and *hMSH2*. This latter trend has especially recently favored in the NGS testing era.

Direct genetic testing can considerably save time and cost in the future (De Keulenaer et al. 2012). They have additional advantages (Rodriguez-Bigas et al. 1997):

- Genetic test results are normally more accurate assessment of cancer risk. If a mutation is identified, predictive genetic testing can be offered to the pedigree members.
- Vigorous surveillance and management can then be reserved for patients with identified mutations and individuals harboring no germline mutation feel relieved and simply follow the ACS recommendations for the general population.
- Genetic testing has no extra physical risk than that of a routine blood test.

BRAF/KRAS testing

The V600E mutation in the *BRAF* gene has been found frequently in sporadic CRCs (Asl et al. 2014). In sporadic patients, MLH1 protein expression may be lost through hypermethylation in the *MLH1* gene promoter. Therefore, tumors which are negative for MLH1 protein with absence of *BRAF* V600E mutation and *MLH1* promoter hypermethylation, are suspected of HNPCC or Lynch syndrome (Ladabaum et al. 2011). For *KRAS*, mutations in codons 12 or 13 of the *KRAS* gene on formalin-fixed, paraffin-embedded tissue or from the primary tumor or a metastasis are investigated. For *BRAF V600E*, a simple PCR amplification followed by a simple technique such as restriction fragment length polymorphism (RFLP) or DNA sequencing the hotspot mutation *BRAF V600E* mutation can be examined in formalin-fixed, paraffin-embedded tissue from the primary tumor or a metastasis (Asl et al. 2014; Soltani et al. 2017).

12.3.3.5 Management and Individualized Medicine

HNPCC patients have the 5-year survival rate of 60% compared with 40–50% for sporadic cases. MSI-positive CRC tumors tend to originate in the proximal colon, are poorly differentiated, and have mucinous or signet ring appearance (Boland and Goel 2010). The tumors, however, are associated with improved survival rates.

The origins of HNPCC tumors are typically adenomas; however, they have a fast adenoma-carcinoma progression sequence compared with the sporadic tumors. Therefore, carriers of germline *MLH1* or *MSH2* mutations should undergo full colonoscopy every 1–2 years from 20–25 years of age or 5 years earlier than the onset of cancer in the family. After the age of 35–40 years, colonoscopy should be carried out annually.

The implementation of CRC testing to identify families with HNPCC provides substantial benefits at acceptable cost (Ladabaum et al. 2011). Up to 30–50% of CRC tumors have a *KRAS* mutation. This group might respond to anti-epidermal growth factor receptor (EGFR) antibody therapy. However, the remaining cases have wild-type *KRAS* in their tumors and do not respond to this therapy (Wilson et al. 2010). In case they are found to have a mutated *BRAF* gene (in 5–10% of tumors) suitable targeted therapy would be planned (Douillard et al. 2013). Mutated *BRAF* confers resistance to anti-EGFR therapy (Bokemeyer et al. 2010). Cetuximab and panitumumab are rather effective in *KRAS* wild-type CRC (Petrelli et al. 2011); Cetuximab plus FOLFIRI (fluorouracil + leucovorin + irinotecan) advances survival and response rate in *KRAS* wild-type compared with FOLFIRI alone (Van Cutsem et al. 2011). In a recent study it was showed that NGS panels could be employed to evaluate mutational load in CRC tumors to identify patients who could potentially respond to treatment with immune checkpoint inhibitors. The immunogenicity of the tumor microenvironment could also be assessed in this way (Stadler et al. 2016).

12.3.4 Familial Adenomatous Polyposis

Familial adenomatous polyposis (FAP) is the most common adenomatous polyposis syndrome. It follows an autosomal dominant inheritance in which hundreds of adenomatous polyps emerge throughout the colon. Untreated patients develop CRC and die by 40 years of age (Talseth-Palmer 2017). In addition, an increased risk exists for the development of other tumors such as fundic gland polyps, adenomatous polyps in the duodenum and small bowel adenomas (Half et al. 2009), desmoid tumors present in Gardner syndrome (a phenotypic variant of FAP) (Juhn and Khachemoune 2010), brain tumors (mainly medulloblastoma, in Turcot's syndrome which is another phenotypic variant of FAP) (Galiatsatos and Foulkes 2006; Half et al. 2009; Talseth-Palmer 2017).

12.3.4.1 Genetics

Germline mutations in the adenomatous polyposis coli (*APC*) gene lead to FAP. Defects in the same gene accounts for the syndromes once thought to be different from FAP but are currently considered to be part of the phenotypic spectrum of FAP (Schulmann et al. 2007). These include Gardner syndrome, Turcot syndrome, and attenuated adenomatous polyposis coli (AAPC). Gardner syndrome includes colonic polyposis typical of FAP, but also osteomas on the skull and the mandible, dental anomalies, and soft tissue tumors (Juhn and Khachemoune 2010). In Turcot syndrome, there is the colonic polyposis typical of FAP, together with central nervous system tumors (medulloblastoma) (Half et al. 2009).

Mutations in the *MUTYH* gene cause autosomal recessive familial adenomatous polyposis (also known as MYH-associated polyposis). In 10–20% of cases suspected with APC but with no APC gene mutation, the *MYH* gene is biallelically mutated (Sereno et al. 2014).

12.3.4.2 Genotype-Phenotype Correlations

As mentioned above, mutations of *APC* can cause FAP, Gardner syndrome, Turcot syndrome, and AAPC. Generally, there have been conflicting genotype-phenotype correlations in different aspects of the phenotypes. The most important established genotype-phenotype correlations are presented below:

- Germline mutations between codons 1250 and 1464 have been associated with profuse polyps (10 or more), whereas mutations in other regions of the APC gene are associated with sparse polyps (fewer than 10) (Nagase et al. 1992a).
- The type or intragenic location of a particular germline mutation is not correlated with extracolonic manifestations such as desmoid tumors (Nagase et al. 1992b).
- Attenuated APC alleles with 5-prime mutations encode APC protein that downregulates beta-catenin, inhibits beta-catenin/T-cell factor-mediated transactivation, and leads to cell-cycle arrest (Heppner Goss et al. 2002).
- Congenital hypertrophy of the retinal pigment epithelium (CHRPE) is caused by truncated proteins which are larger than 50 kD. They are believed to have a dominant-negative effect, resulting in decreased APC function and expression of CHRPE (Wallis et al. 1994).
- 3-prime mutations may be null alleles. The attenuated phenotype observed in AAPC is the result of a haploinsufficiency of the normal APC protein and absence of a truncated protein that would expert a dominant-negative effect (van der Luijt et al. 1996).

12.3.4.3 Screening and Surveilance

- American Society of Clinical Oncology (ASCO) recommends the following screening for people with FAP (Stoffel et al. 2015):
- Sigmoidoscopy or colonoscopy every 1–2 years, starting from 10 to 11 years of age.
- Annual colonoscopy. When polyps are found a colectomy is done.
- Even after colon surgery, surveillance of the lower tract with sigmoidoscopy should continue for every 6–12 months if some rectal tissue remains but every 1–4 years if all rectal tissue has been removed.
- Upper endoscopy at age 25–30 or once colorectal polyps are detected.
- Annual ultrasound of the thyroid may be needed starting at age 25–30.
- If a person has a family history of desmoid tumors or a mutation on the APC gene that is linked with these tumors (Ramaglia et al. 2007).
- CT scan or magnetic resonance imaging (MRI) if a person has a family history of desmoid tumors or an *APC* gene mutation that is associated with these tumors (Ramaglia et al. 2007).

Screening options may change over time as new technologies are emerging.

12.3.4.4 Genetic Counseling and Testing Strategies

Genetic counseling may alter the utilization of genetic testing substantially and may reduce inappropriate testing.

Testing for FAP should be considered for individuals with the following (Benson et al. 2013):

1. Presence of 100 or more polyps;
2. Autosomal dominant inheritance;
3. Possible additional findings, such as CHRP, osteomas, supernumerary teeth, odontomas, desmoids, epidermoid cysts, duodenal and other small-bowel adenomas, gastric fundic gland polyps.

Testing for attenuated FAP (AFAP) should be considered for individuals with the following (Hegde et al. 2014):

1. Presence of <100 adenomas (average 30 polyps; (Individuals with 100 or more polyps occurring at 35–40 years or older may be found to have AFAP);
2. Frequent right-sided distribution of polyps;
3. Adenomas and cancers at an age older than that for classic FAP and other GI manifestations.

It is recommended that FAP/AFAP testing be performed using full sequencing of the *APC* gene. In case no mutation is detected, large gene rearrangements should be investigated.

Testing for MAP should be according to the following criteria (Hegde et al. 2014):

1. CRC diagnosed in an individual younger than 40 years of age;
2. The presence of 10 or more adenomatous polyps in the absence of a germline *APC* gene mutation;
3. Family history of CRC consistent with an autosomal recessive inheritance. This should include CRCs with or without polyps.

Testing for *MUTYH* gene mutations may be considered medically necessary in for Familial FAP when any of the following criteria is met:

1. Individuals with personal history of adenomatous polyposis and negative for *APC* mutations and with a negative family history for adenomatous polyposis; OR
2. Individuals with personal history of adenomatous polyposis whose family history is positive only for sibling(s); OR
3. Asymptomatic siblings if his/her sibling has a known MYH polyposis; OR
4. History of Desmoid tumor.

Testing for MAP should be first performed for the two most common mutations, p.Y165C and p.G382D, before performing the full sequencing of the *MUTYH* gene (Hegde et al. 2014).

12.3.4.5 Management and Individualized Medicine

The main surgical option is the removal of the colorectum including either total colectomy with ileorectal anastomosis) (IRA) or proctocolectomy with ileal pouch–anal anastomosis (IPAA). Choice of the type of colorectal surgery in patients with FAP depends on many factors including the age of the patient, the severity of polyposis, the wish to have children, the risk of developing desmoids and sometimes the site of the mutation in the *APC* gene. IPAA should preferably be performed in expert centers (Vasen et al. 2008).

The site of the mutation in the *APC* gene may predict the risk of developing severe rectal polyposis and the need for subsequent proctectomy if a patient had colectomy with IRA. However, the use of genetic information in the surgical decision making is controversial, because several studies have reported intra familial variation, which might be due to environmental factors or modifier genes. Therefore, future prospective studies should be done to evaluate the utility of this genetic information in surgical practice.

The final decision lies with the patient after being fully informed about the pros and cons of the surgical options. In young patients (<40 years) with advanced duodenal disease (Spigelman stage III/IV), local surgery (duodenotomy and polypectomy)

might be beneficial to postpone major surgery. In older patients with stage IV disease at repeated examinations, there is an indication for duodenectomy.

Patients with unresectable metastatic disease are qualified for targeted therapies. In the setting of CRC, important chemotherapies include using monoclonal antibodies targeting VEGF (Bevacizumab) and EGFR (Cetuximab and Panitumumab) (Ohhara et al. 2016). According to the 2016 European Society for Medical Oncology (ESMO) guidelines (Van Cutsem et al. 2016), targeted therapies can be used in combination with a cytotoxic regimen for patients with unresectable metastatic CRC. Interestingly, in the 2016 ESMO guidelines, the expanded RAS status is a mandatory step before using anti-EGFR therapy. As mentioned before, patients with a *BRAF* mutated tumor might not benefit from anti-EGFR therapy (Van Cutsem et al. 2016)

We are in the new era that treatment of cancer is becoming individualized. Using NGS technologies, the screening of clinically actionable targets for targeted therapies are being discovered (Fontanges et al. 2016). This approach will impact genetics laboratories making them adopt emerging high-throughput diagnostic assays in different types of cancers.

12.4 Conclusions

Different aspects of three main gastrointestinal (GI) cancers including esophageal, gastric and colorectal cancers were tried to be addressed in this chapter including their pathology and classification, genetics, genotype-phenotype correlations, screening and surveillance, genetic counselling and testing strategies, and finally management and individualized medicine, based on the current ideas. As novel technologies such as next-generation sequencing (NGS) are increasingly applied in cancer research and diagnostics, our information on different aspects of cancers will be improved. Molecular-based profiling is predicted to further classify cancer histological subtypes into more categories for which distinct targeted therapies would be developed. Novel technologies should be used in combination with clinical findings to help achieve outstanding results in the management of cancer cases. Fortunately, in this regard, clinical and diagnostic practice guidelines integrating novel findings are being developed by reputable professional bodies and societies.

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Chapter 13
Genetic and Cellular Complexity of Brain Tumors

Fatemeh Karami and Parvin Mehdipour

Abstract Brain tumors are associated with high mortality rate and considered as a leading cause of death among the human cancers. The main molecular mechanisms behind the pathophysiology of brain tumors could be summarized in three major classifications including aberrant signaling pathways, genetic mutations, polymorphisms and epigenetic alterations. Personalized treatment of brain tumor patients based on the specific molecular changes can increase the overall survival of affected individuals. Beside approved different chemotherapy and radiotherapy approaches, psychotherapy can enhance the life expectancy of patients and temporarily increase the success rate of moderating the disease progression.

Keywords Brain tumor · Psychotherapy · Gene · Mutation · Methylation · Pathway

Abbreviations

APG101	Apogenix
bHLH	Basic helix-loop-helix
BBB	Blood brain barrier
Bdnf	Brain-derived neurotrophic factor
BPA	Bisphenol-A
cfDNA	Cell free DNA
CNS	Central nervous system
COX	Cyclooxygenase
CTCs	Circulating tumor cells
CTSC	Circulating tumor stem cells

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DNMT	DNA methyltransferase
DSB	Double strand DNA break
EBV	Epstein-Barr virus
EDCs	Endocrine-disrupting chemicals
EGFR	Epidermal growth factor
EpCAM	Epithelial cell adhesion molecule
ER	Estrogen receptors
ERCCs	Excision repair cross-complementation group
ESR1	Estrogen receptor 1
EZH2	Enhancer of Zeste Homolog
ATM	Mutated in ataxia telangiectasia (A-T)
FGFR1	Fibroblast growth factor receptor 1
G-CIMP	Glioma-CpG island methylator phenotype
GR	Glucocorticoid Receptor
HAT	Histone acetyl transferase
HIF	Hypoxia induced factor
HPV	Human papilloma virus
IGSF4	Immunoglobulin superfamily member 4
IL	Interleukine
Ki67	MKi67 (cellular marker for proliferation; is originated from city Kiel, Germany)
MBCT	Mindfulness-based cognitive therapy
MecP2	Methyl-CpG binding protein 2
MGMT	O(6)-methylguanine DNA methyltransferas
MiRNAs	micro RNAs
MLL1	Mixed lineage leukemia 1
mtDNA	Mitochondrial DNA
Nr3c1	Nuclear Receptor Subfamily 3 Group C Member 1
P53	Protein 53
P63	Protein 63
PARP	Poly (ADP-ribose) polymerase
PCNA	Proliferating cell nuclear antigen
RASSF1A	Ras association domain family 1 isoform A
ROS	Reactive oxygen species
SMG-1	Suppressor with morphogenetic effect on genitalia 1
SNPs	Single nucleotide polymorphisms
SSRI	Selective serotonin reuptake inhibitor
TACC1	Transforming acidic coiled-coil 1
TCAs	Tricyclic antidepressants
TNF	Tumor necrosis factor
WT1	Wilms tumor 1
XRCC	X-ray repair cross-complementation group (XRCC)

13.1 Background

There are a variety of different types of primary brain tumors including benign and malignant originated from brain parenchyma and the other structures around it. Brain tumors are considered as the main cause of tumor induced mortality in either age ranges of infants or adults (Lacy et al. 2012). According to the last report, the overall incidence rate of all types of brain tumors was calculated as 25.95 per 100,000 person in every year (de Robles et al. 2015). They are also reported as 1.4% of new cancer cases which include 2.6% of cancer induced death (McNeill 2016). Although all types of central nervous system (CNS) malignancies are the most frequent solid tumors among children, the rate of different heterogeneous types of primary brain tumors varies between male and female, age range and populations around the world (Table 13.1) (de Robles et al. 2015). The frequency of different types of brain tumor in adults includes meningioma (36%), glioblastoma and pituitary tumors (15%), other types of glioma and other types of brain tumors (10%), nerve sheath tumors (8%), lymphoma and ependymal tumors (2%) and embryonal tumors (1%) (McNeill 2016). This order of frequency increases which includes the higher rates of embryonal tumors, malignant glioma and pylocyctic astrocytoma (15–17%) and lower rates of meningioma (2%) and pituitary tumors (4%) among children.

The main risk factors of primary brain tumor include ionizing radiation, allergic reactions and genetic predisposition factors (McNeill 2016).

13.1.1 Genetic Factors

Brain tumors are among some of human cancers which are revealed to be associated with familial cancer syndromes such as Li-Fraumeni and Turcot's syndrome. Besides, in the frame of some other familial neurological diseases neurofibromatosis and tuberous sclerosis could be mentioned as well (Malkin 1994). The major genes which have been found to be involved in those familial cancer diseases include *TP53*, *CHEK2, NF1*, *NF2, TSC1* and *TSC2*. Although some Mendelian inheritance as well as autosomal recessive pattern has been detected in families affected by glioma, familial brain tumors may share the same either environmental or genetic risk factors (Louis et al. 2007).

Table 13.1 The age and gender preferences in the most important types of brain tumors

Brain tumor's type	Median age (Y)	Gender preference
Embryonal tumors	9	Female (Gessi et al. 2009)
Pituitary tumors	12	Female
Glioma	7	Male
Meningioma	9	Female

13.2 Molecular Pathophysiology

Although there are many common aberrant molecular pathways in different cancers, genetic alterations profiles may be different in various primary and secondary types of brain tumors and even within different tumor grades. Many cohort studies have been performed to clarify the molecular pathogenesis of different grades of brain pathology as well as Cancer Genome Atlas (TCGA) which has studied the glioblastoma multiform (GBM). Based on the fact that glioma is known as the most frequent type of brain tumors in adults including astrocytomas and oligodendrogliomas and as the main histological and pathological subtypes, most of the molecular studies have been focused on this type of tumors (Louis et al. 2007).

According to neuroglial lineage tree, there are primary progenitor cells which have the capability of self renewing and can differentiate into astrocytes and oligodendrocytes. Therefore, in this section of chapter, the major focus of molecular pathogenesis of brain tumors will be on low and high grades of glial tumors.

The major signaling pathways which have been found in different subtypes of glial tumors include: p53 Pathway, Rb Pathway, Receptor Tyrosine Kinases and its downstream pathways consisting of Ras/Raf/MAPK, PI3K/PTEN/AKT/mTOR signaling pathways (McLendon et al. 2008). So far, P53 is considered as a common element involved in the most important pathways, not only in brain tumors but also in almost all types of cancers. It is a well-known tumor suppressor gene which is located on chromosome 17 (17p13.1) and has critical role in cell cycle, apoptosis and genomic stability (Carson et al. 1995). P53 pathway can be affected by either *p53* gene mutations or alterations in other molecules regulating function of p53. The *MDM2/MDM4* genes are the most important antagonists of *p53* that can modulate its expression directly through binding to *p53* gene promoter. Amplification of those genes has been shown to be associated with defective p53 pathway (Reifenberger et al. 1993). In contrast, inactivating mutation or deletion in *Ink4a/Arf* as an antagonist of p53 were detected in glioma in different assays which induces downregulation of p53 pathway (Jen et al. 1994; Nishikawa et al. 1995; Hodges et al. 2017). It is worth to note that increase in frequency of *p53* mutations from lower grades of astrocytoma to higher grades (anaplastic astrocytoma and GBM) supports the critical role of *p53* in evolution of brain tumors from primary to secondary malignant changes (Fig. 13.1) (Nasser and Mehdipour 2017).

Furthermore, Rb is also involved in another important pathway which is deregulated in many types of cancers as well as brain tumors. Like p53, Rb has pivotal role in control of cell cycle and cell proliferation using its various negative and positive modulators. Actually, Rb regulates cell proliferation through a binding process to an important transcription factor known as E2F. When inactivating alteration such as hypermethylation or mutation occurs, E2F can separates and activates multiple downstream molecules which drive other cell proliferation pathways (Henson et al. 1994). Lower mRNA expression of *p53* and *Rb* genes have been detected in higher grades of astrocytoma (Kheirollahi et al. 2011a, b). *Rb* mutations have been found in about 30% of high grades of astrocytoma

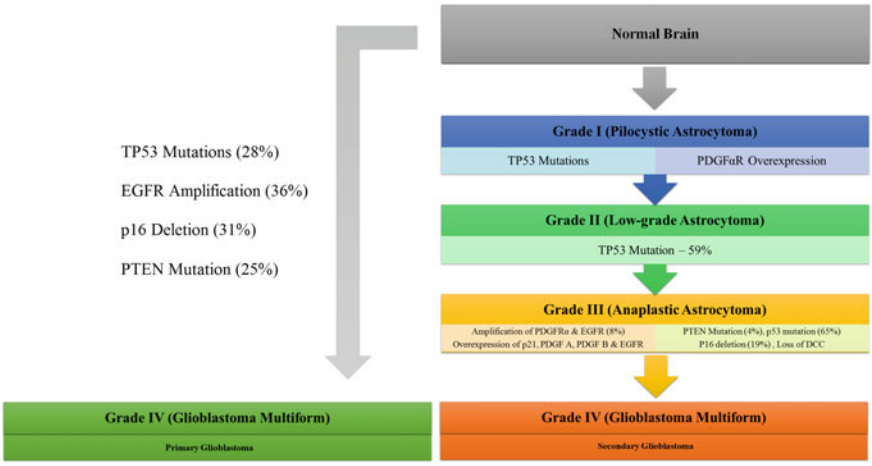

Fig. 13.1 Molecular alterations and differentiation in gliomas and genetic pathways in glioblastomas. This figure is adopted from Nasser and Mehdipour (2017)

(Henson et al. 1994). In addition to Rb, amplification of the oncogenes of this pathway such as *CDK4/6* and *Cyclin D2* has been frequently observed in glial tumors. All the loss of function alteration including deletion, mutation and change in the gene expression has been demonstrated in tumor suppressor genes (*p16, p15* and *p27*) within the Rb pathway, as well (Jen et al. 1994; Faria et al. 2007; Gao et al. 2015). Surprisingly, genetic alteration of either Rb itself or any other Rb pathway elements was always observed in glioma and therefore could be considered as a novel target of chemotherapy.

The other category of pathways with critical role in pathogenesis of both low and high grades brain tumors are Receptor Tyrosine Kinases related signaling pathways (Suzuki et al. 2015). In this regard, there are many mouse and human models indicating the role of tyrosine kinase receptors such as Platelet derived growth factor (PDGF) and epidermal growth factor (EGFR) in initiating cancer cell proliferation and glial tumor evolution. PDGF and EGFR are cellular homologous of viral oncogenes v-sis and v-ErbB, respectively (Connolly et al. 2017). PDGF is one of the growth factors that plays major role in regulating the cellular proliferation and migration, embryonic development and the process of angiogenesis especially in glial and oligodendrocyte cells (Barres et al. 1992; Heldin 1992). The remarkable alterations in EGFR pathway have been extensively studied in multiple cell lines, mouse models and human assays. However, putative role of this pathway's components in initiation and migration of glioma especially in the grade 4, glioblastoma multiform (GBM) is highlighted (Azuaje et al. 2015). It was found that overexpression of PDGF and EGF can lead to glial tumor cell progression and driving angiogenesis pathways (Plate et al. 2012). However, resistant to targeting EGFR pathway has deviated the focus of researchers toward other interacting signaling

pathways that facilitates the resistance of brain tumors to chemotherapy and even radiotherapy (McLendon et al. 2008; Azuaje et al. 2015).

The other important defective pathway of glioma is Ras/Raf/MAPK which has critical role in cancer cell proliferation. Two mechanisms have been identified to induce over activity of Ras/Raf/MAPK pathway including loss of functional mutations in neurofibromatosis factor 1 (*NF1*, a RAS-GTPase inhibitor) and *BRAF* gene aberrations (V600E mutation or *BRAF* fusion) (Zhang et al. 2016a, b). The *NF1* gene mutations rarely occurs and recently a novel NFIA:RAF1 fusion has been found in Pilocytic astrocytoma (PA) patients which was associated with increased Raf1 expression (Yde et al. 2016).

PI3K/PTEN/AKT/mTOR (AKT) is an intracellular pathway which is directly involved in regulation of cell cycle, cell proliferation and apoptosis (Nasser and Mehdipour 2017) (Fig. 13.2). The major activator of this pathway is PI3 K which phosphorylates AKT that in turn induces transmembrane signaling to activate and inhibit other downstream molecules such as CREB and p27, respectively (Peltier et al. 2007; Rafalski et al. 2011; King et al. 2015). PTEN and GSK3B are the most important suppressor of AKT pathway and inactivating *PTEN* mutation as well as the loss of chromosome 10 which was frequently seen in higher grades of astrocytoma and glioma (Rios et al. 2014). EGFR, accompanied by AKT and PDGF signaling pathways are also involved in angiogenic process through activation of vascular endothelial growth factor (VEGF)/VEGF receptor pathway. Blocking VEGF pathway has recently received great and novel attentions in targeted therapy of glioma which has been associated with increase in patient's survival and decrease in mortality rate (Momeny et al. 2017; Wang et al. 2017).

Based on the molecular alterations seen in various grades of brain tumors specially glioma, there is a corresponding molecular classification of brain tumors which led to more influential management including targeted base therapy of patients. However, this classification may be transient and will be modified in near future following current complementary studies on molecular probing of brain tumors. Furthermore, the molecular classification of tumors was initially aroused from this observation that a specific type of tumor may have multiple RNA/DNA and protein profiles among different patients with the same pathology grade (Delgado-Lopez et al. 2017). Many gene expression transcriptomics assays have been performed on glioma patients around the world which share some common expression profile used in molecular classification (Li et al. 2009; Kloosterhof et al. 2013; Guan et al. 2014). Molecular classification not only could assist the oncologist to select the best choice of treatment and management of brain tumor patients, but also can determine the patient's prognosis and survival more effectively than the classic pathologic classification.

The initial molecular classification is known as mesenchymal owing to enhanced expression of genes responsible for mesenchymal stem cells trans-differentiation and cancer cell migration through epithelial mesenchymal transition (EMT) and activation of angiogenic process. Nuclear factor κB (NF-κB) was identified as one of the inducer of mesenchymal subtype formation which is itself regulated by mixed lineage kinase 4 (MLK4), a serine threonine kinase. Recently, it was found

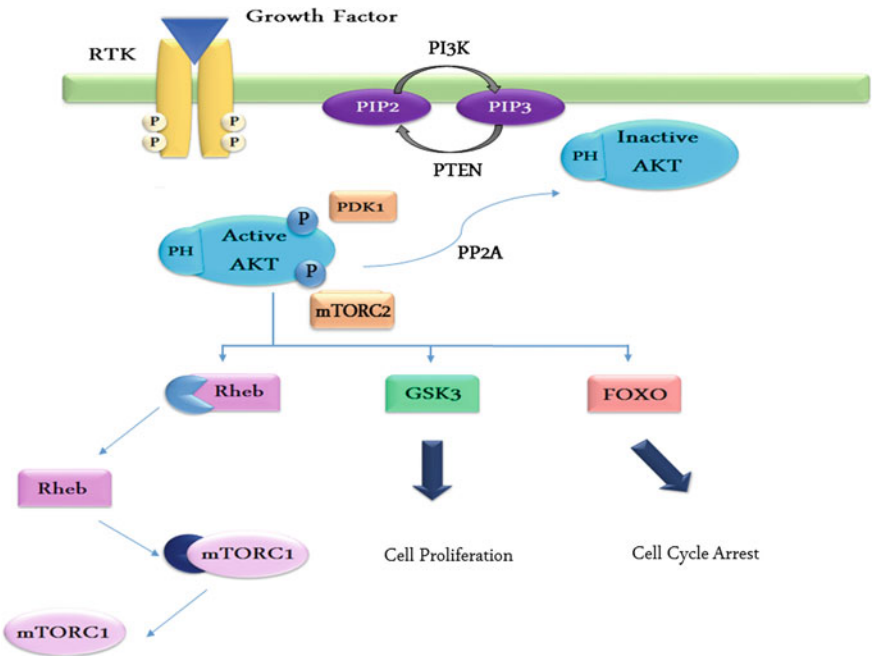

Fig. 13.2 Signaling pathway of PI3K-Akt in brain tumors. This figure is adopted from Nasser and Mehdipour (2017)

that NF1 can establish the mesenchymal status of glioma cells through modulating the CCL5/CCL5-receptor signaling and inhibition of AKT/mTOR signaling pathway (Pan et al. 2017). Mesenchymal subtype has been shown to be associated with radioresistance and worse prognosis and sometimes is accompanied with primary grade 4 of astrocytoma, GBM (Liffers et al. 2015; Kim et al. 2016).

The second subtype of glioma is named as proneural tumors which is surprisingly associated with the expression profile of normal neural cell growth and development. In contrast of mesenchymal subtype, proneural tumors have good prognosis and is associated with secondary type of GBM. *IDH1* and *p53* mutations (54%), *PDGFR* amplification are the remarkable molecular characteristics of proneural subtype. It is worth to note that *NF1* loss of function mutation following proneural changes can direct the tumor evolution towards transformation of proneural glioma cell to mesenchymal subtype of tumor (Ozawa et al. 2014). The classic subtype of glial tumors is identified by high frequency of *EGFR* amplification and chromosome 7. Interestingly, *p53* mutations were not found in this subtype and interestingly, the affected patients have a remarkable high survival period in response to radiotherapy and chemotherapy compared to other subtypes (Verhaak et al. 2010). Common gene mutations of all types of brain tumors especially higher grades will be described in the following section of chapter.

13.3 Common Mutations in Brain Tumor

In spite of some common molecular pathophysiology, molecular alterations in all types of brain tumors, glioma is particularly distinctive between adults and pediatrics. This is a vital alarm in choosing the best option of clinical management including treatment for brain tumor patients in different age of onset. Herein, we describe the most important mutations occur in pediatrics glioma by providing a brief discussion on the differences of mutational profile in adults.

Isocitrate dehydrogenase (*IDH*) is one of the most important genes that its isoform 1 has found to be mutated in around 70% of low grade glioma and secondary GBM (Watanabe et al. 2009). *IDH1* gene mutations are more important in adult glial tumors and its frequency is considerably lower in pediatrics tumors (0–17%). The main function of IDH is oxidative decarboxylation of isocitrate to generate α-ketoglutarate and CO_2 in the context of the citric acid cycle (Corpas et al. 1999). In addition to brain tumors, *IDH1/2* mutations were also found in approximately 20% of acute myeloid leukemia (AML) patients with normal cytogenetic finding (Wang et al. 2015a, b). *IDH1/2* mutations are less frequent in childhood glial tumors (<14 years old) and the overall frequency of *IDH1* alterations is considerably higher than *IDH2* (Hartmann et al. 2009). The c.395G>A (R132H) missense variant is the most common mutation of *IDH1* gene while the most common mutation of *IDH2* gene in adult brain tumor patients is found to be c.516G>C (R172S) (Moriya et al. 2014). It is worth to note that, those missense mutations lead to an alternative production of *D*-2-hydroxyglutarate (*D*-2HG) which itself induces degradation of hypoxia-inducible factor 1α (HIF-1 α) and then changes the DNA and histones methylation pattern toward activation of some oncogenes and inactivation of some tumor suppressor genes (Molenaar et al. 2014). Although it is less frequent, when the *IDH1/2* mutations are detected in pediatrics, so due to the enhanced probability of tumor progression toward higher grades, an on time monitoring is required to be performed (Jaeckle et al. 2011). In contrast, detection of those mutations in adults indicates good prognosis and an influential chemotherapy.

The other key gene frequently mutated in brain tumors is *BRAF* which is a serine/threonine-protein kinase and is known as v-Raf murine sarcoma viral oncogene homolog B. The BRAF has essential roles in regulation of cell growth and differentiation through modulating the MAP kinase/ERKs signaling pathway (Daum et al. 1994). As it was previously noted in Ras/Raf/MAPK pathway in above section, c.1799T>A (V600E) is the most frequent mutation of *BRAF* gene (40% of glioma) which leads to activation of MEK without prior phosphorylation of RAS (Schindler et al. 2011). MEK activation could be alternatively occurs through loss of regulatory domain of *BRAF* gene in *BRAF* fusion genes as well as *BRAF-KIAA1549* as the most prevalent type of fusion. The *BRAF-KIAA1549* fusion has been found in diffuse fibrillary astrocytomas, pilomyxoid astrocytomas and

Pilocytic astrocytomas (PAs), as well (Faulkner et al. 2015). Although the rate of *BRAF* fusion and mutations is also much less in pediatrics glioma (<2%), the detection of c.1799T>A mutation in affected children could be a suitable molecular marker for grade I of astrocytoma to be differentiated from higher grades (Schiffman et al. 2010).

P53 is another most frequent mutated gene especially in adult brain tumors (40–80%) (Kim et al. 2010). It was shown that *p53* mutations are usually associated with aberrantly expressed *ATRX* gene and *IDH1* gene mutations (Kannan et al. 2012). GBM patients with ATRX-/p53-/IDH+ protein profile had the longest survival time amongst other possible protein combinations (Kannan et al. 2012; Chaurasia et al. 2016). The general prevalence of p53 mutations among the affected children with low grade glioma is lower than adults (around 5%) while its frequency significantly increases in higher grades (Pollack et al. 2001; Mascelli et al. 2016). It was demonstrated that *p53* mutations could lead to overexpression of *MGMT* gene as a part of DNA repair machinery of cell and thereby increase the chance of resistance to chemotherapy and therefore decrease the possible event-free survival (Wang et al. 2014; Zhang et al. 2015; Mascelli et al. 2016).

CDKN2A/p16 is the other tumor suppressor gene with major responsibility at G1 checkpoint of cell cycle which is transcribed from both p16INK4a and p14ARF loci (Ivanchuk et al. 2001). *CDKN2A* is usually found to have a deletion particularly in higher grades of brain tumors in both adults and pediatrics. It was frequently reported that *CDKN2A* deletion is associated with poor patient prognosis and survival which may be independently considered as a molecular marker of prognosis in either low or high grades of gliomas (Reis et al. 2015; Sibin et al. 2015). Of note, *CDKN2A* deletion is almost accompanied with V600E *BRAF* mutation which was found to be associated with higher probability of tumor progression and lower patient's survival in pediatrics glioma (Mistry et al. 2015). Directed mutagenesis on neural progenitor cells has demonstrated that V600E *BRAF* mutation was not sufficient for initiating astrocytoma. Therefore, as a complementary stage, further *p16* deletion made neural progenitors more aggressive (Horbinski et al. 2012). Interestingly, regarding the combination therapy targeting, both *p16* and *BRAF* alterations have significantly increased the survival and prognosis of affected patients (Huillard et al. 2012). *H3F3A* encoding the Histone H3.3, one of the basic nuclear proteins which is involved in nucleosome conformation of eukaryote chromatin structure (Wells et al. 1987). *H3F3A* mutation (p.Lys27Met) was initially found in 78% of pediatric Diffuse Intrinsic Pontine Glioma (DIPG) (Wu et al. 2012). G34R and K27M are the most frequent Histone H3.3 mutations which have been detected in hemispheric high-grade and diffuse low grade glioma, respectively (Orillac et al. 2016; Ryall et al. 2016). Unfortunately, the available investigations defining the survival of patients harboring Histone H3.3 alterations are limited to some case report studies. However, it was demonstrated that carrier patients may have progressive clinical presentations especially in those with diffuse gliomas and the disease course would be actually more aggressive than patients with only BRAF mutations (Gessi et al. 2015, 2016).

The role of *MYB/MYBL1* (*MYB proto-oncogene like 1*) amplification was primarily detected in 60% of pediatric low-grade gliomas patients which was replicated in two further studies among 23–28% of the same patients (Tatevossian et al. 2010; Ramkissoon et al. 2013; Zhang et al. 2013). MYB is belonged to one of the regulatory protein family having tight control on cell proliferation and differentiation through transcription. All of this family members have a C-terminal negative regulatory domain that truncation of it can cause constitutive activation of MYB proto-oncogene (Biedenkapp et al. 1988; Oh et al. 1999; Zhang et al. 2013). Furthermore, truncation of regulatory domain usually occurs as the byproduct of several types of gene rearrangements (Bandopadhayay et al. 2014). MYB–QKI is one of the well known of those rearrangements which can lead to carcinogenesis not only by activation of *MYB* oncogene activity but also results in loss of tumor suppressor function of *QKI* gene and enhancer induced ectopic expression of fusion protein (Jain et al. 2017).

The same other protocongene, *MYCN*, is a member of MYC family which by encoding a basic helix-loop-helix (bHLH) has critical role in expression of genes involved in brain growth and development. The *MYCN* gene amplification has been replicated in some Neuroblastoma patients and is found to be associated with poor prognosis and shorter survival (Tanaka et al. 2004; Wang et al. 2013; Niu et al. 2015). In an assay on different grades of brain tumor we have demonstrated *MYCN* gene amplification which was concordant with corresponding protein expression. However, disease free survival was found to be associated with protein expression but not gene amplification which may indicate the importance of protein analysis in analyzing the impact of gene aberrations on determining prognosis and survival of affected individuals (Estiar et al. 2017).

Fibroblast growth factor receptor 1 (FGFR1) is specific for fibroblast growth factor family and belonged to cell surface membrane receptors group which function as a receptor tyrosine kinase (Itoh et al. 1990). Similar to other proto-oncogene genes, it can be activated through several mechanisms including point mutation, gene rearrangements and gene amplification (Katoh and Nakagama 2014). Although the general frequency of *FGFR1* gene aberrations has shown to be low in brain tumors, its mutations and fusion have been described in 3% of low grade glioma (Singh et al. 2012; Di Stefano et al. 2015; Lasorella et al. 2017). The final result of either mutation or fusion of *FGFR1* would be over activity of MAPK pathway and subsequent uncontrolled cell proliferation and transformation (Jones et al. 2013). The most common type of gene fusion of *FGFR1* gene have been reported to be included transforming acidic coiled-coil 1 (*TACC1*) *and TACC3* genes which has been frequently reported in other cancers as well (Stransky et al. 2014). Transforming acidic coiled-coil protein family play role in regulation of cell cycle through interaction with centrosome and microtubule which by binding to histone acetyltransferase (HAT) may also has pivotal roles in epigenetics (Gangisetty et al. 2004). In addition, the N546K and K656E are the most important hot spots mutations of *FGFR1* which mainly found in pilocytic astrocytoma patients (Jones et al. 2013; Zhang et al. 2013; Becker et al. 2015). The importance

of genotyping *FGFR1* gene aberrations may lead to the worse prognosis and significantly decreased overall survival duration in low grade astrocytoma patients (Becker et al. 2015).

Mutated ataxia telangiectasia (A-T) (*ATM*) gene is one of the major tumor suppressor genes and cell cycle checkpoint molecule which has been primarily found as a cancer associated genes owing to the high cancer probability of A-T patients (Savitsky et al. 1995). It is also involved in double strand DNA break and thereby has crucial roles in keeping genomic status stable (Shiloh 2003). Three hit hypothesis was reported for the first time in an astrocytoma patient who was carrier of D1853N polymorphism as a first hit, followed by IVS 38-63T –> A and IVS 38-30 A –> G as the second and third hits, respectively (Mehdipour et al. 2008). Expression analysis of *Rb, p53* and *ATM* genes in various grades of brain tumor tissue samples have revealed that mRNA levels have significantly decreased in higher grades (Kheirollahi et al. 2011a, b). Of note, ATM can regulates the activity of p53 through decreasing p53 degradation by MDM2 phosphorylation and p53 in turn modulates the Rb function via indirect inhibiting transcription of CDK4/6 kinases as Rb suppressor (Fig. 13.3) (Nasser and Mehdipour 2017). We also described that 73% of brain tumor tissues have shown to be methylated for *ATM* promoter which was in direct correlation with reduced protein expression (Mehdipour et al. 2015). Interestingly, the promoter of *MCPH1* gene was methylated in almost all of the tumor tissues, as well (Karami et al. 2015). MCPH1 functions in upstream of ATM, phosphorylates it to recruit the repair system on double strand DNA break (DSB) site. Simultaneous methylation and decreased protein expression of ATM and MCPH1 which has been also demonstrated in breast cancer samples may indicate the common mechanism of methylation within the DSB system (Bhattacharya et al. 2013).

Ki-67 or MKI67 is actually a nuclear protein which is an essential factor for cell proliferation and expression of rRNA genes. Limited expression of this protein in only proliferating cells is a good reason to name it as a proliferation marker in various types of cancer (Bullwinkel et al. 2006). It was described that Ki-67 may control cell proliferation through regulation of histone modification and thereby prevention of heterochromatin complex formation (Sobecki et al. 2016). In the initial assessment of Ki-67 in brain tumor studies, it was revealed that combinational application of Ki-67 and 53 labeling index can help to differentiate different grades of brain tumors and prognosis of affected patients (Tihan et al. 2000; Shaffrey et al. 2005; Thotakura et al. 2014). It was also demonstrated that high Ki-67 expression was associated with lymphatic metastasis of glioma cells (Miao et al. 2015).

Telomerase activity as a hallmark of cancer cell proliferation has been extensively studied in brain tumors in comparison with telomere length. We demonstrated that in spite of normal telomere length within brain tissue, it had larger repeating expansion in lower grades of tumor compared to higher grades which was concordant with telomerase activity as a tumor evolution (Kheirollahi et al. 2011a, b, 2013; Mehdipour et al. 2011).

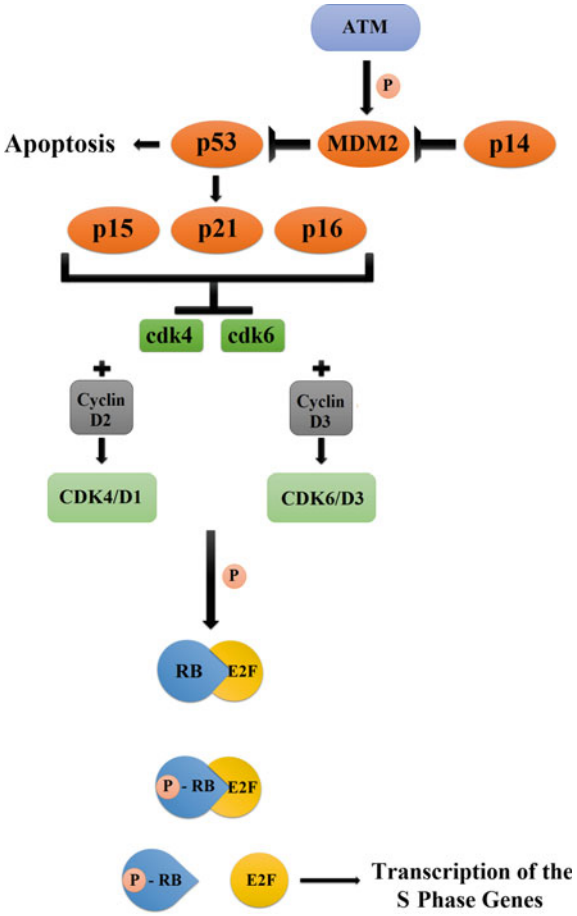

Fig. 13.3 An interactive scheme between Rb and p53 pathways. This figure is adopted from Nasser and Mehdipour (2017)

13.3.1 Single Nucleotide Polymorphisms and Brain Pathology

The most important single nucleotide polymorphisms (SNPs) which have been found to be significantly associated with brain tumors were found within X-ray repair cross-complementation group (*XRCC*), excision repair cross-complementation group (*ERCCs*), *MGMT* and Poly (ADP-ribose) polymerase (*PARP*) (Table 13.2) (Liu et al. 2017).

Table 13.2 The most important SNPs which have been found in glioma

Gene	Polymorphism	Significant population
XRCC1	rs1799782 rs25487 rs25489	Caucasian Caucasian Caucasian
XRCC2	rs3218536	Asian
XRCC3	rs861539	Caucasian
XRCC4	rs3734091 rs1805377	Asian Asian
ERCC1	rs3212986 rs11615	Caucasian Asian
ERCC2	rs13181	Caucasian
ERCC4	rs1800067	Asian
ERCC5	rs17655	Caucasian
MGMT	rs2308321 rs12917	Caucasian
PARP1	rs1136410	Caucasian

13.4 Common Methylation Aberrations

The most important epigenetic mechanism includes DNA methylation and histone modifications. Since the most important epigenetics processes is change in DNA methylation status, either within promoter or gene body. The aberrations of DNA methylation have been an attractive targets of brain tumor studies and will be described in this section in details. In the other hand, due to the specific enzymes complexes and methylation regulatory pathways, methylation would be a promising target of cancer treatment as well as brain tumors (Louis et al. 2007). Besides target of treatment, DNA methylation alterations can occur couple of years prior the initiation of tumor and therefore can be considered as an early biomarker for on time interventions.

Similar to other cancers, inactivating promoter methylation usually occurs in genes involved in regulation of cell cycle and proliferation such as tumor suppressor, DNA repair, cell cycle checkpoint and apoptosis. Different methylation pattern among various grades of glioma is also notable (Kuo et al. 2016; Moyon et al. 2017). Interestingly, it was found that *IDH1* and *IDH2* mutations were associated with specific promoter gene methylation throughout the genome called as glioma-CpG island methylator phenotype (G-CIMP) in 78 and 98% of cases, respectively (Noushmehr et al. 2010; Duncan et al. 2012; Turcan et al. 2012). This observation may be indicating a coordinate changes between genetic and epigenetic aberrations driving the tumor evolution. It is a very important idea since the presence of a few brain tumor cells with different epigenetic profile can cause resistance to chemo or radiotherapy and therefore predicting the patients' prognosis and survival (Mazor et al. 2016).

One of the most important genes methylated in brain tumors is *AGT* encoding O(6)-methylguanine DNA methyltransferase (MGMT) enzyme which has pivotal

role in repairing nitrosourea induced DNA alkylation. Promoter methylation status of *MGMT* gene has great impact on the treatment option of glioma patients. Patients harboring hypermethylated *MGMT* promoter are more sensitive to chemotherapeutic agent, temozolomide, whereas other patients are only treated by radiotherapy (Weller et al. 2014; Ansari et al. 2017). Of note, *MGMT* gene promoter methylation is also a determining factor in making decision on tumor surgery resection along by considering the size and location of tumor and age of patient (Park et al. 2013). Moreover, it was shown that *MGMT* gene promoter methylation is associated with good prognosis and longer survival of GBM patients (Montano et al. 2011).

Cell death 95 ligand (CD95L) is the other important gene which demonstrated that its promoter methylation status has a crucial role in response to APG101 chemotherapeutic agent (Merz et al. 2015).

In finding correlation between gene promoter methylation and survival of patients, *Estrogen receptor 1 (ESR1)*, Immunoglobulin super family member 4 (*IGSF4*) and Ras association domain family 1 isoform A (*RASSF1A*), promoter methylation was found to be associated with short survival of oligodendroglial tumors patients (Kuo et al. 2016).

13.4.1 Environmental Elements

Environmental elements in brain tumor progression can be divided into two main categories including micro- and macro-environmental factors. Micro-environment refers to the changes occurs within the niche, main location of tumor growth while macro-environmental factors is related to the external factors existing in the entire pre-, post- birth and geographical living territories of individuals.

One of the amazing microenvironmental factor which makes tumor growth and progression through epigenetic changes is found to be hypoxia. This hypothesis is rather premature in brain tumors and requires further complementary investigations. However, it was described that, hypoxia activates an interactive feedback circle including Hypoxia induced factor (HIF) complexes and mixed lineage leukemia 1 (MLL1) with histone methyltransferase activity and multiple epigenetic effects in GBM cells niche (Heddleston et al. 2012; Huang et al. 2016).

Among external or macro-environmental factors, one of the frequently assessed target is revealed to be long term exposure to heavy metals such as arsenic, nickel, lead, and cadmium. The general mechanism of brain tumorgenesis is as the result of exposure to the mentioned elements which cause generation of reactive oxygen species (ROS) and free radicals leading to altered methyltransferase enzymes activity and thereby methylation. Each of heavy metals have identified to cause epigenetic alterations in different genes (Caffo et al. 2014). In addition, *N*-nitrose compounds with unique target of DNA mutation known as DNA alkylation is notable as well. They are present in tobacco smoke and dietary compound and their effects on DNA are repaired by contribution of MGMT enzyme and *N*-alkyladenine-DNA glycosylase (AAG)-initiated base excision repair (Fahrer et al.

2015). These agents are debatable in two aspects of methylation, first impact on; (1) MGMT methylation and gene inactivation, and (2) genomic methylation consequences of *N*-nitrose compounds. MGMT loss of function leads to increased frequency of mutations especially if the cell is still before the second round of replication, since then double strand break (DSB) and mismatch repair (MMR) systems may end up the cell fate toward apoptosis (Christmann et al. 2011). Although there is a limited studies in brain tumor field, it was described that *N*-nitrose compounds can cause DNA hypomethylation through making DNA inaccessible to DNA methyltransferase enzymes (Tomita et al. 2010).

Endocrine-disrupting chemicals (EDCs) as well as Bisphenol A (BPA, found almost in plastics for bottles and containers) are amongst the other environmental exposures which have been under broad cancer investigations. It was established that prenatal exposure to BPA, caused enhanced expression of all DNA methyltransferases in children and following change (s) in methylation pattern of estrogen receptors (ERs; ERα and ERβ), estrogen-related receptor-γ genes, Nuclear Receptor Subfamily 3 Group C Member 1 (*Nr3c1)* and brain-derived neurotrophic factor (*Bdnf*). The results of such epigenetic changes will be appeared couple of years later by reflection of different brain dysfunction and disorders (Kundakovic et al. 2013, 2017). To our knowledge, there is no study indicating the correlation among BPA, DNA methylation and risk of brain tumor. Duan and colleagues demonstrated that the urinary level of BPA was associated with risk of meningioma (Duan et al. 2013). Further studies are required to clarify the exact correlation between BPA exposure and risk of brain tumors especially for higher grades as well.

Among infectious pathogens which were defined to be associated with head and neck cancer, Epstein-Barr virus (EBV) infection has shown to induce methylation and loss of function of *RASSF1A*, *TSCL1, EDNRB*, and death-associated protein kinas and *p16* genes during initial phases of nasopharyngeal carcinoma (Alibek et al. 2013). Methylation profile including *CDH18*, zinc finger factor 33 (*ZNF733*), *ALDH1A2*, *OSR2*, *GATA4*, *GRIA4*, *IRX4* and *CTNND2* genes has been added to human papilloma virus (HPV) induced epigenomic changes in replicated studies on head and neck squamous cell carcinomas and oropharyngeal cancer (Wilson et al. 2013; Sano et al. 2016; Degli Esposti et al. 2017). Gubanova and colleagues demonstrated that HPV positive cells with downregulated suppressor with morphogenetic effect on genitalia 1 (*SMG-1*) had more radio sensitivity compared to HPV negative cells (Gubanova et al. 2012).

13.4.2 Dietary Factors

The major effect of dietary elements on human genomic and epigenomic is modulation of several micro RNAs (miRNAs) throughout body which by their imbalance can have adverse effect on risk of different cancers (Ross et al. 2011). The most important dietary components with major contribution in cancer through

influencing the DNA methylation include folate, choline, retinoic acid, vitamin D, vitamin E, selenium, omega-3 fatty acids, butyrate, phytochemicals and resveratrol (Dauncey 2013). Most of the epigenetic effects of dietary factors have been assessed on brain function and development. As an instance, hypermethylation effects of α linoleic acid on inflammatory *COX 1/2* and *Mecp2* genes have demonstrated to be associated with decreased risk of ovarian cancer and normal brain development (Eilati et al. 2013; He et al. 2014). However, novel approaches have been recently great attentions to give high ketogenic diet as well as diet enriched by *β*-hydroxybutyrate to the glioma patients. The power of this strategy includes providing more energy to healthy cells and to decrease the risk of metastatic and angiogenic processes of tumor cells (Woolf et al. 2016; Puchalska et al. 2017).

13.4.3 Psychological Factors

Stress with indirect effect of exercise or any other emotionally effective factor as well as depression and social environment are the main psychological factors that may have critical impact on the cancer pathophysiology through different mechanisms as well as increase in inflammatory signals including IL-6, IL-1β, IL-10, TNF-α, IL-1β, IL-4 and interferon-γ (Marsland et al. 2017). It was found that anxiety causes significant difference in global genomic hypermethylation and expression level of *EZH2, IL-6* and *DNMT1/3A* genes between anxious and normal individuals. In addition stress and social defeat has shown to be associated with promoter methylation of Glucocorticoid Receptor (GR) and BDNF genes (Vialou et al. 2013; Archer 2016; Nestler 2016).

Glucocorticoid receptor has a crucial role in controlling of cellular metabolic status and proliferation and therefore affect on pathophysiology of tumorgenesis by transcriptional modulation of several target genes (Nakatani et al. 2016). *GR* gene has been found to be methylated in breast cancer and to the best of our knowledge its status remained to be elucidated in different types of brain tumors (Nesset et al. 2014). Since BDNF molecule can pass through blood brain barrier (BBB), concordant methylation pattern of its promoter in brain tissue and peripheral blood may be a reliable non-invasive biomarker of brain tumor progression if the correlation between BDNF methylation and brain pathologies will be clarified (Kang et al. 2015). Further studies required to find the correlation between aforementioned epigenetic changes in response to stress and risk of brain tumors.

13.5 Cell Free DNA Biomarkers

In recent decade's non-invasive or semi-non invasive approaches have received great attentions in screening, diagnosis and therapeutic follow up of various types of diseases particularly human cancers. The best non-invasive human sources include saliva, urine and stool. Peripheral blood and cerebral spinal fluid (CSF) are considered as semi-non invasive sampling strategy. Non-invasive approaches are more important in far access and large tumor tissues as well as large brain tumors that makes biopsy so difficult with sometimes inevitable and irreparable side effects. Cell free DNA (cfDNA) and circulating tumor cells (CTCs) which are so called as liquid biopsy are the most amazing non-invasive tools in detection of tumor tissue genetic aberrations before and after the treatment schedules. It is of note that liquid biopsy would be a better representative of overall genetic profile of a heterogeneous tumor than a tissue biopsy which can be removed from every part of tumor with different pathologic and genetic signatures (Gerlinger et al. 2012). The first trial of implication of cfDNA in nervous system disease was diagnosis in Herpes-simplex-virus (HSV) DNA in CSF from infected patients (Rowley et al. 1990).

In spite of remarkable application of cfDNA in management of brain tumor patients and their families, scarcity of cfDNA in serum is a major limitation which would be negligible beyond high throughput sequencing technologies (Wang et al. 2015a, b).

Since cfDNA is present in serum of healthy individuals in lower concentrations, it could be a valuable source for comparison of cfDNA changes such as quantity, copy number, microsatellite repeats, new mutations and methylation changes for either diagnosis or treatment responses (Wang et al. 2004; Schwarzenbach et al. 2011). One of the initial cfDNA studies in the scope of brain tumors has demonstrated that the promoter methylation of *MGMT, p16, DAPK*, and *RASSF1A* genes was similar in both cfDNA and GBM tissues. Moreover, it was found that MGMT promoter methylation of cfDNA could be a well predictive marker of response to Carmustine, a major chemotherapy agent used in treatment of brain tumors (Balana et al. 2003). Liu and colleagues also have found concordant promoter hypermethylation of *MGMT, p16INK4a, TIMP-3*, and *THBS1* genes in tumor tissues of glioma patients and their CSF and blood cfDNA (Liu et al. 2010).

There are two main debates in using of cfDNA in diagnosis and follow up of brain tumor patients including mtDNA mutations and sources of non-invasive detection. Although mitochondrial DNA (mtDNA) mutations could be detected in cfDNA, non-overlapping results between tumor tissue and CSF needs further assessments to the reason will be elucidated. Owing to low permeability of blood brain barrier to cfDNA and the importance of the sensitivity of used molecular technique, it is thought that CSF maybe a better source of cfDNA detection than serum and urine (Bettegowda et al. 2014). Pan W et al. have described that although

the mean of cfDNA concentration is higher in plasma, CSF is a better choice for mutation analysis especially in lower grades of CNS tumors (Pan et al. 2015). Further studies are warranted to define the best source of non-invasive genetic analysis of brain tumors.

13.6 Circulating Tumor Cells (CTCs)

Detection of CTCs using glial fibrillary acidic protein (GFAP) specific antibody in blood samples of GBM patients has opened a novel window toward early diagnosis and treatment. Given that it was a coincidence between appearance of CTCs in circulation and EGFR amplification, it was suggested that the CTCs release into the peripheral blood may herald of GBM metastasis and therefore can be evaluated as a promising early biomarker of brain tumor progression (Muller et al. 2014). This suggestion was based on previous study which has shown that a small subset of CTCs called circulating tumor stem cells with major stem cells characteristics can pass through vascular barriers and drives cancer metastasis (Li et al. 2014). Moreover, dramatic changes in CTCs level before and after radiotherapy maybe a good indicator of possible tumor recurrence (Macarthur et al. 2014). The other recurrence related promising application of peripheral CTCs detection in brain tumor patients is in pseudoprogression therein chemotherapy and radiotherapy induce remarkable local brain inflammation which mimics tumor recurrence or progression (Brandsma et al. 2008).

One of the most important challenges in using of CTCs in management of brain tumor patients is the absence of epithelial cell adhesion molecule (EpCAM) surface marker which is commonly used in trapping CTCs (de Wit et al. 2015). Immune targeting of glial fibrillary acidic protein, CTCs detection based on telomerase activity and co-targeting of SOX2, Tubulin β-3, EGFR, A2B5, c-MET as the specific glioma markers are the main strategies in trapping circulatory glioma cells (Macarthur et al. 2014; Muller et al. 2014; Sullivan et al. 2014). Although a direct correlation between CTCs quantity and poor prognosis and short survival of patients has been described in most of other cancers, there is a limited data investigating correlation between CTCs detection and survival and prognosis of brain tumor patients warranting more studies (Sullivan et al. 2014; Fina et al. 2017; Kroigard et al. 2017).

13.7 Novel Horizons in Brain Tumor Treatment

According to the standard treatment strategy of brain tumor patients, chemotherapy using temozolomide followed by radiotherapy and maintenance dose of temozolomide for at least 6 months has increased the overall survival up to the maximum of 2 years (Bush et al. 2017). It was demonstrated that one of the main reason

of treatment failure in brain tumor patients may be due to the tumor heterogeneity and obscure transformation of tumor cells from one pattern to another one with different genetic and epigenetic profiles (Szopa et al. 2017). The other obstacle against drugs access into the brain tumor tissue is the selectivity characteristics of BBB (Trippier 2016). Successful treatment of a brain tumor case needs urgent genetic and epigenetic personalized profiling and implication of novel tools as well as nanotechnology to overcome on aforementioned barriers. As mentioned above, the efficacy of all the EGFR inhibitors has been improved when only the patients with *EGFR* amplification were selected to be undergone adjuvant therapy (Wachsberger et al. 2013).

Among the best novel approaches, immunotherapy maybe the most hopeful strategy which has been frequently trialed in management of brain tumor patients (Weathers et al. 2015). Active immunotherapy with different types of vaccine had shown to have the best results in their initial trials. HLA-restricted Wilms tumor 1 (WT1) 9-mer peptide vaccine is one of the successful peptide vaccine in treatment of WT1/HLA-A*2402-positive recurrent GBM patients with 33.3% rate of progression free survival (Izumoto et al. 2008). However, the results of DNA vaccines were associated with longer survival time in GBM patients and investigations are still continued to be absolutely approved (Hdeib et al. 2015). To get the best achievement in immunotherapy, considering a combination of various approaches and strategies may pave the way towards achieving a reliable opportunity of longer survival time and less side effects for patients (Farber et al. 2017).

13.7.1 Implication of Psychotherapy in Management of Brain Tumors

In all of the cancer types, disease progression and arising aching, annoying symptoms, signs of anxiety and stress are warning events for cancer patients to be worry about their future and how will their disease will be end up. Owing to late onset of clinical signs in brain tumor patients, almost when the surgery would not be a helpful decision, anxiety, hallucination, psychosis and mood deficits are the major psychological side effects which can affect the outcome of treatment. In addition, arising some cognitive and neurological insufficiencies can increase the psychological stress in spite of overall disease improvement (Bergo et al. 2016). Several strategies have been implicated to improve the quality of life of various types of brain tumor patients including antidepressant drugs, interpersonal therapy, exercise plans, mindfulness-based therapy and even severe psychological treatment options (Boele et al. 2015). In children affected with brain tumors, play based procedures during radiotherapy not only decreased the need for sedation and

avoiding its inevitable side effects but also it had a great effect on general cost of therapy (Grissom et al. 2016).

Antidepressant drugs such as tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitor (SSRI) are commonly prescribed for cancer patients to treat the depression especially at end stages of disease. However, the use of antidepressant drugs in brain tumor patients have shown to be effective in progress of treatment, as well. It was shown that fluxetin, a SSRI, caused decrease in glioma cell growth and enhanced tumor cells death through induction of Ca^{2+} release (Liu et al. 2015). This finding could be in line with other reports indicating that long term using of TCAs and SSRI is significantly associated with decreased risk of glioma (Pottegard et al. 2016).

Mindfulness-based therapy is the other approved psychotherapy which has been extensively used in management of psychological disorders associated with various diseases as well as cancers. In a simple and brief description, mindfulness strategies insists on drifting away all of annoying and negative thought and enhancing mind power towards all positive corners of life. There are many studies indicating the effective role of mindful therapy in improving the general physical and mental health of breast cancer women (Zhang et al. 2016a, b). Mindfulness-based stress reduction (MBSR) is the most prevalent type of mindfulness training strategy used in cancer patients which is usually followed by another type called as mindfulness-based cognitive therapy (MBCT) (Williams et al. 2008). Foley and colleagues used MBCT to alleviate the anxiety and depression symptoms in different cancer patients and demonstrated significant improvement in quality of life of affected patients (Foley et al. 2010). To our knowledge, there is no study to investigate the effect of MBCT on cognitive status of brain tumor patients and future assays are required to clarify the role of MBCT and other types of mindfulness practice in quality of life of those patients.

Exercise plans may have the most psychotherapy strategy used in management of brain tumor patients' neurocognitive symptoms while have shown to be the successful element of treatment plan among colon cancer patients (Armstrong and Gilbert 2008; Rogers et al. 2008; Day et al. 2016). Choice of exercise plan should be defined according to the grade of disease and general status of brain tumor patients to be the most beneficial (Cormie et al. 2015). Although it has been reported that the physical activity can control the oxidative stress pathways through increasing the level of natural antioxidants as well as Glutathione, further studies are warranted to clarify the detailed mechanism of action (Hall et al. 2013).

13.8 Protein Expression as a Directive Paradigm

Different alterations in Molecular Genetic, Molecular Pathophysiology, and impact of environmental factors have been discussed in brain tumors. Besides, we had a brief look at the cell free DNA biomarkers, and CTCs. Furthermore, we referred to

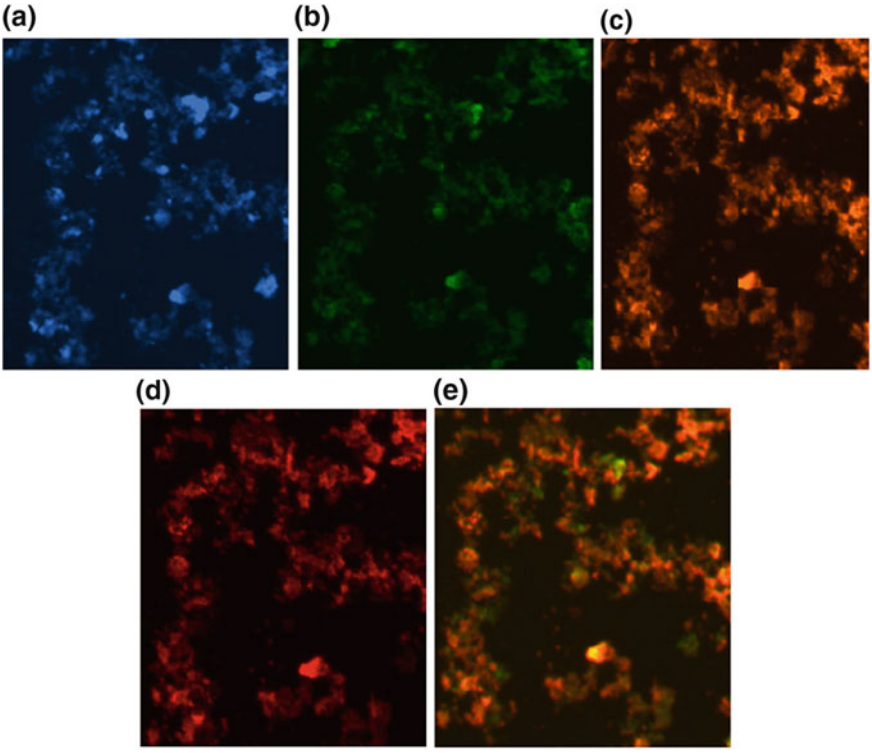

Fig. 13.4 Protein expression status of Ki-67, P14 and P63 in Meningioma. **a** Tumor cells with dapi; **b** same cells conjugated with Ki-67; **c** same cells reflective of heterogenic of P14 protein; **d** same tumor cells indicative of protein expression of P63 with a heterogenic pattern; and **e** co-expression of Ki-67/P14/P63 with a harmonic interaction in majority of cells tumor cells were conjugated with: FITC *green*; Rpe *Orange*; Pe-cy5 *Texas red*. Magnification: ×100. This image is adopted from P. Mehdipour's archive

therapeutic aspects of brain tumors by emphasizing on immunotherapy, complementary, multi-approaches strategies and psychological aspects which may be useful for the personalized strategy in the patients with brain tumors. To achieve such goal, the strategic program is required in which the end point cellular production play a crucial role. So, by focusing on protein expression, the heterogenic pattern of such territory would lead to plan for the most reliable clinical managements including the most influential, target based and personalized approaches. In this regard, different triangle profiles of protein expression to highlight the cellular heterogeneity in different types of brain tumors (Figs. 13.4, 13.5, 13.6, 13.7, 13.8, 13.9, 13.10, 13.11 and 13.12).

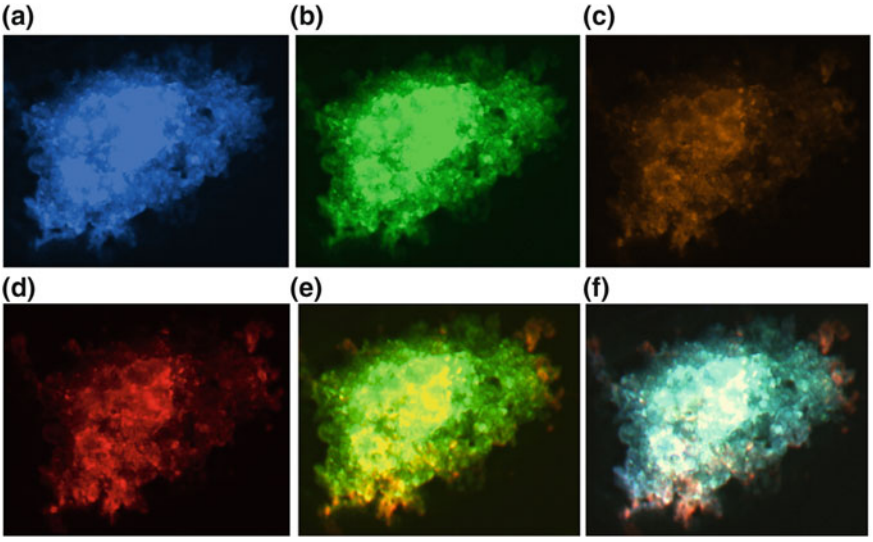

Fig. 13.5 Protein expression of Ki-67, P14 and P63 in GBM. **a** GBM cells with dapi; **b** same cells conjugated with FITC, reflecting high pattern of Ki-67 protein; **c** same cells with Rpe reflective of low- and lack of expression in p14; **d** same cells with Pe-cy5 and heterogenic pattern of P63 protein; **e** conjugation of Ki-67, P14 and P63 indicative of a remarkable and opposing interaction between Ki-67 and P63; **f** merged image of dapi/Ki-67/P14/P63. GBM: Glioblastoma multiform. Tumor cells were conjugated with: FITC *green*; Rpe *Orange*; Pe-cy5 *Texas red*. Magnification: ×100. This image is adopted from P. Mehdipour's archive

13.8.1 Protein Expression Status of Ki-67, P14 and P63 in Different Brain Tumors

13.8.1.1 Protein Expression of Profile Ki-67, P14 and P63 in Meningioma

As this profile shows, the Ki-67 is not reflective of a high proliferative status and only few cells have low protein expression, but the images of P14 and P63 are reflective of major cells with high expression accompanied by the lack of expressed cells (Fig. 13.4a–e).

13.8.1.2 Protein Expression Status of Ki-67, P14 and P63 in GBM

The heterogenic pattern of protein expression is observable in Ki-67 and P63. The highest expression is traceable in Ki-67 followed by P63 and low expression is reflected in P14. In spite of such diversity within the brain cells, proliferative factor played a crucial role in progression of this tumor (Fig. 13.5).

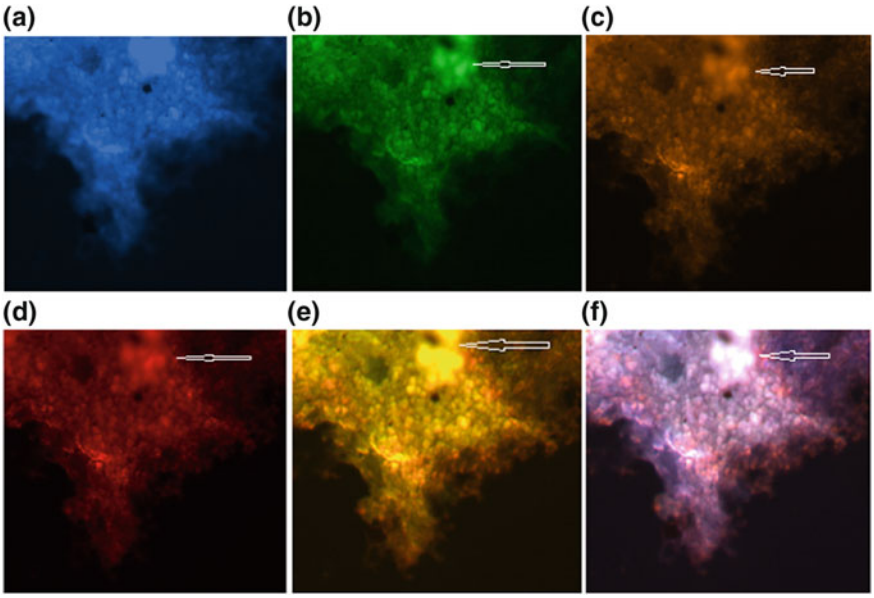

Fig. 13.6 Protein expression status of Ki-67, P14 and P63 in Oligodendroglioma. **a** Tumor cells with dapi; **b** same cells conjugated with FITC, reflecting a heterogenic pattern of Ki-67 protein; **c** same cells with Rpe reflective of low- and lack of expression in p14; **d** same cells with Pe-cy5 with a heterogenic pattern of P63 protein; **e** merged image of Ki-67, P14 and P63, indicative of an interaction between three proteins in the minor clone; **f** merged image of dapi/Ki-67/P14 and P63. Tumor cells were conjugated with: FITC *green*; Rpe *Orange*; Pe-cy5 *Texas red*. Magnification: ×100. This image is adopted from P. Mehdipour's archive

13.8.1.3 Protein Expression Status of Ki-67, P14 and P63 in Oligodendroglioma

Heterogenic expression pattern of Ki-67, P14, and P63 is observed and co-expression of these three proteins is revealed to be remarkable in the minor clone of cells. Dual co-expression-of Ki67/P14, Ki67/P63 and P14/P63 is notably diverse which is reflective of a personalized characteristics of cellular function (Fig. 13.6a–f).

13.8.1.4 Protein Expression of P53, ATM and P63 in Meningioma

These three genes with normal function have: (1) Critical roles; and (2) Beneficial impacts on the health status of cancer patients. However, as the following images show, these targets reveal to have a heterogenic pattern of protein expression in common with a harmonic co-expression as well (Fig. 13.7a–f).

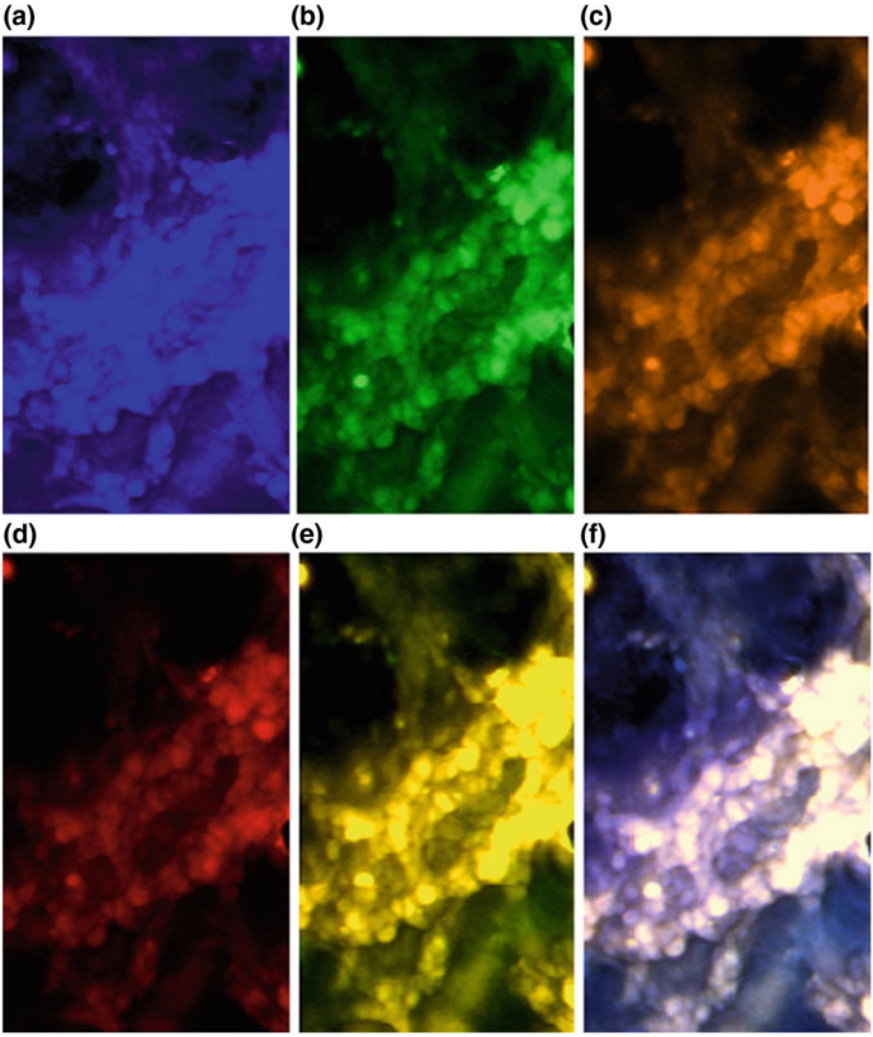

Fig. 13.7 Protein expression status of P53, ATM and P63 in Meningioma. **a** Tumor cells with dapi; **b** same cells reflects image of P53 protein conjugated with FITC; **c** same cells with Rpe reflective of low- and lack of expression in ATM; **d** same cells with Pe-cy5 with a heterogenic pattern of P63 protein; **e** merged image of P53, ATM and P63, indicative of an interaction between three proteins in minor clone of cells; **f** merged image of dapi/P53, ATM and P63. Tumor cells were conjugated with: FITC *green*; Rpe *Orange*; Pe-cy5 *Texas red*. Magnification: ×200. This image is adopted from P. Mehdipour's archive

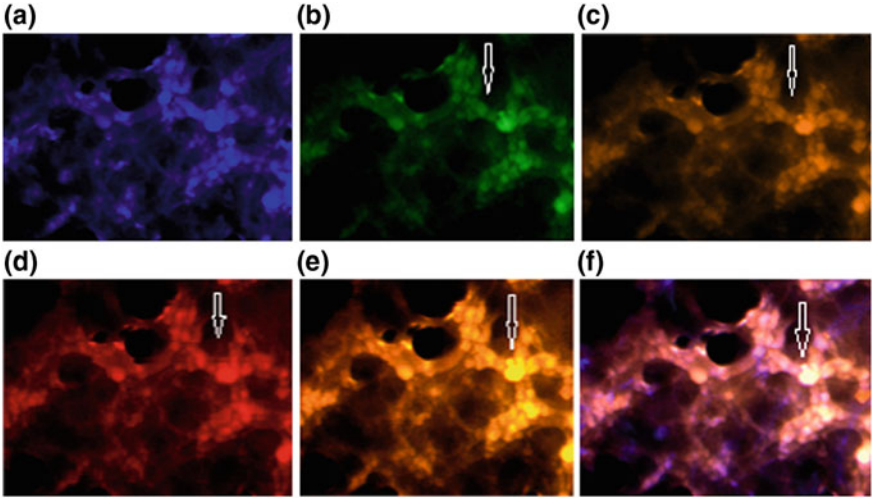

Fig. 13.8 Protein expression status of P53, ATM and P63 in Astrocytoma. **a** Tumor cells with dapi; **b** same cells conjugated with FITC, reflecting expression mode of P53 protein; **c** same cells with Rpe presenting expression mode of ATM protein; **d** same cells with Pe-cy5 with a heterogenic pattern of P63 protein; **e** merged image of P53, ATM and P63, indicative of an interaction between three proteins in the minor clones. **f** Merged image of dapi, P53, ATM and P63. All three genes presenting the heterogenic pattern of protein expression. Tumor cells were conjugated with: FITC *green*; Rpe *Orange*; Pe-cy5 *Texas red*. Magnification: ×200. This image is adopted from P. Mehdipour's archive

13.8.1.5 Protein Expression Status of P53, ATM and P63 in Astrocytoma

By considering an astrocytoma with poor prognosis, the provided images of pP53, ATM and P63 is expected to be low, however, a minor clone with normal/high expression is observable for all three proteins (Fig. 13.8a–f).

13.8.1.6 Protein Expression of P53, ATM and P63 in Oligodendroglioma

Lack of protein expression in both P53 and ATM and high level expression of P63 in few cells is observable. In P63 the expression pattern is heterogenic and only a minor clone of tumor cells reflect high expression which may be considered as a benefit for the patient (Fig. 13.9).

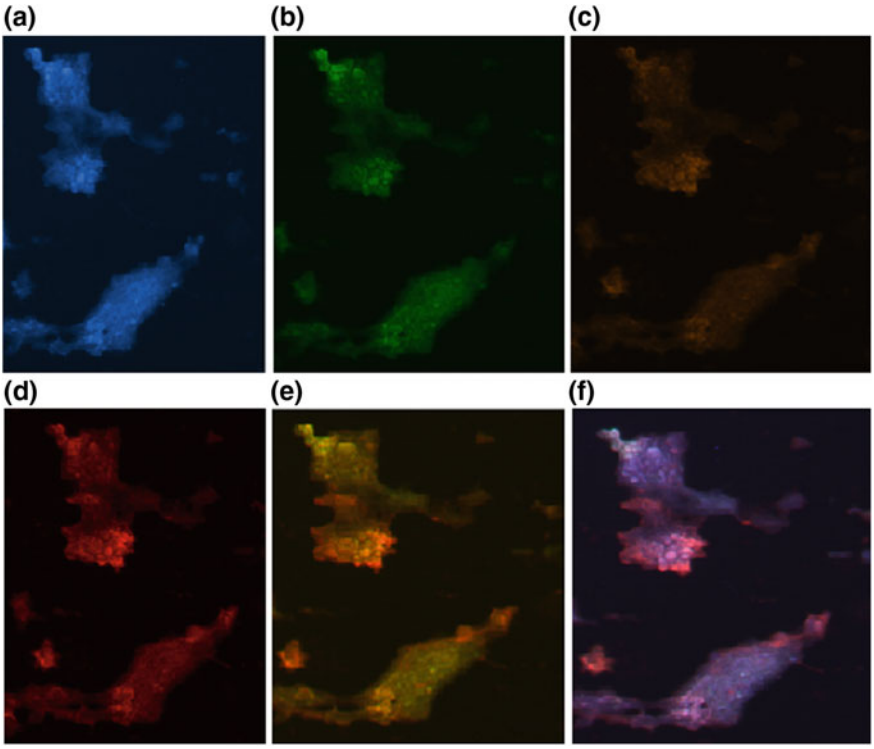

Fig. 13.9 Protein expression status of P53, ATM, and P63 in Oligodendroglioma. **a** Tumor cells with dapi; **b** same cells conjugated with FITC, reflecting lack of expression and very few cells with low expression in P53 protein; **c** same cells with Rpe presenting lack of expression of ATM protein; **d** same cells with Pe-cy5 with a heterogenic pattern of P63 protein; **e** merged image of P53, ATM and P63, is indicative of an interaction between P53 and P63 in the minor clone of cells. **f** Merged image of dapi, P53, ATM and P63. All three genes presenting the heterogenic pattern of protein expression. Tumor cells were conjugated with: FITC *green*; Rpe *Orange*; Pe-cy5 *Texas red*. Magnification: ×100. This image is adopted from P. Mehdipour's archive

13.8.1.7 Protein Expression of P53, ATM and P63 in Meningioma

A heterogenic pattern of protein expression is found in P53, ATM and P63, but the clones with high expression as the minor clones include more cells with P63, followed by ATM and P53 with a limited cell population (Fig. 13.10a–e). It worth's to emphasize on the key role of P63 in regulation of cell proliferation. As this figure shows, co-expression is found to be between ATM and P63 (Fig. 13.10d), and the isolated minor cells reflect low expression of P53 as a sole in Fig. 13.10e.

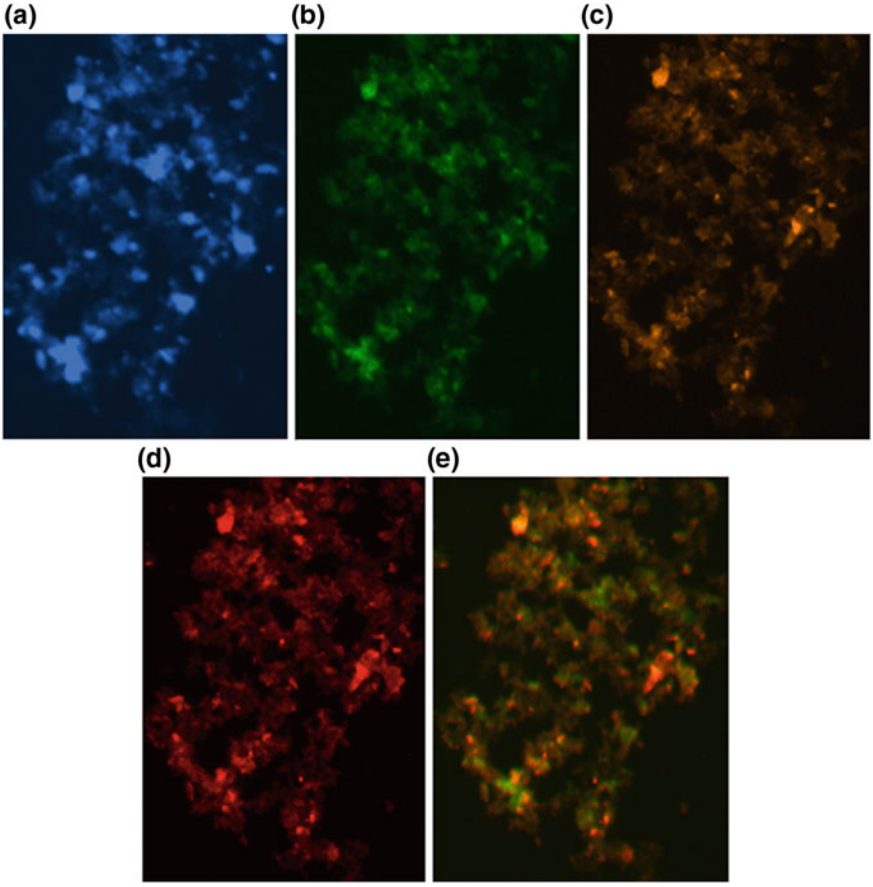

Fig. 13.10 Protein expression status of P53, ATM and P63 in glioblastoma multiform. **a** GBM cells with dapi; **b** same cells conjugated with FITC, reflecting lack and low expression in P53 protein; **c** same cells with Rpe reflect lack of expression in ATM; **d** same cells with Pe-cy5 with heterogenic pattern of P63 protein; **e** conjugation of P53, ATM and P63 indicative of a cooperation and opposing interaction between ATM and P63. *GBM* Glioblastoma multiform. Tumor cells were conjugated with: FITC *green*; Rpe *Orange*; Pe-cy5 *Texas red*. Magnification: ×100. This image is adopted from P. Mehdipour's archive

13.8.1.8 Protein Expression Status of Pcna, Cyclin D2, and Cyclin E in Meningioma

The heterogenic pattern of protein expression is observable in pcna, cyclin D2 and cyclin E, but the highest expression in pcna and cyclin D2 and the lowest expression is observed in cyclin E. In spite of such diversity within the brain cells, proliferative factor in cells with high protein expression played a crucial role in progression of this tumor (Fig. 13.11a–f).

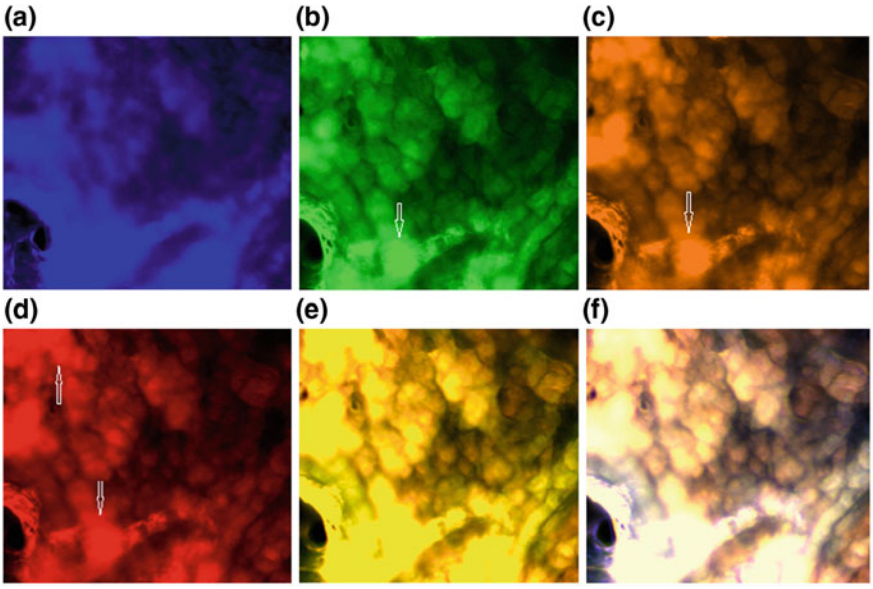

Fig. 13.11 Protein expression status of pcna, Cyclin D2, and Cyclin E in Meningioma. **a** Tumor cells with dapi; **b** same cells conjugated with FITC, reflecting clone of cells with high protein expression of cyclin E; **c** same cells with Rpe reflective of heterogenic expression of pcna; **d** same cells with Pe-cy5 also with a heterogenic pattern of Cyclin D2 protein, **e** merged image between Cyclin E/pcna/Cyclin D2; **f** merged image between dapi/Cyclin E/pcna/Cyclin D2 in Meningioma. Magnification: ×400. This image is adopted from P. Mehdipour's archive

13.8.1.9 Protein Expression Status of Pcna, Cyclin D2, and Cyclin E in Astrocytoma

High protein expression of cyclin E, and heterogenic pattern of protein expression for pcna and Cyclin D2 is traceable in this profile of an astrocytoma tumor (Fig. 13.12a–e). As this figure shows, the majority of cells had highest expression of cyclin E, and harmonic co-expression is due to an interaction between cyclin E, pcna and cyclin D2 (Fig. 13.12e).

As an overview, regardless to more invasiveness or non-malignant status of brain tumors, the mode of protein expression is a challenging item and it is not easy to generalize the manner of expression. But, it is rather logic to individualize the expression status, at least within the group of patients who have diagnostic characteristics in common.

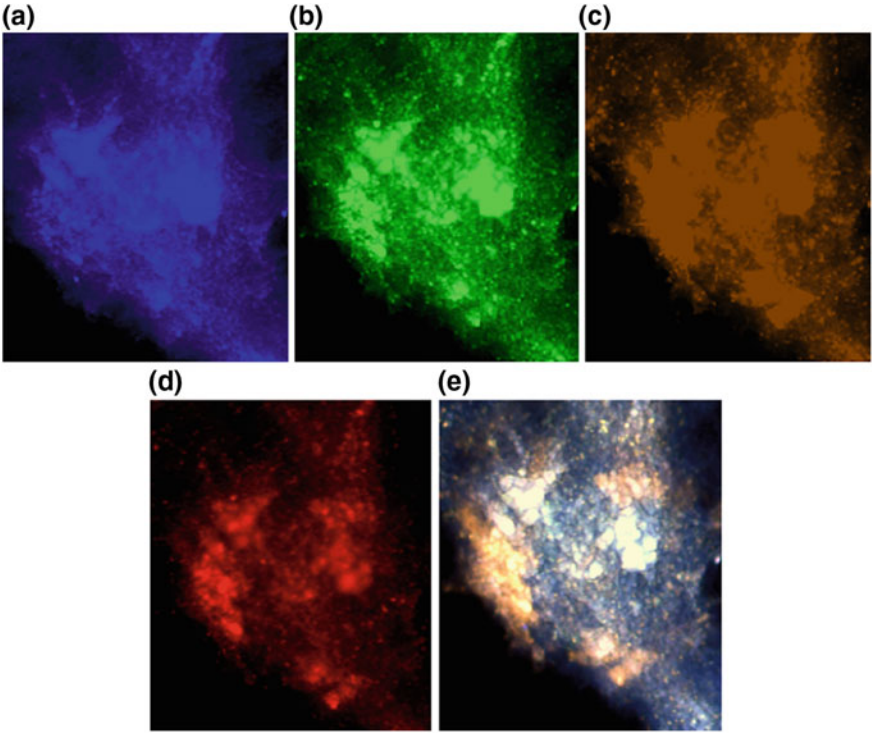

Fig. 13.12 Protein expression status of Pcna, Cyclin D2, and Cyclin E in astrocytoma. **a** Tumor cells with dapi; **b** same cells conjugated with FITC, reflecting high protein expression of protein cyclin E in majority of cells; **c** same cells with Rpe reflective of highest expression in pcna; **d** same cells with Pe-cy5 also with a heterogenic pattern of cyclin D2 protein, and **e** merged image between dapi/Cyclin E/pcna/Cyclin D2 in Meningioma. Magnification: ×100. This image is adopted from P.Mehdipour's archive

13.9 Conclusion

Taken together, although scrutinizing the molecular pathogenesis of brain tumors can have pivotal contribution to finding the best approaches in management of affected individuals, psychological supportive plans may play critical roles in control of disease progression. Based on the special structure of brain, the best psychotherapy program is required to be selected with caution. Besides, the health care providers are obligated to consider the most suitable personalized approach based on the pathological diagnoses including the tumor grade and the most reliable biomarkers. Furthermore, the impact of protein expression assay is an essential and an influential strategy through which the faint of all molecular alterations, could be traced at genomics and somatic levels. Through such strategy, most appropriate clinical managements including the therapeutic innovation would be provided.

13.10 Summary

High grades of brain tumors are among the cancer list which are associated with high mortality and morbidity rate. In spite of several performed genetic studies further investigations warranted to determine the precise molecular pathophysiology of brain tumors to find early detection biomarkers in order to increase survival time. Even though, treatment usually initiates in late stages of disease, selection of the most appropriate option of therapies based on molecular alterations found in peripheral blood and brain tissue of the patients may have dramatic effects on enhancing the survival and prognosis of patients. Moreover, incorporation of supportive psychological treatment options within the general plan not only would have their own beneficial effects on overall health status but also may enhance the efficacy of chemo and radio therapies. Owing to high rate of mortality and very low level of life expectancy in brain tumor patients, further studies are immediately needed in field of neurooncology.

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Chapter 14
Genetic, Hematologic and Psychological Aspects of Leukemia

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Abstract Current advances in diagnostic methods, our understanding of its molecular basis and therapeutics have made leukemia one of the most exciting and rapidly changing fields in oncology. Owing to the routine use of sensitive and innovative molecular and cytological techniques to diagnose or prognosticate leukemia and applying novel targeted agents for leukemia therapy, the study of leukemia has been always at a forefront of cancer research. Moreover, patients with leukemia in their "cancer trip" should adapt to a sequence of crises including crisis of hearing diagnosis, intensive therapy such as surgery, chemotherapy, radiotherapy or combination of them, and survivorship crisis. These crises may result in mental health problems including adjustment disorder, anxiety and mood disorders in vulnerable individuals. Psychiatric problems can also be caused directly by adverse effects of chemotherapy and radiotherapy. In this chapter expert clinicians and researchers have expressed in details the cytogenetic, molecular and hematological aspects of different types of leukemia and have attempted to briefly cover the major topics in the field of psycho-oncology. We hope this chapter will provide concise and up-to-date information of leukemia for clinicians and researchers interested in this field.

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Abbreviations

AML	Acute myelogenous leukemia
AML-MRC	AML with myelodysplasia-related changes
AP	Accelerated phase
APL	Acute promyelocytic leukemia
ARID5B	AT rich interactive domain 5B
BCR	Breakpoint cluster region gene
BET	Bromodomain and extra-terminal domain
BM	Bone marrow
BRAF	B-raf proto-oncogene
CALR	Calreticulin gene
CD	Cluster of differentiation
CDKN2A	Cyclin dependent kinase inhibitor 2A
CEBPA	CCAAT/enhancer binding protein alpha
CEBPE	CCAAT/enhancer binding protein epsilon
CLL	Chronic lymphocytic leukemia
CML	Chronic myeloid leukemia
CMML	Chronic myelomonocytic leukemia
CN-AML	Cytogenetics normal AML
CNL	Chronic neutrophilic leukemia
CR	Complete remission
DNMT3A	DNA (cytosine-5)-methyltransferase 3A
ET	Essential thrombocythemia
ETPs	Early T-precursor subtype
FAB	French–American–British
FISH	Fluorescence in situ hybridization
FLAG-IDA	Fludarabine, cytarabine, G-CSF and idarubicin
FLT3	Fms-like tyrosine kinase 3
FLT3-ITD	Fms-like tyrosine kinase 3-internal tandem duplication
G-CSF	Granulocyte-colony stimulating factor
HDACi	Histone deacetylase inhibitors
HLA	Human leukocyte antigen
HSP90	Heat shock protein 90
IDH-1	Isocitrate dehydrogenase 1
IDH-2	Isocitrate dehydrogenase 2
IGHV	Immunoglobulin heavy chain variable region
IKZF1	Ikaros family zinc finger protein 1

IPSS	International prognostic scoring system
IPSS-R	Revised IPSS score
IW CLL	International workshop on chronic lymphocytic leukemia
JAK2	Janus kinase 2
JAK3	Janus kinase 3
JMML	Juvenile myelomonocytic leukemia
KMT2A	Lysine methyltransferase 2A
KRAS	Kirsten ras oncogene homolog TP53:
MBL	Monoclonal B-cell lymphocytosis
MDS	Myelodysplastic syndrome
MLPA	Multiplex ligation-dependent probe amplification
MPNs	Myeloproliferative neoplasms
MRD	Minimum residual disease
NPM1	Nucleophosmin 1
NRAS	Neuroblastoma RAS viral (v-ras) oncogene homolog
OS	Overall survival
PBS	Peripheral blood smear
Ph	Philadelphia
PMF	Primary myelofibrosis
PV	Polycythemia vera
RAR-α	Retinoic acid receptor–α
RFS	Relapse-free survival
RQ-PCR	Real-time quantitative RT-*PCR*
RT-PCR	Reverse transcriptase PCR
RUNX1	Runt-related transcription factor 1
SLL	Small lymphocytic lymphoma
STAT3	Signal transducer and activator of transcription 3
TET2	Tet methylcytosine dioxygenase 2
TKIs	Tyrosine kinase inhibitors
TPO	Thrombopoietin
TRM	Treatment-related mortality
TYK2	Tyrosine kinase 2
WBC	White blood cells

14.1 Introduction to Leukemia

The hematologic system consists of blood and the tissues involved in hematopoiesis including bone marrow and reticuloendothelial system (RES). Existing in a fluid state, the blood has been distinguished from the other organs. Different types of blood cells and cell fragments are floating in a liquid phase called plasma. The cellular components are: Red Blood Cells (RBCs), White Blood Cells (WBCs) and platelets.

Leukemia is a malignant disorder of the blood causing too many white blood cells to be produced and affecting the marrow. Leukemia involves the production of abnormal white blood cells—the cells responsible for fighting against different infections. However, the abnormal cells in leukemia fail to function as normal white blood cells. The leukemia cells continue to grow and divide, eventually crowding out the normal blood cells. The ultimate result is the inability of immune system to fight infections, control bleeding, and transport oxygen.

14.1.1 What Is Leukemia?

Leukemias, which are cancers of the bone marrow and blood, are the most frequent childhood cancers. Depending on the type of leukemia they originate from a distinct blood cell. In normal conditions, the cells grow and divide to generate new cells while the body needs them. The cells have a certain life time after which they undergo different mechanisms of cell death and become replaced by new cells. Occasionally, this process fails to function properly. In a malignant condition, an uncontrolled cell division occurs while the body is not in demand, and the old cells refuse to die when they should.

Leukemia is a malignancy involving the blood-forming tissues of the marrow, spleen and lymph nodes. It is characterized by an out of control production of abnormal, immature blood cells. There are different types of childhood leukemia. The most frequent type is acute lymphoblastic leukemia (ALL) in which too many underdeveloped infection-fighting white blood cells or lymphocytes accumulate in the blood and marrow of the child. ALL is the most frequent type of leukemia in children and the most frequent form of all types of malignancies in children.

The marrow is a spongy tissue located inside many large bones of the body which is the main site where lymphocytes and other blood cells are generated. The bone marrow produces three types of blood cells: Red blood cells contain hemoglobin which carry oxygen and other materials to the tissues throughout the body; platelets that help to form blood clots following the bleeding events; and white blood cells which are responsible to fight against different foreign invaders.

Acute lymphoblastic leukemia is the most frequent malignancy of childhood, representing nearly one-third of all pediatric malignancies. In the U.S., approximately 2000 new cases of ALL are diagnosed annually. The prevalence peaks

between the 2 and 5 years old children, with a little higher rate in males. The annual incidence of ALL among children younger than 15 is 33 cases/million for whites, and 15 cases/million for blacks. The precise cause of most cases of leukemia remains unknown.

14.1.2 Risk Factor for Leukemia

Although the initial causes for development of leukemia in most patients is yet to be fully determined, many factors are associated with increased risk of developing the leukemia. These factors are listed following:

- Age
- Prior Chemotherapy
- Ethnicity/Gender
- Inherited Syndromes (such as Down syndrome)
- Ionizing Radiation
- Infection by certain viruses
- Cigarette smoking.

The relative effects of these and other risk factors in any given case of cancer is variable.

14.2 Types of Leukemia

Rather than the disease course and severity, leukemia is also classified according to the cell types affected in the malignancy. Leukemia involving myeloid cells is referred to as myelogenous leukemia. The cells from myeloid lineage are immature blood cells which definitely differentiate into granulocytes or monocytes. Leukemia involving lymphocytes is called lymphocytic leukemia. Taken together, based on the above mentioned facts there are four major types of leukemia:

14.2.1 Acute Myelogenous Leukemia (AML)

AML, the most frequent form of leukemia, is a malignancy affecting the myeloid lineage which can occur in children and adults. According to National Cancer Institute (NCI), about 21,000 new cases of AML are diagnosed annually in the United States.

14.2.2 Chronic Myelogenous Leukemia (CML)

CML is most likely to occur in adults, with 7000 rate of annual incidence.

14.2.3 Chronic Lymphocytic Leukemia (CLL)

CLL is most likely to affect people over the age of 55 and rarely occurs in children. About 15,000 new cases of CLL are diagnosed annually. A very rare subtype of CLL with microscopic view of cancerous lymphocytes is referred to as hairy cell leukemia.

14.2.4 Acute Lymphocytic Leukemia (ALL)

ALL occurs mostly in children with annual incidence of approximately 6000 cases.

14.3 Acute Myeloid Leukemia

14.3.1 Overview

Acute myelogenous leukemia (AML) is the consequence of a sequence of somatic mutations in primitive hematopoietic stem cells. Exposure to radiation, chronic exposure to high-doses of benzene, and chronic inhalation of tobacco smoke increase the rate of the disease. Obesity has been found to be an endogenous risk factor. A small but increasing proportion of cases develop AML following a history of lymphoma, a non-hematologic malignancy, or an autoimmune disorder, exposure to intensive chemotherapy, especially with alkylating agents or topoisomerase II inhibitors. The diagnosis of the myelogenous form of acute leukemia is confirmed particularly by identification of myeloperoxidase activity in blast cells or by identifying characteristic cluster of differentiation (CD) antigens on the blast cells (e.g., CD13, CD33). Because the leukemic stem cell is capable of imperfect differentiation and maturation, in addition to myeloblasts or promyelocytes the clone may contain cells that have the morphologic or immunophenotypic features of erythroblasts, megakaryocytes, monocytes, eosinophils, or rarely, basophils or mast cells. Certain cytogenetic alterations are more frequent in AML; these abnormalities include t(8;21), t(15;17), inversion 16 or t(16;16), trisomy 8, and deletions of all or part of chromosome 5 or 7. A translocation involving chromosome 17 at the site of the retinoic acid receptor-α (RAR-α) gene is uniquely associated with acute

promyelocytic leukemia. AML is usually treated with cytarabine and an anthracycline antibiotic, although other agents may be added or substituted in poor-prognosis, older, refractory, or relapsed patients. The exception to this approach is the treatment of acute promyelocytic leukemia with all-trans-retinoic acid, arsenic trioxide, and sometimes an anthracycline antibiotic. High-dose chemotherapy and either autologous stem cell infusion or allogeneic hematopoietic stem cell transplantation may be used in an effort to treat relapse or patients at high risk to relapse after chemotherapy treatment.

14.3.2 Definition

AML is a cancer of the myeloid lineage, characterized by the overproduction of abnormal WBCs that accumulate in the bone marrow and interfere with the normal hematopoiesis (Fig. 14.1). AML is the most frequent acute leukemia in adults, and its rate increases with age. Though AML is a relatively rare disease, accounting for roughly 1.2% of cancer-related deaths in the U.S. (Jemal et al. 2002), its prevalence is expected to increase by the age.

AML is the most frequent acute leukemia affecting adults, accounting for approximately 80% of cases in this group (Yamamoto and Goodman 2008). In the U.S., the rate of AML ranges from three to five cases per 100,000 populations. In 2015, an estimated 20,830 new cases were diagnosed, and over 10,000 patients died from this disease (Siegel et al. 2015). The frequency of AML increases with age, from $\sim$1.3 per 100,000 populations in patients younger than 65 years old, to 12.2 cases per 100,000 populations in those over 65 years. Although advances in the treatment of AML have led to significant improvements in outcomes for younger patients, prognosis in the elderly who account for the majority of new cases remains poor (Shah et al. 2013). Even with existing treatments, as much as 70% of patients

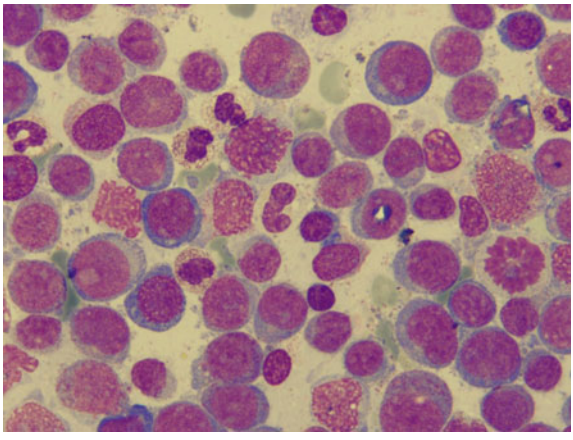

Fig. 14.1 Acute myeloblastic leukemia, M2 subtype: bone marrow aspirates showing myeloblasts and immature and mature myeloid cells. High power view (Used with permission from A. Ghavamzadeh: A color atlas of morphologic Hematology)

in 65 years of age or older will die of their disease within 1 year of diagnosis (Meyers et al. 2013).

AML can arise in individuals with an underlying hematological disease, or due to exposure to alkylating agents, topoisomerases II or radiation as a consequence of prior therapy (Sill et al. 2011). However in most of cases, it appears as de novo leukemia in previous healthy cases. Irrespective of its etiology, AML pathogenesis involves the uncontrolled proliferation and abnormal differentiation of specific clonal population of myeloid precursor and stem cells. Well-defined chromosomal aberration, such as t(8;21)(q22;q22), (AML1/ETO) or t(15;17)(q24;q21) PML/RARA in acute promyelocytic leukemia (APL) result in the formation of chimeric transcripts, which alter the normal development process of myeloid stem cell. Molecular alterations have also been implicated in the development of leukemia such as AML. In fact, somatic mutations are detected in >97% of patients (Patel et al. 2012) often in the absence of chromosomal aberrations (Network 2013).

Animal model studies at the first of the century led to the development of a two-hit model of leukemia induction (also known as leukemogenesis), which recommend a conceptual framework for categorizing the different mutations related to AML (Gilliland and Griffin 2002). Regarding this model, mutations in class I which result in the activation of pro-proliferative pathways must occur in combination with class II mutations which led to impairment in normal hematopoiesis in development of leukemia (Takahashi 2011; Kihara et al. 2014). Frequent class I somatic mutations, such as FLT3/ITD (internal tandem duplications), K/NRAS, TP53 and c-KIT are detected in ~28, 12, 8 and 4% of cases, respectively (Network 2013). Solid and hematological disorder studies have also introduced the function of signal transducer and activator of transcription 3 (STAT3) in uncontrolled proliferation and abnormal survival (Cook et al. 2014; Ghoshal et al. 2008; Yamada and Kawauchi 2013). Increased tyrosine kinase activity of STAT3 whether due to cytokines secretion increment, such as IL-6 (Schuringa et al. 2000) or somatic mutations in tyrosine kinases receptor such as, FLT3 duplications (Spiekermann et al. 2003) or less frequently JAK2 (Steensma et al. 2006) is seen in up to 50% of AML cases which signifies a bad prognosis. Considerable class II mutations include NPM1 and CEBPA, which are found in virtually 27 and 6% of cases, respectively, and confer a good prognosis (Network 2013). Recently, Genetic alterations involved in epigenetic regulation process have emerged as a third class of mutations, with downstream effects on both cellular proliferation and differentiation. These include mutations in the DNA-methylation associated genes DNMT3A, TET2, and IDH-1 and IDH-2 (Network 2013; Patel et al. 2012) which are found in >40% of cases of AML.

The majority of clinical features of AML reveal the accumulation of leukemic cells, immature myeloid cells within the BM, peripheral blood smear (PBS) and uncommonly in other tissues. Most of the patients present an association of high leukocyte count and evidences of BM failure such as anemia and thrombocytopenia. Weight loss, fatigue and anorexia are common complaints; while organomegaly are not typically seen. If the patients remain untreated, secondary infections or bleeding, they usually expire within few months of diagnosis.

The diagnosis of acute leukemia is confirmed by the presence of $\geq 20\%$ blasts in the BM or PB (Döhner et al. 2010). AML is further diagnosed by testing for myeloperoxidase activity, immunophenotyping or documenting the presence of Auer rods for characterization of myeloid origin of these cells. The last finding comprises azurophilic granules, frequently needle-shaped cytoplasmic inclusion bodies that are usually seen in APL, acute myelomonocytic leukemia and most of AML with t(8;21). AML diagnosis can also be confirmed in an extramedullary tissue infiltration, or a documented chromosomal abnormality such as t(8;21), inv (16) or t(15;17) in the suitable clinical setting, regardless of the blast percentage.

14.3.3 Classification

The first attempt to distinguish different types of AML was described by the French–American–British (FAB) classification system. According to the morphological characteristics and cyto-chemistry staining of the malignant cells, FAB defines eight subtypes (M0 through M7). In 2001, as part of an attempt to incorporate advances made in AML diagnosis and management, the World Health Organization (WHO) introduced a new classification system followed by a revised version in 2008 (Vardiman et al. 2009). Later in 2016 a new revised version was released, the WHO classification of AML discriminates itself by integrating genetic data with morphology, flow cytometry information and clinical manifestation to describe six main disease entities: AML with recurrent genetic abnormalities; AML with myelodysplasia-related features; therapy related AML; AML not otherwise specified; myeloid sarcoma; and myeloid proliferation related to Down syndrome (Table 14.1) (Arber et al. 2016). Among AML cases with recurrent genetic abnormalities, 11 subclasses are further defined based on distinct chromosomal aberrations. As part of the 2008 revision of the WHO, the provisional entities AML with mutated NPM1 and AML with mutated CEBPA were introduced (Vardiman et al. 2009). While as part of the 2016 revision of the WHO AML with BCR-ABL1 and AML with mutated RUNX1 were introduced (Arber et al. 2016). Genetic aberrations also apprise the AML diagnosis with myelodysplasia-related changes: along with a history of MDS or dysplastic cells in two or more myeloid cell lineages (multilineage), the presence of myelodysplasia-related cytogenetic aberrations such as monosomy 5 or 7, and deletion of 5q or 7q represent the AML patients with myelodysplasia-related features.

14.3.4 Common Chromosomal Aberrations

The karyotype of leukemic cells has been the mostly focused independent prognostic indicator which determine therapy response and patient's overall survival (Byrd et al. 2002). It was reported that nearly 55–78% of adults and 77–85% of

Table 14.1 Classification of AML and related neoplasm

FAB classification	AML with minimal differentiation (M0) AML without maturation (M1) AML with maturation (M2) Acute promyelocytic leukemia (M3) Acute myelomonocytic leukemia (M4) Acute monoblastic/monocytic leukemia (M5) Acute erythroid leukemia (M6) Acute megakaryoblastic leukemia (M7)
WHO classification: acute myeloid leukemia (AML) and related neoplasms	AML with recurrent genetic abnormalities AML with t(8;21)(q22;q22.1); RUNX1-RUNX1T1 AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22);CBFB-MYH11 APL with PML-RARA AML with t(9;11)(p21.3;q23.3); MLLT3-KMT2A AML with t(6;9)(p23;q34.1);DEK-NUP214 AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); GATA2, MECOM AML (megakaryoblastic) with t(1;22)(p13.3;q13.3);RBM15-MKL1 Provisional entity: AML with BCR-ABL1 AML with mutated NPM1 AML with biallelic mutations of CEBPA Provisional entity: AML with mutated RUNX1 AML with myelodysplasia-related changes Therapy-related myeloid neoplasms AML, NOS AML with minimal differentiation AML without maturation AML with maturation Acute myelomonocytic leukemia Acute monoblastic/monocytic leukemia Pure erythroid leukemia Acute megakaryoblastic leukemia Acute basophilic leukemia Acute panmyelosis with myelofibrosis Myeloid sarcoma Myeloid proliferations related to Down syndrome Transient abnormal myelopoiesis (TAM) Myeloid leukemia associated with Down syndrome Blastic plasmacytoid dendritic cell neoplasm Acute leukemias of ambiguous lineage Acute undifferentiated leukemia Mixed phenotype acute leukemia (MPAL) with t(9;22)(q34.1;q11.2); BCR-ABL1 MPAL with t(v; 11q23.3); KMT2A rearranged MPAL, B/myeloid, NOS MPAL, T/myeloid, NOS

children with AML harbor an abnormal karyotype (Byrd et al. 2002; Raimondi et al. 1999). More than 100 balanced chromosomal aberrations (translocations, insertions, and inversions) have been described and cloned, with evidences proposing these abnormalities as critical initiating events in AML pathogenesis (Mitelman et al. 2011). Most leukemia investigators have classified AML subtypes based on the pretreatment karyotype. This is rooted in the fact that karyotype has the potential of predicting the therapy response, relapse risk, and overall survival (OS). The cytogenetic risk groups can be also categorized based on treatment outcomes: favorable, intermediate, and unfavorable; which comprise 20, 50, and 30% of AML cases, respectively. AML classification was developed more than two decades ago based on the outcomes of large prospective clinical trials by the Southwest Oncology Group (SWOG), the Medical Research Council (MRC), and Cancer and Leukemia Group B (CALGB) (Slovak et al. 2000; Byrd et al. 2002; Grimwade et al. 2001) (Table 14.2). A summary of the most prevalent chromosomal abnormalities are discussed below.

14.3.4.1 Low Cytogenetic-Risk AML

t(8;21)(q22;q22)

One of the well characterized chromosomal abnormality which occurs in adult patients with de novo AML and accounts for 7–8% of all aberrations is a translocation between band 22 of the long arm of chromosome 8 and band 22 of the long arm of chromosome 21 [t(8;21)(q22;q22)] (Fig. 14.2) (Byrd et al. 2002). This chromosomal aberration brings consequences such as de-regulation of core binding factor (CBF). CBF is a heterodimeric transcription factor (TF) which acts as a regulator for hematopoiesis. This TF comprises an α-subunit, functioning as DNA binding domain, and a β sub-unit, which facilitates this link (Speck 2001).

AML1 gene encodes the α-subunit, while the β-subunit is encoded by CBF-β gene. At molecular levels, this translocation is defined by the AML1 gene fusion to band 21q22 of gene ETO, mapped at band 8q22 (AML1/ETO also known as RUNX1/CBFA2T1). This fusion gene acts as a transcriptional repressor, blocking the normal processes of hematopoiesis. AML patients with t(8;21) are characterized by specific clinical and biological properties which emerge as a typical morphology (M2 FAB-subtype).

Interestingly, the t(8;21) is occasionally associated with other cytogenetic deregulations including the loss of sex chromosome, deletions at chromosome 9 and trisomy 8. Nevertheless, the clinical outcome for patients with AML with t(8;21) is particularly favorable, with high CR rate, long CR duration and a good OS, especially after a post-remission therapy using high-dose cytarabine (Bloomfield et al. 1998; Byrd et al. 1999). It was reported in a large meta-analysis study by the German Acute Myeloid Leukemia Intergroup 191/410 that newly diagnosed AML patient between 16–60 years old harbor the t(8;21) translocation. CR in 87% of cases with a 3-year DFS was shown to be 60% and in 191 patients

Table 14.2 Cytogenetic classifications of acute myeloid leukemia

Group	CALGB	MRC	SWOG
Favorable	t(15;17) inv(16)/t(16;16)/del(16) t(8;21)	t(15;17) with any abnormality inv(16)/t(16;16)/del(16q) with any other abnormality t(8;21) with any other abnormality	t(15;17) with any abnormality inv(16)/t(16;16)/del(16q) with any other abnormality t(8;21) without del(9q) or complex karyotype
Intermediate	Normal karyotype	Normal karyotype +8, −Y, +6, der(12p) 11q23 abnormality del(9q) or del(7q) without other abnormality Complex karyotypes (≥ 3 but <5 abnormalities) All abnormalities of unknown prognostic significance	Normal karyotype +8, −Y, +6, der(12p)
Unfavorable	Other abnormalities	−5/del(5q) − 7 inv(3q), del(9q), 17p abnormality t(6;9) t(9;22) Complex karyotypes with ≥ 5 abnormalities	−5/del(5q). −7/del(7q) inv(3), 17p abn, 20q, +13, t(6;9) t(9;22) 11q23 abnormality (8;21) with del(9q) or complex karyotype Complex karyotypes with ≥ 3 abnormalities
Unknown	–	–	All other clonal karyotypes with <3 chromosomal abnormalities

CALGB cancer and leukemia group B; *MRC* medical research council; *SWOG* southwestern oncology group

with a 3-year OS was 65%. The authors also analyzed various prognostic factors potentially associated with CR rate, DFS and OS. It was reported that the only independent prognostic factors showing a negative effect on OS are platelet count $\leq 28 \times 10^9$/L, WBC count $\geq 25.4 \times 10^9$/L and loss of Y chromosome as an adjunctive chromosomal abnormality in male patients (Schlenk et al. 2004).

inv(16)(p13q22) and t(16;16)(p13;q22)

Other current cytogenetic alterations occurring in 4–9% of de novo AML cases are a pericentric inversion of chromosome 16 [inv(16)(p13q22)] (Fig. 14.3) and

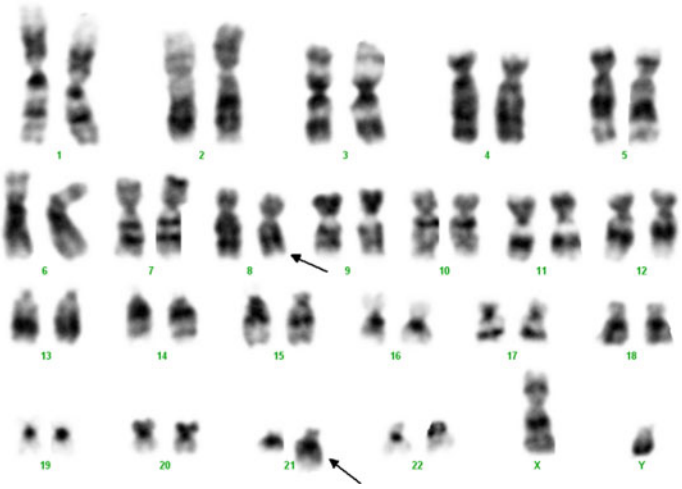

Fig. 14.2 The G-banded karyotype of the metaphases analyzed in the AML patient's bone marrow cells shows 46, XY, t(8;21)(q22;q22). *Arrows* indicate abnormal chromosomes. The figures adopted from cytogenetic lab of hematology, oncology and stem cell transplantation research center, Tehran University of Medical sciences, Dr. Yaghmaie's Archives

translocation between band 12 of the short arm of chromosome 16 and band 22 of the long arm of the same chromosome [t(16;16)(p12q22)] (Byrd et al. 2002; Marcucci et al. 2005; Schlenk et al. 2004). These chromosomal aberrations were indicated to cause de-regulation of the core binding factor (CBF), characterized by CBFβ gene fusion (located on 16q22 coding β-subunit of CBF), and MYH11 gene (located on 16p13 coding heavy chains of smooth muscle). While breakpoints in MYH11 gene are still unidentified, the breakpoints of CBF gene have been characterized to be at exons 5 or, more rarely, at exon 4. The CBFβ/MYH11 fusion gene function in leukemogenesis is not clarified; however, it was discussed that this fusion could contribute to the CBFβ oligomerization, resulting in loss of its function and thereby loss of CBF function.

In 50% of AML cases, Inv(16) and t(16;16) were shown to be associated with other chromosomal abnormalities, more commonly with trisomy 8, 21 or 22 (Schlenk et al. 2004; Mrózek et al. 1997). AML patients harboring inv(16) or t (16;16) are tightly associated with a peculiar morphologic characteristics with FAB-subtype M4eo described by the presence of atypical eosinophils in bone marrow, and occasionally with M5 or M2 FAB-subtypes. Clinical perspectives have shown that AML with inv(16)/t(16;16) emerges with high CR rate and favorable clinical outcome. Having inv16 is also considered as a good prognostic factor. It was revealed by a large meta-analysis of the German Acute Myeloid Leukemia Intergroup that 201 AML patients with newly diagnosed inv(16)/t(16;16) were 16 to 60 years old. CR was reported in 89% of cases with a 3-year DFS to be

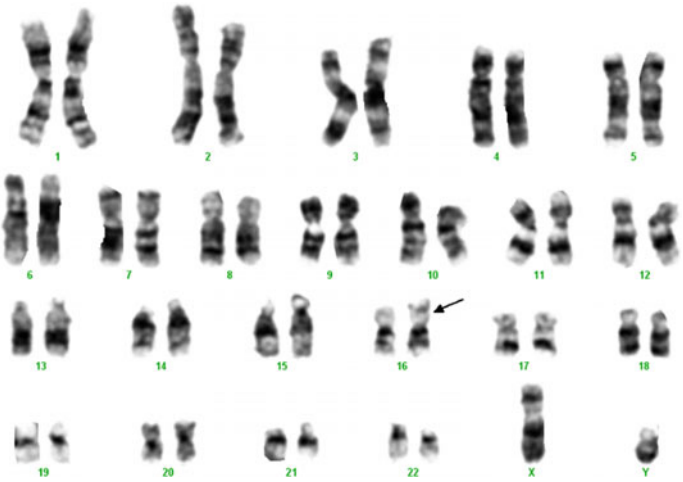

Fig. 14.3 The G-banded karyotype of the metaphases analyzed in the AML patient's bone marrow cells shows 46, XY, inv (16)(p13q22). *Arrow* indicate abnormal chromosome. The figures adopted from cytogenetic lab of hematology, oncology and stem cell transplantation research center, Tehran University of Medical sciences, Dr. Yaghmaie's Archives

58% and a 3-years OS of 74% was calculated. In this study, early death or death in aplasia was demonstrated to be significantly associated with higher WBC count and older ages (Schlenk et al. 2004).

t(15;17)(q22;q21)

Based on the AML classification made by WHO, a distinct genetic category was characterized to harbor translocation (15;17) (Fig. 14.4) which is a current abnormality associated with AML (Arber et al. 2016). In FAB classification; generally, t(15;17) is pathognomonic for AML-M3, characterized by the presence of abnormal promyelocytes, which is also associated with disseminated intravascular coagulation (DIC). T(15;17) has been detected in 5–8% of AML cases, more likely in middle-aged adults; however, has been reported in adults of any age (Tallman et al. 1997). t(15;17) is a consequence of PML gene fusion in 15q24 with the RARA gene in 17q12, an abnormality which is normally detected through cytogenetic and FISH analyses (Chauffaille et al. 2001). In the cases with early diagnosis, AML patients with PML/RARA gene fusion responds well to ATRA therapy (Mi et al. 2012). In 40% of cases, t(15;17) has been also reported to be combined with additional karyotypic alterations, most commonly with trisomy 8. Variant RARA translocations also occur commonly in acute promyelocytic leukemia. These subtypes may include complex translocations or masked RARA

translocations without clear chromosomal aberrations. Also Variant translocations of t(15;17) have been commonly seen among which the t(11;17) occurs frequently. t(5;17) (q35;q12) was also reported as a recurrent translocation in which the RARA locus is also involved and responds well to ATRA therapy (Adams and Nassiri 2015).

14.3.4.2 AML with Intermediate Cytogenetic Risk

Normal Karyotype

Approximately, 40–50% of newly diagnosed AML patients lack a clonal abnormality in 20 or more analyzed bone marrow cells in metaphase stage, which defined as AML with normal karyotype and known as the largest subset of AML cases based on the cytogenetic risk (Byrd et al. 2002; Grimwade et al. 1998; Baldus et al. 2007). It is classified as intermediate risk by all the most significant cooperative groups. AML with normal karyotype comprise a heterogeneous group with remarkable variability in treatment response, achievement of CR, relapse rate, DFS and OS. It is also characterized by a concomitant heterogeneity in terms of molecular properties of AML with normal cytogenetic; indeed, over the last decade, extensive studies have revealed that the AML prognosis is associated with presence or absence of particular gene mutations and/or alterations in gene expression profiles (Baldus et al. 2007).

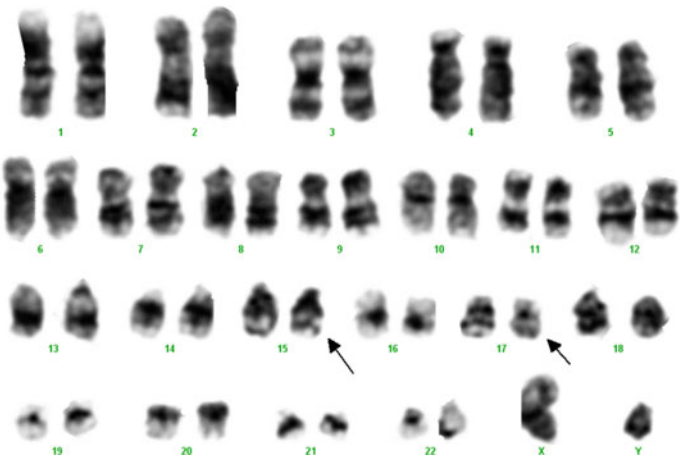

Fig. 14.4 G-banded karyotype obtained from an acute myeloid leukemia subtype M3 case at diagnosis: t(15;17)(q22;q21). *Arrows* indicate abnormal chromosomes. The figures adopted from cytogenetic lab of hematology, oncology and stem cell transplantation research center, Tehran University of Medical sciences, Dr. Yaghmaie's Archives

t(9;11)(p22;q23)

Among the AML patients having 11q23 aberrations, the most frequent abnormality is a translocation between short arm of chromosome 9, band 22, and long arm of the chromosome 11, band 23, [t(9;11)(p22;q23)] (Fig. 14.5); which constitutes about 30–40% of 11q23 AML (Tamai et al. 2008; Zhang and Rowley 2006). The resulting gene rearrangement is a fusion of MLL gene, located on chromosome 11, and AF9 gene on chromosome 9 (Zhang and Rowley 2006; Langer et al. 2003).

AF9 gene with a molecular size of more than 110 kb consists of 10 exons with two different breakpoints associated with t(9;11), which occurs in de novo or therapy-related AML patients, respectively. The MLL-AF9 fusion gene immortalizes hematopoietic progenitors and blocks the differentiation in monocytic lineage.

This chromosomal alteration is associated with peculiar clinical findings, distinct from the other forms of 11q23 AML. Particularly, AML patients with t(9;11) emerge commonly in younger ages (median age of 38–40 years), a higher hemoglobin value and platelet count, extramedullary infiltration resulting in clinical manifestations such as hepatomegaly, splenomegaly, adenopathy and skin localization. In FAB classification, the AML patients with t(9;11) are categorized in M5 FAB-subtype (Tamai et al. 2008). Several evidences have proven that this translocation is associated with a better prognosis compared with other aberrations affecting chromosome 11 and MLL gene (Grimwade et al. 1998). Therefore, this AML subtype has been classified in intermediate cytogenetic risk group based on all the international cooperative study groups.

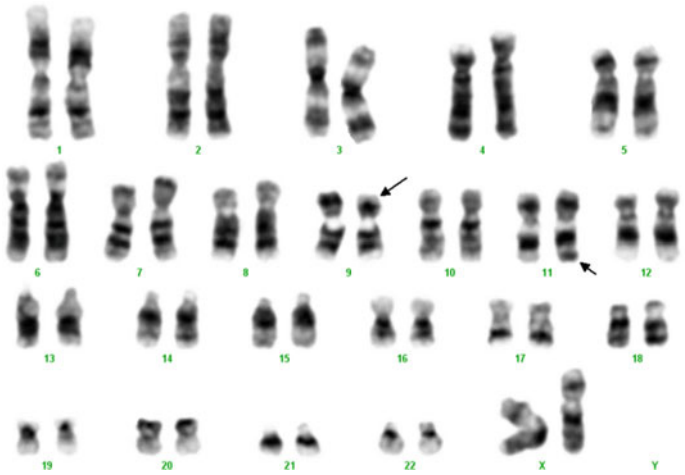

Fig. 14.5 The G-banded karyotype of the metaphases analyzed in the AML patient's bone marrow cells shows 46, XX, t(9;11)(p22;q23). *Arrows* indicate abnormal chromosomes. The figures adopted from cytogenetic lab of hematology, oncology and stem cell transplantation research center, Tehran University of Medical sciences, Dr. Yaghmaie's Archives

Trisomy of Chromosome 8

The most common numeric abnormality in AML which is reported either as single or combined aberration is trisomy of chromosome 8 (+8). Six percent of newly diagnosed AML with single abnormal chromosomal aberration, and 10% of newly diagnosed AML cases with combined cytogenetic abnormality have been detected to present trisomy of chromosome 8 (Schoch et al. 1998). This alteration has been shown to be a typical signature for myeloid malignancies, regarding its high prevalence in MDS and its relative expression in the patients with myeloproliferative disorders.

It has been suggested that +8 incidence is higher in elders, affecting 11% of cytogenetically abnormal AML patients over 81–90 years old. An increased incidence of +8 has been also observed to be associated with gender, geography-related varieties and a precedent exposure to toxic agents. Although the pathogenetic role of +8 remains unidentified; a possible role of global over-expression of genes in chromosome 8 has been suggested. Also it was proposed that +8 can deregulate MYC gene in band 24 of the long arm of the chromosome 8 (8q24). Nevertheless, this appears to be a simplistic mechanism since previous studies have shown that +8 is responsible for deregulation of various genes and experiments based on microarray analysis have indicated that +8 appears to be related with a global gene expression.

Trisomy of Chromosome 11

In both de novo and secondary AML or MDS, trisomy of chromosome 11 (+11) has been detected, which is considered as the third most current isolated chromosomal abnormality in de novo AML patients. It is more commonly associated with other cytogenetic aberrations. Apparently, AML with trisomy 11 do not present as peculiar clinical and immunophenotypic features. It has a poor clinical course with a CR rate of 43% in patients undergone intensive chemotherapy course, and a median OS of 2 months (Sierra et al. 2005).

Trisomy of Chromosome 13

Trisomy of chromosome 13 (+13) has been found as an isolated abnormality in 2.5% of newly diagnosed AML cases (Byrd et al. 2002). It is a rare but recurring numeric chromosome aberration. Although the molecular mechanism underlying leukemogenic function of this trisomy is still not determined, it has been proposed that it may be associated with over-expression of one or more genes on chromosome 13. A well-known candidate deregulated gene in this term is FLT3 gene on chromosome 13 which is expressed in immature hematopoietic progenitors (Dicker et al. 2007). Although does not exist a common agreement about the prognostic significance of trisomy 13, according to the European Leukemia Net

(ELN) classification, AML patients with Trisomy of chromosome 13 are currently classified in the Intermediate genetic group (Mrózek and Bloomfield 2006; Döhner et al. 2010).

Trisomy of Chromosome 21 (Down Syndrome)

With an incidence of 1 in 700 births, the Down syndrome (DS) is considered as the most prevalent human aneuploidy. DS affects the hematopoietic system, so that the children with DS currently show macrocytosis, deregulation in platelet count and an elevated prevalence of leukemia (Malinge et al. 2009). Based on evidences, the children with DS have approximately 20 times higher chance to get acute lymphoblastic leukemia (ALL) than general population; however, acute megakaryoblastic leukemia (AMKL, FAB-subtype M7 AML) is more common in patients with DS, with 500 times higher incidence than general population. AMKL is defined by peculiar clinical and pathogenetic characteristics with a good prognosis and 80% cure rate (Malinge et al. 2009; Rao et al. 2006). However, there is no significant difference between the occurrence of myeloid leukemia in people with DS of different ages (regarding the clinical features and outcome) from AML in patients without DS.

14.3.4.3 High Cytogenetic-Risk AML

Complex Karyotype

An AML with complex karyotype is characterized by the presence of 3 or more cytogenetic aberrations in bone marrow cells which does not include inv(16), t(16;16), t(8;21), t(15;17) and t(9;11) (Fig. 14.6) (Byrd et al. 2002; Grimwade et al. 1998).

As a result, it is proposed that having a t(9;21)(p22;q23) or any balanced rearrangements affecting band 11q23 or any primary balanced cytogenetic aberrations is not included in the category of AML with complex karyotype (Rücker et al. 2006). It has been shown in a multicenter study that 13% of AML patients over 55 years old harbor 5 or more chromosomal abnormality; while in only 6% of AML patients under 55 years old (including children) this complex karyotype has been observed (Grimwade et al. 2001). It is more likely that a complex karyotypes occur in secondary AML (nearly two fold higher de novo AML), which may be due to the aggressive accumulation of chromosomal abnormalities caused by antecedent treatment using alkylating agents, radiotherapy or other hematologic disorders (Grimwade et al. 2001; Schoch et al. 2005).

It is evidenced that a complex karyotype abnormality in bone marrow blasts of AML patients is highly associated with a poor prognosis, irresponsiveness to intensive induction treatments, high relapse rate, a poorer DFS and OS (Byrd et al. 2002; Slovak et al. 2000; Grimwade et al. 1998).

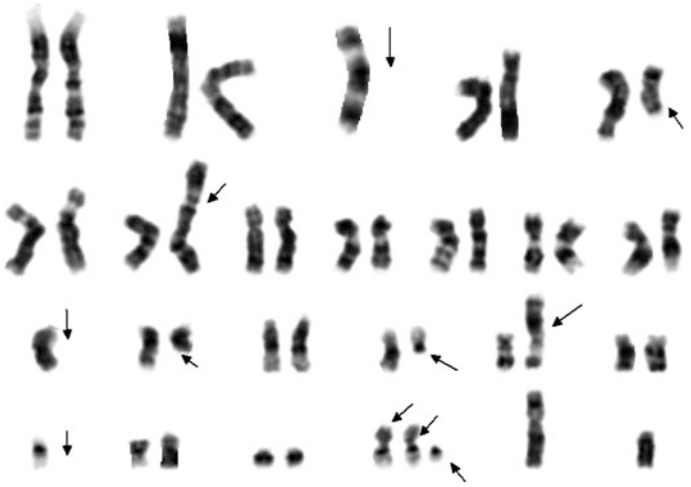

Fig. 14.6 G-banded karyotype obtained from an acute myeloid leukemia case at diagnosis: An abnormal male complex karyotype complement with the following chromosomal abnormalities: 44, XY, −3, del(5)(q13q33), der(7)t(7;13)(p22;q12), −13, del(14)(q22q31), del(16)(q11.2), der(17)t(14;17)(q11.2;p11.2), −19, add(22)(p11.2) × 2, +del(22)(q11.2)[20]. *Arrows* indicate abnormal chromosomes. The figures adopted from cytogenetic lab of hematology, oncology and stem cell transplantation research center, Tehran University of Medical sciences, Dr Yaghmaie's Archives

inv(3)(q21;q26) and t(3;3)(q21;q26)]

In approximately 2.5% of AML patients a paracentric inversion of chromosome 3 leads to a rearrangements in the long arm of chromosome 3 [inv(3)(q21;q26)] occurs (Fig. 14.7). Moreover, a translocation between the long arms of both homologous chromosomes 3 [t(3;3)(q21;q26)] is found in AML patient with a same rate (Byrd et al. 2002).

The 3q26 region possesses chromosomal breakpoints disseminated over several hundred kilobases in EVI1 gene region (Ecotropic Viral Integration Site 1). Nevertheless, chromosomal breakpoints in the 3q21 region are restricted to a smaller DNA segment (100 kilobases) encoding RPN1 (Ribophorin 1) gene (Suzukawa et al. 1994). It has been suggested that the ectopic expression of EVI1 gene through RPN1 which functions as an enhancer for EVI1 expression, is responsible for the leukemogenic effect of 3q21q26 rearrangements (Suzukawa et al. 1994). Interestingly, this mechanism has been mainly detected in lymphoid leukemias and lymphomas and rarely in the pathogenesis of myeloid malignancies. The proto-oncogene EVI1 which encodes a DNA binding zinc finger protein serves as a transcription suppressor or activator. EVI1 has been indicated to have an aberrant expression in leukemic cells, and lines of studies have suggested that erythroid and granulocytic development is interfered by EVI1 ectopic expression in immature hematopoietic cells (Lahortiga et al. 2004).

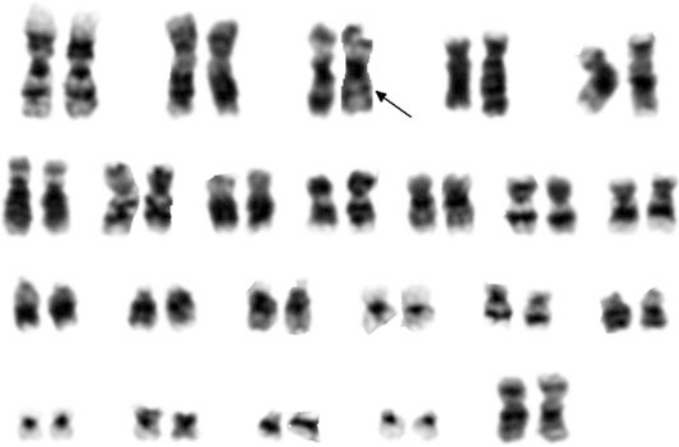

Fig. 14.7 The G-banded karyotype of the metaphases analyzed in the AML patient's bone marrow cells shows 46, XX, inv(3)(q21q26). *Arrow* indicate abnormal chromosome. The figures adopted from cytogenetic lab of hematology, oncology and stem cell transplantation research center, Tehran University of Medical sciences, Dr. Yaghmaie's Archives

t(6;9)

Approximately, 0.5–4% of AML cases are found to harbor translocation between short arm of chromosome 6, band 23, and long arm of chromosome 9, band 34 [t(6;9)(p23;q34)] (Fig. 14.8) (Chi et al. 2008). Historically, this chromosomal abnormality was associated with M2 FAB-subtype with basophilia in bone marrow and peripheral blood (PB). However, recent studies have also reported that in M1 or M4 FAB-subtypes with or without basophilia it is detected in bone marrow blasts. This translocation leads to a chimeric rearrangement of DEK gene, located on the short arm of chromosome 6 (6p23), and CAN or NUP214 genes on band 34 of the long arm of chromosome 9 (9q34). Physiologically, CAN gene codes for a nuclear pore complex protein allowing the accurate transfer of messenger RNA and various proteins through the nuclear membrane. The fusion gene causes an over expression of CAN gene, accelerating leukemogenesis via alterations in protein transfer through the nuclear pores which ultimately blocks the cell cycle in G0 phase (Chi et al. 2008; Garcon et al. 2005). The DEK and CAN breakpoints are clustered in introns which allow the molecular techniques such as qRT-PCR or Southern blotting to detect the fusion gene. It is assumed that the poor prognosis AML patients with t(6;9) may be due to the formation of chimeric DEK-CAN fusion gene (Oyarzo et al. 2004).

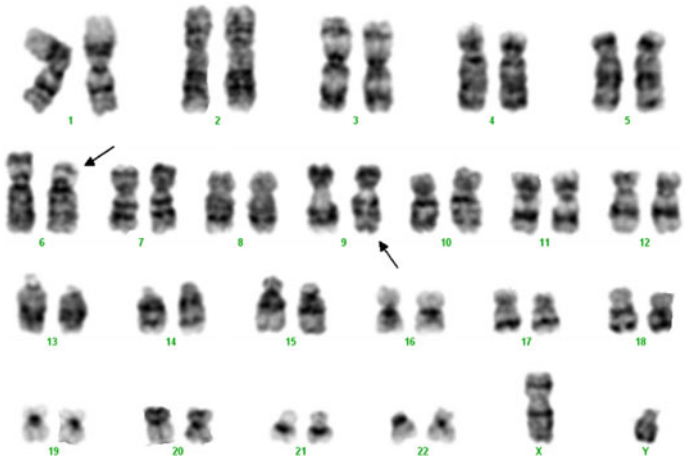

Fig. 14.8 The G-banded karyotype of the metaphases analyzed in the AML patient's bone marrow cells shows 46, XY, t(6;9)(p23;q34). *Arrows* indicate abnormal chromosomes. The figures adopted from cytogenetic lab of hematology, oncology and stem cell transplantation research center, Tehran University of Medical sciences, Dr. Yaghmaie's Archives

t(8;16)(p11;p13) and inv(8)(p11q13)

Generally, in 0.5% of FAB-subtypes AML M4, M5a and M5b, the balanced translocation between the band 11 of the short arm of chromosome 8 and band 13 of the short arm of chromosome 16 [t(8;16)(p11;p13)] (Fig. 14.9) is detected as a rare chromosomal abnormality (Gervais et al. 2008). Also in this case, AML with t (8;16) could be de novo or secondary to another hematological malignancies or to exposure to toxic agents (i.e. chemotherapy or radiotherapy for another neoplastic disease). MOZ (monocytic acute leukemia zinc finger) is the gene involved in 8p11 rearrangement, a gene of 17 exons currently named MYST3 (MYST histone acetyltransferase 3). A nuclear protein with a histone acetyltransferase activity, that acts as a transcriptional regulator is encoded by MYST3.

CBP gene localized in the short arm of chromosome 16 is another gene that involved most frequently in t(8;16) and encodes for CREB-binding protein (CREBBP). CREBBP is a nuclear protein with acetyltransferase activity which plays a role in transcriptional regulation by its interaction with DNA, and is essential in embryogenesis, cell differentiation, apoptosis and proliferation (Borrow et al. 1996). MYST3 rearrangements are Also seen in others translocations involving the chromosome 8 in AML such as: t(8;19)(p11;q13), t(8;22)(p11;q13), inv(8)(p11q13) and t(8;20)(p11;q13) (Chaffanet et al. 2000). Even with intensive induction chemotherapy, this form of AML has a very poor prognosis with fatal clinical course.

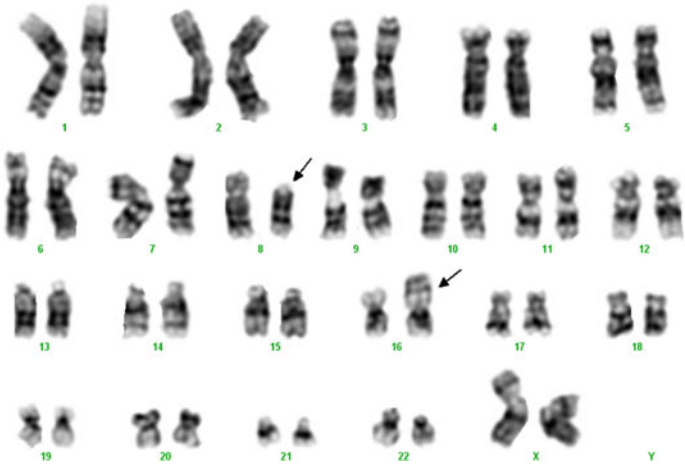

Fig. 14.9 The G-banded karyotype of the metaphases analyzed in the AML patient's bone marrow cells shows 46, XX, t(8;16)(p11;p13)]. *Arrows* indicate abnormal chromosomes. The figures adopted from cytogenetic lab of hematology, oncology and stem cell transplantation research center, Tehran University of Medical sciences, Dr. Yaghmaie's Archives

t(3;5)(q25;q35)

A rare chromosomal aberration is the balanced translocation between the long arm of chromosome 3 and the long arm of chromosome 5 which occurs in <1% of AML patients and more frequently in MDS of young adults (Grimwade et al. 2001). Variable breakpoints for this translocation have been reported, however, the breakpoints are most commonly described as t(3;5)(q25;q35) (Fig. 14.10) (Arber et al. 2003). This chromosomal rearrangement results in a fusion of the nucleophosmin gene (NPM, localized in chromosome 5) with myeloid leukemia factor 1 (MLF1) gene on chromosome 3. The NPM gene is involved in ALK-NPM fusion observed in anaplastic-large-cell lymphoma and a fusion partner with RARA in a small percentage of cases of acute promyelocytic leukemia. MLF1 is a gene expressed normally in various tissues. NPM/MLF1 fusion gene encodes for a protein mostly expressed in the nucleus and specially in the nucleolus which binds to the myeloid nuclear differentiation antigen, a nuclear protein important for development of human myelomonocytic cells (Arber et al. 2003). The real effect of this chimeric gene in the pathogenesis of MDS/AML is not clear. The evaluation of the prognostic role of t(3;5) and its role in leukemogenesis requires further studies. AML patients with t(3;5) are mostly young (median age of 36 years) and characterized by multi-lineage dysplasia and related to FAB-subtypes M4 or M5. For the high rate of relapse, prognosis is poor despite intensive chemotherapy. Therefore, AML patients with t(3;5) are possible candidates for allogeneic HSCT.

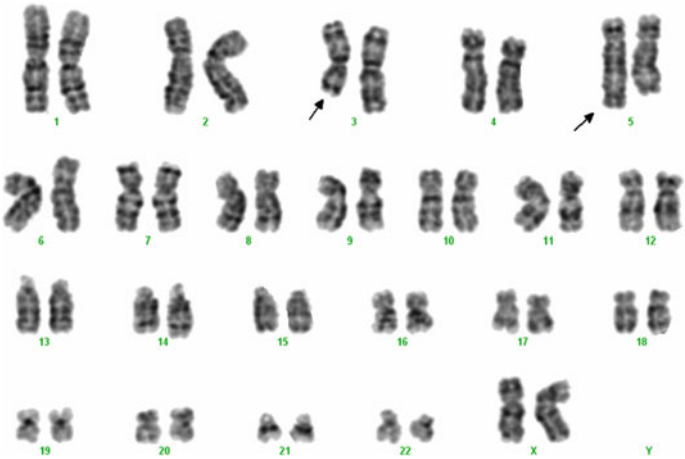

Fig. 14.10 The G-banded karyotype of the metaphases analyzed in the AML patient's bone marrow cells shows 46, XX, t(3;5)(q25;q35). *Arrows* indicate abnormal chromosomes. The figures adopted from cytogenetic lab of hematology, oncology and stem cell transplantation research center, Tehran University of Medical sciences, Dr. Yaghmaie's Archives

MLL Rearrangements [t(6;11), t(11;19) and t(10;11)]

It has been occurred in nearly 4–10% of AML patients, aberrations in the band 23 of the long arm of chromosome 11 (11q23). Specially 11q23 rearrangements is present in about 40–50% of childhood AML, 5% of adult de novo AML and 80% of adult secondary AML (i.e. in therapy-related AML, especially after treatment with topoisomerase II inhibitors). Firstly in 1979, the 11q23 chromosomal aberration was described in patients affected by acute lymphoblastic leukemia (ALL). Until now, more than 80 chromosome loci have been described as partner site of reciprocal translocations involving band 11q23 and the majority of these translocations involve the mixed-lineage-leukemia (MLL) gene. MLL is a gene of 36 exons, encoding a nuclear protein acting as a gene expression regulator in early embryonic development and hematopoiesis (Tamai et al. 2008); actually the exact function of MLL gene is not known. Nearly all the breakpoints in MLL gene occur in an 8.3-kb region, named breakpoint cluster region (BCR) and encompassing exons 8–14. In treatment-related adult AML, the genomic breakpoints have tendency to cluster in the 3′ portion of BCR, near exon 12, whereas in adult patients with de novo AML have tendency to occur in the 5′ portion of BCR between exons 9 and 10 (Zhang and Rowley 2006). MLL gene translocations leads to the production of a chimeric protein in which the amino-terminal portion of MLL gene is fused to the carboxy-terminal portion of the partner fusion gene. Therefore, the normal cellular differentiation processes, favoring leukemogenesis might be altered by these gene fusions (Ayton and Cleary 2001). AML with 11q23 rearrangements, with the only exception of t(9;11) are characterized by poor prognosis and worse clinical outcome.

The various sub-types of 11q23 AML rearrangements show similar clinical features at diagnosis as: frequent anemia, high WBC counts and thrombocytopenia, diffuse bone marrow infiltration by myeloid blasts and M2, M4 or M5FAB-subtype and extramedullary disease in nearly one third of patients. In a study conducted by the German Acute Myeloid Leukemia Intergroup, WBC count was obviously higher in AML patients with t(6;11) with a median WBC count of 55.5 $\times$ 10^9/L (Krauter et al. 2009). Moreover, some translocations are most frequently associated to peculiar clinical characteristics: t(6;11) is often related to AML with multilineage dysplasia, whereas t(11;19) is typically related to a biphenotypic leukemia. A lower CR rate and shorter DFS and OS characterize11q23 AML. As a result, this setting of patient is considered as high risk AML patients and should be candidate to allogeneic HSCT. The most frequent common 11q23 aberrations are t(9;11), t(6;11), t(10;11) and t(11;19). Translocations involving chromosome 9 and 11 are described in another section of this review (i.e. intermediate cytogenetic risk AML).

t(11;19)(q23;p13.3)

In 10–20% of all AML patients having 11q23 aberrations, the translocation between band 23 of the long arm of chromosome 11 and the band 13.3 of the short arm of chromosome 19 has been occurred (Fig. 14.11). The breakpoint in the MLL gene occurs within a 8.3-kb genome region, including exon 5 through 11. This change decides the formation of a fusion gene encoding for the chimeric protein MLL/ENL, which acts as activator of transcriptional processes and is responsible of the leukemogenesis (Strout et al. 1999).

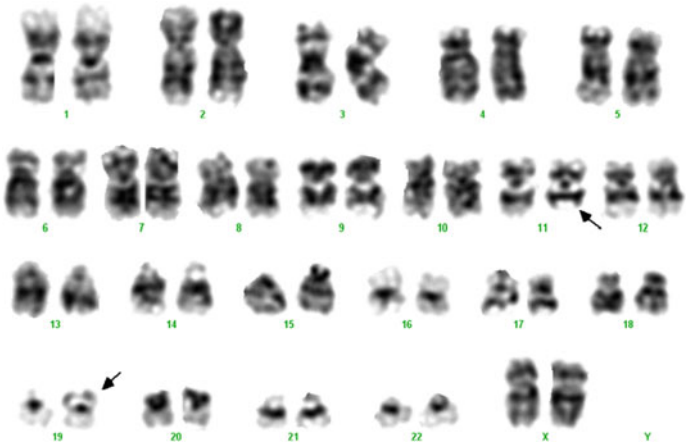

Fig. 14.11 The G-banded karyotype of the metaphases analyzed in the AML patient's bone marrow cells shows 46, XX, t(11;19)(q23;p13.3). *Arrows* indicate abnormal chromosomes. The figures adopted from cytogenetic lab of hematology, oncology and stem cell transplantation research center, Tehran University of Medical sciences, Dr. Yaghmaie's Archives

t(6;11)(q27;q23)

In about 15–20% of 11q23 AML patients, Translocation between band 27 of the long arm of chromosome 6 and the band 23 of the long arm of chromosome 11 has been occur (Fig. 14.12).The incidence of this translocation in a recent study was 19% in 11q23 AML patients aged less than 60 years (Krauter et al. 2009).

It is the AF6 gene on chromosome 6, which is involved in this translocation, it is a 140 kilobases gene localized on chromosome 6q27 and composed by 32 exons. The t(6;11) results in formation of a fusion gene MLL-AF6 in which gene AF6 exon 2 is fused to exon 6 or 7 of MLL gene. This MLL-AF6 fusion gene is responsible of the leukemogenesis through the deregulation of HOX genes. HOX genes that are normally regulated by MLL multi protein complex are important for the regulation of cell proliferation (Daser and Rabbitts 2004). So MLL-AF6 fusion gene determines a deregulation of this gene which contribute to leukemogenesis.

t(10;11)(p12;q23)

It represents about 5–8% of all 11q23 AML cases. The formation of MLL-AF10 chimeric gene is induced by this translocation which involves MLL and AF10 genes. 109-kDa protein is encoded by AF10 gene which is localized on the short arm of chromosome 10 (Caudell and Aplan 2008).

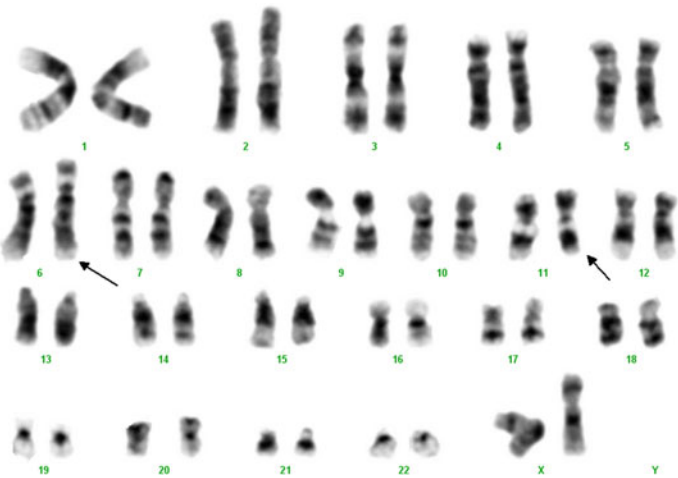

Fig. 14.12 The G-banded karyotype of the metaphases analyzed in the AML patient's bone marrow cells shows 46, XX, t(6;11)(q27;q23). *Arrows* indicate abnormal chromosomes. The figures adopted from cytogenetic lab of hematology, oncology and stem cell transplantation research center, Tehran University of Medical sciences, Dr. Yaghmaie's Archives

The real function of this protein is unknown, but structural and functional data suggest its role as transcriptional factor. Another translocation t(10;11)(p13;q14-21) (Fig. 14.13) not involving the MLL gene has been seen. The genes involve in this translocation are: the CALM (clathrin assembly lymphoid myeloid) gene localized on chromosome 11q14-21 and encodes a protein with multiple domains involved in endocytosis, the second gene is AF10, localized on chromosome 10p12-13. This translocation results in formation of a chimeric gene CALM/AF10 and also is responsible for the leukemogenesis by an unclear pathogenetic mechanism (Caudell and Aplan 2008). This translocation seems to be most frequently associated with T-cell ALL, specifically T-cell ALL of either γ/δ or immature phenotype and have been rarely observed in AML.

t(9;22)(q34;q11)

In about 1% of AML patients, translocation between band 34 of the long arm of chromosome 9 and band 11 of the long arm of chromosome 22 [t(9;22)(q34;q11)] has been found (Slovak et al. 2000). This translocation is similar to that observed in CML and Ph + ALL and produces the fusion gene BCR-ABL that encodes for the chimeric proteins p210 or p190, with high tyrosine-kinase activity.

To know whether t(9;22) AML is a de novo AML or if it is a blastic phase of a precedent and unknown CML is hard.

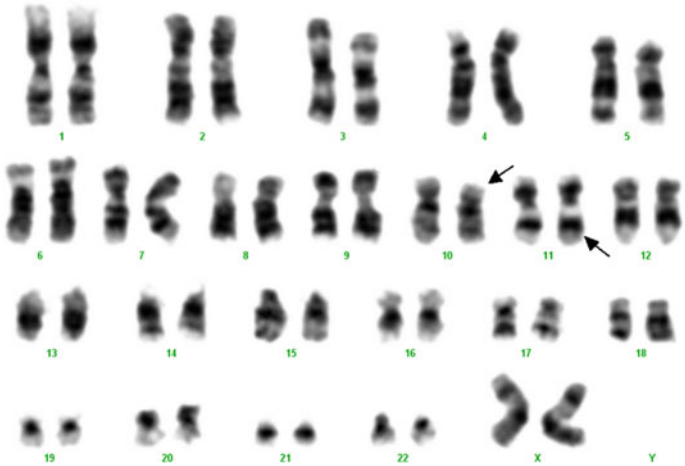

Fig. 14.13 The G-banded karyotype of the metaphases analyzed in the AML patient's bone marrow cells shows 46, XX, t(10;11)(p12;q23). *Arrows* indicate abnormal chromosomes. The figures adopted from cytogenetic lab of hematology, oncology and stem cell transplantation research center, Tehran University of Medical sciences, Dr. Yaghmaie's Archives

Monosomy and Deletion of Chromosome 7

In nearly 4–5% of newly diagnosed AML, they are found monosomy of the chromosome 7 (−7) and deletion of the long arm of the chromosome 7 (7q−) as single chromosomal aberration.

It is estimated by multicenter clinical trials that the incidence of these chromosomal aberrations was 7.8% among 1213 newly diagnosed AML patients aged 15–86 years and 6% among 1612 newly diagnosed AML patient with median age of 35 years respectively (Byrd et al. 2002; Grimwade et al. 1998). AML with chromosomal 7 aberrations represent a heterogeneous group; frequently are related to others chromosomal aberrations and form a complex karyotype. In all AML cases with chromosome 7 aberrations, Monosomy 7 and deletion of 7q are present as single chromosomal alteration only in 35 and 33% respectively (Hasle et al. 1999). The exact pathogenetic role of −7 and 7q− in leukemogenesis is not fully understood. So far, it has been hypothesized that an important tumor suppressor gene is present in the long arm of the chromosome 7 and some authors offers the HIC (human I-mfa domain containing also called MyoD family inhibitor domain containing, MDFIC) gene as a possible candidate in leukemogenesis (Woo et al. 2009); however, further investigations are required to evaluate the role of this gene in the development of AML. The role of gene EZH2 in the pathogenesis of 7q− myeloid disorders has been shown by recent studies. EZH2 gene encodes the catalytic subunit of the polycomb repressive complex 2 (PCR2), a histone methyltransferase involved in stem cells renewal by epigenetic suppression of some others genes. According to some evidences, mutations of EZH2 gene lead to histone methyltransferase inactivity, which act as a tumor suppressor for myeloid malignancies (Ernst et al. 2010). Frequent multilineage dysplasia in bone marrow cells and worse clinical course with low rate of CR (20–30%) and low DFS and OS characterize AML patients with chromosome 7 aberrations, especially in AML patients with −7 or patients with 7q− in the context of a complex karyotype.

Monosomy and Deletion of Chromosome 5

Monosomy of chromosome 5 (−5) and deletion of the long arm of the chromosome 5 (5q−) represents approximately 6–9% of all the chromosomal abnormalities among newly diagnosed AML patients (Byrd et al. 2002).

Similarly to the aberrations of chromosome 7, these chromosomal alterations are frequently observed in patients previously exposed to alkylating agent or to other leukemogenic factor favoring multilineage dysplasia in bone marrow cells followed by MDS and finally by a secondary AML. Also in this case, there is not any general agreement among the international cooperative groups about how to classify AML with chromosome 5 aberrations. Although −5 AML is universally considered as an unfavorable cytogenetic risk, data are controversial for 5q− AML.

When considering patients with diagnosis of AML, The rate of detection of −5/5q− as single alteration was significantly lower. Alterations of chromosome 5

were linked to chromosome 7 abnormalities and more frequently (in 90% of cases) with a complex karyotype (Lessard et al. 2007). Frequent multilineage dysplasia in bone marrow cells and poor clinical course with low response rate to induction chemotherapy and high relapse rate are all the characteristics of AML patients with chromosome 5 aberrations.

14.3.5 Molecular Genetic

14.3.5.1 FLT3 (Fms-like Tyrosine Kinase 3)

FLT3 is a receptor tyrosine kinase family that is involved in hematopoiesis and frequently mutated in AML. Two common mutations that occur in *FLT3* gene: an internal tandem duplication (ITD) in the juxtamembrane domain and a point mutation of the tyrosine kinase domain (TKD) (Thiede et al. 2002). Both mutations lead to constitutive activation of receptor; however only the *FLT3* ITD is certainly related to a poorer prognosis of AML patients (Fröhling et al. 2002). *FLT3* ITD mutation occur in approximately 20% of all AMLs, but the *FLT3* ITD mutation is more frequent occur in AML patients with t(15;17) and AML with a normal karyotype (Fröhling et al. 2002). AML with a normal karyotype and *FLT3* ITD mutation has an adverse prognosis.

In AML patients, FLT3 ITD mutations are variable in the size and the ratio of *FLT3* ITD mutation to wild type allele. Studies have shown that patients with a higher *FLT3* ITD ratio have a poorer prognosis than patients with a lower ratio (Yohe 2015). However, allelic ratio does not include in recent risk stratification of AML patients (De Kouchkovsky and Abdul-Hay 2016). About 14–25% of *FLT3* ITD positive patients have more than one *FLT3* ITD mutation. However most studies have not shown a prognostic value of having multiple *FLT3* ITD mutations. The *FLT3* ITD size can be variable from a few base pairs to over thousand base pairs. In some studies it has been shown a correlation between size and patients prognosis (Yohe 2015).

Despite the differences in sequence, mutations seem to remain in-frame. In a study 91 unique insertion of the *FLT3* mutation were detected in AML patients showing the insertion site is highly variable. Nearly 30% of *FLT3* ITD occur in the first tyrosine kinase domain outside the juxtamembrane domain.

It has been shown that at least some of these *FLT3* ITD in the TKD1 domain result in constitutive activation (Breitenbuecher et al. 2009). A number of recent studies have shown worse prognosis with insertion in the TKD1 domain, but another study in 2012 did not (Schnittger et al. 2012; Schlenk et al. 2014; Kayser et al. 2009). So further studies are needed to determine whether specific mutations have different prognostic impacts or not.

For *FLT3* ITD positive AML with a normal karyotype, it is recommended allogeneic transplant; however, there is a high risk of relapse even with this transplant. Targeting *FLT3* ITD mutations with *FLT3* inhibitors is also interesting.

Unfortunately, success in this area has been limited till now. Coexistence or development of *FLT3* TKD mutations, activation of downstream signaling molecules, up-regulation of *FLT3*, or activation of other pathways are the possible reasons.

The less frequent *FLT3* TKD *FLT3* TKD mutation also results in constitutive activation of *FLT3* occur in about 10% of AML cases (Martelli et al. 2013). However, the *FLT3* TKD has not clearly been shown to have an effect on prognosis despite a seemingly similar mechanism of action.

14.3.5.2 NPM1 (Nucleophosmin 1)

NPM1 encodes a phosphoprotein that normally moved between the nucleus and cytoplasm and plays a key role in ribosome biogenesis, centrosome duplication during mitosis, and cell proliferation and apoptosis through regulation of p53 and p19Arf signaling pathway (Falini et al. 2007). Loss of the nucleolar localization signal and gain of a nuclear export signal ultimately leading to cytoplasmic localization of this protein caused by mutations in *NPM1* occur in the C-terminus of the gene. A 4 base pair insertion *is the most* common mutation of NPM1 gene in AML cases. In approximately 30% of all AML and 50–60% of cytogenetic normal AML, *NPM1* mutations were found therefore it is the most common genetic mutation in AML. *NPM1* seldom occurs with *CEBPA* gene mutation but frequently co-exist with *FLT3*, *DNMT3A*, and IDH (Yohe 2015).

Similar to the core-binding factor leukemias, the presence of an *NPM1* mutation in normal karyotype AML patients in the absence of a *FLT3* ITD mutation provide a good prognosis (Schlenk et al. 2008).

14.3.5.3 CEBPA

CEBPA (CCAAT/enhancer binding protein α) is an intronless gene that encodes a transcription factor and play an important role in myeloid differentiation. Mutations of CEBPA gene are found in approximately 10% of AML patients. Mutations of this gene are more common in AML with a normal karyotype or with 9q deletions. About two-thirds of cases with CEBPA mutations carry 2 mutations simultaneously (biallelic) and the remaining cases harbor single heterozygous mutations (monoallelic). AML patients with Normal karyotype harboring isolated biallelic CEBPA mutations have a better prognosis, while a monoallelic mutation does not likely confer the same favorable prognosis (Pastore et al. 2014). A recent meta-analysis showed that in long term follow-up, biallelic CEBPA mutations provide a longer overall survival compared with monoallelic CEBPA mutations. Mutations can occur across the whole gene, but cluster in two main hotspots: N-terminal or C-terminal. Biallelic mutations usually include one C-terminus (in-frame insertions/deletions) and one N-terminus (out-of-frame insertions/deletions) mutation and results in the absence of expression of

normal CEBPA (Mueller and Pabst 2006). The truncating N-terminal mutations lead to a shortened CEBPA protein that has a dominant negative effect. The C-terminal mutations lead to decreased dimerization or DNA binding (Wouters et al. 2009).

14.3.5.4 KIT

KIT is a receptor tyrosine kinase that plays a vital role in the regulation of cell proliferation, differentiation, and survival. Gain-of-function mutations of KIT occurring in the extracellular domain (exon 8) or in the kinase domain (exon 17) occur in 2–14% of all AML cases (Kihara et al. 2014). The incidence of KIT mutations being found in about 7–46% of cases is higher in core-binding factor leukemia, carrying t(8;21)(q22;q22) or inv(16)(p13q22)/t(16;16)(p13;q22) (Beghini et al. 2004). It is widely accepted that the presence of KIT mutations in CBF-AML patients is associated with a worse prognosis and can transform CBF-AML patients from favorable-risk AML to intermediate-risk AML (Park et al. 2011). There are conflicting results on prognostic significance of KIT mutations. While some studies have shown that the KIT mutation is significantly associated with decreased overall survival (OS) in CBF-AML patients, other studies have shown that KIT mutations have no important influence on CBF-AML clinical outcomes (Cairoli et al. 2013).

14.3.6 Epigenetic

14.3.6.1 DNA Methylation

Aberrant DNA methylation patterns are characteristics of acute myeloid leukaemia (AML) and are found in AML of all French–American–British (FAB) subtypes and throughout all cytogenetic risk groups (Mehdipour et al. 2015). Several genes, including p15, MDR1, ER, and HIC1, have been shown to be inactivated by hypermethylation in AML cases. The p15 gene, an inhibitor of cyclin-dependent kinsase-4 and a negative regulator of the cell cycle, is hypermethylated in the majority of patients with AML. *p15* (*CDKN2B*) is a tumor suppressor gene that its ablation in mice lead to lymphoproliferative disorders and tumours (Mehdipour et al. 2015).

It has been shown that clinical outcomes of AML patients could be influenced by methylation patterns for instance, hypermethylation of p15 has been shown to be associated with a poor outcome whereas hypermethylation of ER has been found to be associated with better survival. The simultaneous hypermethylation of several genes in AML has been demonstrated by several groups. A study determined the methylation status of eight genes in 20 AML patients and detected aberrant methylation in 19 samples. Moreover 15/20 samples carried two or more hypermethylated genes.

Another study showed that hypermethylation of p15, p16, CACNA1G, MINT1, MINT2, MDR1, THBS1 and PTC1 is relatively infrequent (6–31% of patients) whereas hypermethylation of MYOD1, PITX2, GPR37 and SDC4 is frequent in AML (47–64% of patients). In this study, an opposite correlation was seen between age of patients and frequency of CpG island methylation in AML. In another study the methylation status of the promoter-associated CpG islands from 11 cancer-related genes was analyzed in 60 adult AML patients. The results of this study showed that in 70% of the AML patients at least one gene was methylated at diagnosis and SOCS-1 (45%), p15 (31%) and (RARb2) (20%) were methylated with higher frequency. There exists evidence to suggest that AML-associated fusion proteins may have an important role in establishing specific DNA methylation patterns in AML cases.

For instance, PML/RARα and AML1/ETO resulting from t(15;17) and t(8;21), respectively, induce transcriptional repression of target genes by recruitment of HDACs and DNMTs. These data demonstrate how genetic changes may be linked with epigenetic changes to induce leukaemogenesis process.

A recent large study has shown that promoter hypermethylation of the *RARB2* (Acid Receptor Beta) gene is a frequent event in AML patients and is correlated with the presence of CBFbeta-MYH11 fusion transcripts.

This gene has been found to be silenced in several types of solid tumors, however further studies are needed to determine its role in leukaemias.

Interestingly, in a study hypermethylation of *BRCA1* promoter and reduced expression of this gene was reported in therapy-related AML (t-AML).

BRCA1 has an important role in the double strand DNA break repair. Hypermethylation of BRCA1 was found in 76% of t-AML cases and 31% of de novo AML cases.

Diagnosis of AML

AML is an aggressive, clonal myeloid malignancy with maturation arrest of granulopoiesis, leading to an accumulation of myeloid progenitors in marrow and/or blood. According to the current WHO classification, myeloblasts must comprise at least 20% of nucleated cells in bone marrow or blood to establish a diagnosis of AML. This cutoff is admittedly arbitrary and has been as high as 30% in the prior FAB classification scheme. Conversely, some myeloid malignancy with smaller numbers of myeloblasts (such as acute erythroid leukemia and some therapy-related myeloid leukemia) may display aggressive behavior in spite of lower blast counts. In establishing a diagnosis of AML, it is critical to obtain a precise blast count performed on at least 500 nucleated bone marrow cells and at least 200 PB leukocytes. In instances of a ‘dry tap’, it is acceptable to perform a myeloblast count on an air-dried touch preparation. The blast count in well-prepared aspirate smears or touch preparations usually correlates with the blast estimate in the bone marrow biopsy and the blast percentage obtained by flow cytometry. However, the ‘gold standard’ remains the aspirate smear blast count: neither flow cytometry nor biopsy blast estimate should be used in lieu of the

aspirate blast count, unless the aspirate smears are compromised or otherwise appear to be not representative (Hasserjian 2013).

There are some exceptions to the requirement of 20% myeloblasts in blood or bone marrow to establish a diagnosis of AML:

- In cases with the cytogenetic abnormalities t(15;17), inv(16)/t(16;16), or t(8;21), a diagnosis of AML can be made irrespective of blast count.
- In cases with monocytic differentiation, promonocytes (primitive monocytic cells with features intermediate between monocytes and monoblasts) are included along with blasts in the blast count.
- In cases where erythroid elements comprise at least 50% of the bone marrow nucleated cells and blasts
- Comprise more than 20% of the nonerythroid cells, a diagnosis of acute erythroleukemia (erythroid-myeloid subtype) is made.
- In cases of pure erythroid leukemia, undifferentiated erythroblasts replace the marrow and comprise

More than 80% of the marrow cells count, even though myeloblasts are not increased.

Myeloid lineage of blasts may be established by the identification of Auer rods, presence of myeloperoxidase (MPO) cytochemical staining or, more commonly, by demonstrating expression of myeloid markers such as CD13, CD33, CD117, or MPO by flow cytometry. Leukemias with monocytic differentiation show folded or indented nuclei and relatively abundant pale basophilic cytoplasm, often express the monocytic enzyme nonspecific esterase by cytochemistry, and express one or more monocytic markers (CD14, CD64, CD11c, or CD11b) by flow cytometry (Hasserjian 2013).

14.3.7 Epidemiology

AML accounts for 15–20% of the acute leukemia in children and 80% of the acute leukemias in adults. It is somewhat more frequent in males. Little difference in occurrence is seen between individuals of African or European ethnic at any age. A slightly lower occurrence is seen in persons of Asian ethnic (Yang and Zhang 1991).

AML is the main form of leukemia during the neonatal period but represents a small proportion of cases during childhood and adolescence. In the U.S. each year about 20,000 new cases of AML occur annually, representing about 35% of the new cases of leukemia. Each year in the U.S. about 12,000 patients with AML die as a consequence of the AML. The occurrence rate of AML is about 1.5 per 100,000 in infants less than 1 year of age, decreases to approximately 0.4 per 100,000 children ages 5 to 9 years, increases gradually to about 1.0 persons per 100,000 population until age 25 years, and afterward increases exponentially until the rate reaches about 25 per 100,000 persons in octogenarians. The exception to this exponential

age-related increase in incidence is APL, which does not change greatly in incidence with age (Vickers et al. 2000)

14.3.8 Treatment

Cases with eligibility to achieve CR first undergo induction therapy. Unfortunately, in CR phase minimal residual disease often persists, and relapse will unavoidably occur if treatment is discontinued. Thus, in order to eradicate minimal residual disease and achieve lasting complete remission a favorable response to induction therapy should be followed by consolidation therapy. The pivot of induction therapy consists of the '7 + 3' regimen, which combines 7 continuous days of cytarabine infusion with 3 days of anthracycline. It is usually offered to cases with an intermediate to good prognosis and a low risk of TRM for example, younger cases with better performance state, normal serum creatinine, albumin and platelet count (Estey 2014). Induction therapy studies using either daunorubicin at 60 or 90 mg/m^2, or idarubicin at 12 mg/m^2 have shown similar rates of CR and survival (Estey 2014, Gong et al. 2015). Some of cases with DNMT3A and KMT2A mutations, which demonstrate an adverse prognostic factor, may however benefit from higher doses of daunorubicin (Patel et al. 2012). Cytarabine standard dose consists of 100–200 mg/m^2 daily administered as a continuous infusion over 7 days. However other researches have shown superior efficacy at higher doses, this added benefit is small and accrued at the cost of increased toxicity (Estey 2014; Löwenberg 2013; Löwenberg et al. 2011) generally induction regime with high-dose cytarabine is reserved for refractory cases. Fludarabine, cytarabine, G-CSF and idarubicin (FLAG-IDA) combination, which was traditionally used for relapse treatment, has also been shown to be a rational alternative to standard induction therapy and results in similar CR rates and OS overall but higher rates of CR after a single course (Burnett et al. 2013).

14.4 Myeloproliferative Neoplasms

14.4.1 Overview

The myeloproliferative neoplasms (MPNs), unlike MDS, usually show end stage of myeloid cell proliferation in the PB (Dickstein and Vardiman, 1995). MPNs disorder include polycythemia Vera(PV), essential thrombocythemia(ET), chronic myeloid leukemia (CML), primary myelofibrosis (PMF), chronic neutrophilic leukemia(CNL), chronic eosinophilic leukemia, and mast cell disease.

The myelodysplastic/myeloproliferative neoplasms (MDS/MPN) include disorders where both proliferative and dysplastic features are present. These include

chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia (JMML), atypical CML (aCML, BCR-ABL1 negative), MDS/MPN with ring sideroblasts and thrombocytosis, and unclassifiable MDS/MPN.

14.4.2 Definition

CML: CML is a multipotential hematopoietic stem cell disorder characterized by anemia, very leukocytosis with shift to the left, basophilia, often thrombocytosis (Fig. 14.14a, b), and splenomegaly. In more than 95% of CML patients the hematopoietic cells contain a reciprocal translocation between chromosomes 9 and 22 with classic morphologic findings, referred to as the Philadelphia (Ph) chromosome. On the long arm of chromosome 22 defines a rearrangement of the breakpoint cluster region gene (BCR) and is present even in the 10 percent of patients without an obvious 22q abnormality by Giemsa chromosome banding. The disease can be progressive and to undergo clonal evolution into an accelerated phase and/or a blast phase, known as acute leukemia, highly refractory to treatment, which had been a common event prior to the introduction of tyrosine kinase inhibitors (TKIs) in 2001(Apperley 2015).

14.4.3 Classification

The aim of this section is to summarize the major changes in the revised WHO classification of myeloproliferative neoplasms to provide the rationale for those changes.

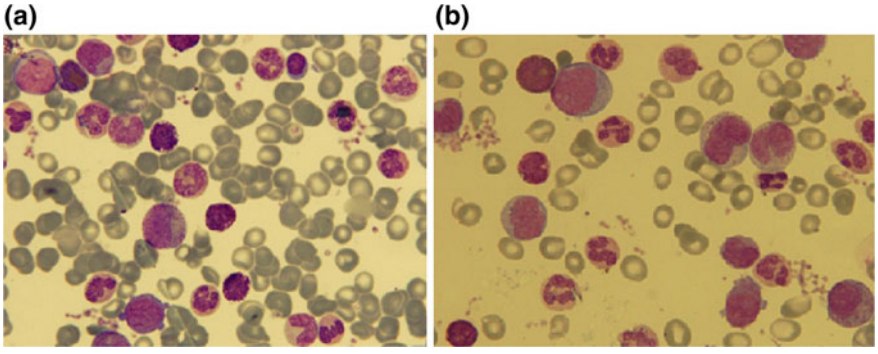

Fig. 14.14 Chronic myeloid leukemia: peripheral blood films showing various stages of myeloid cells. **a** Contains three basophils. High power view (**a** and **b**) (Used with permission from A. Ghavamzadeh: A color atlas of morphologic Hematology)

Table 14.3 WHO classification of myeloproliferative disorder

Chronic myeloid leukemia (CML), *BCR-ABL1*$^{+}$
Chronic neutrophilic leukemia (CNL)
Polycythemia Vera (PV)
Primary myelofibrosis (PMF) · PMF, prefibrotic/early stage · PMF, overt fibrotic stage
Essential thrombocythemia (ET)
Chronic eosinophilic leukemia, not otherwise specified (NOS)
MPN, unclassifiable
Mastocytosis

Table 14.3 lists the major subtypes of myeloid neoplasms according to the updated (2016) WHO classification (Arber et al. 2016)

14.4.4 Common Chromosomal Aberrations

Chronic Myeloid Leukemia (CML) is a myeloproliferative disorder characterized by a balanced chromosomal translocation, t(9;22)(q34;q11.2) (Fig. 14.15), involving a fusion of ABL from chromosome 9q34 with the BCR gene on chromosome 22q11.2. This rearrangement is known as the Philadelphia chromosome leads to generation of a BCR-ABL fusion oncogene translated into a Bcr-Abl oncoprotein. BCR-ABL is a constitutively active tyrosine kinase that through downstream pathways such as RAS, RAF, JUN kinase, MYC and STAT promotes growth and replication of leukemic cells. Generally, all CML cases have a t(9;22), at least at the molecular level, with the presence of a BCR/ABL gene rearrangement. The identification of this abnormality is important for the diagnosis of the disease and for treatment purposes.

Around 5–10% of CML patients may harbor variant types of Ph characterized by the involvement of another chromosome in addition to chromosome 9 or 22 (Fig. 14.16) (Heim et al. 1985).

Variant Ph is simple when only one additional chromosome is involved or complex when two or more than two chromosomes, besides chromosomes 9 and 22, are involved.

The generation mechanism of variant Ph and the biological differences between classic and variant Philadelphia chromosomes are not fully understood. A recent studies showed that 59 genes including differently expressed in patients harboring classic Ph compared with those harboring variant Ph chromosomes. Some of these genes including *TR1B1*, *PTK2B* and *C5AR1* were involved in the MAPK pathway, which has a key role in CML pathogenesis. However, it has been showed that variant Ph chromosomes does not impact on clinical outcome of CML patients.

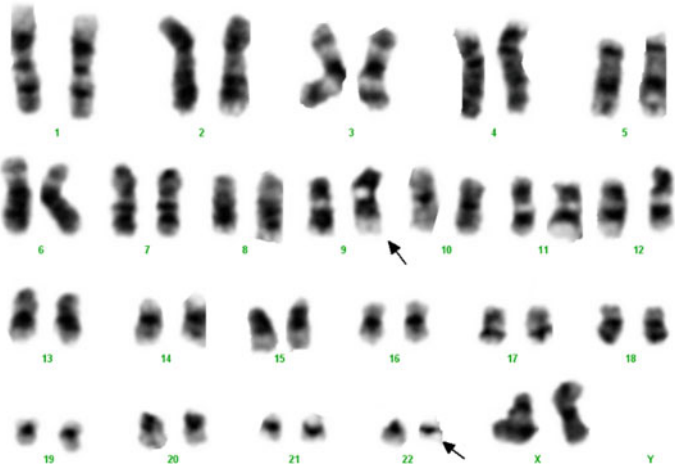

Fig. 14.15 Cytogenetic analysis of a Female with Chronic Myeloid Leukemia by G-banding identified 46, XX, t(9;22)(q34;q11.2). *Arrows* indicate abnormal chromosomes. The figures adopted from cytogenetic lab of hematology, oncology and stem cell transplantation research center, Tehran University of Medical sciences, Dr. Yaghmaie's Archives

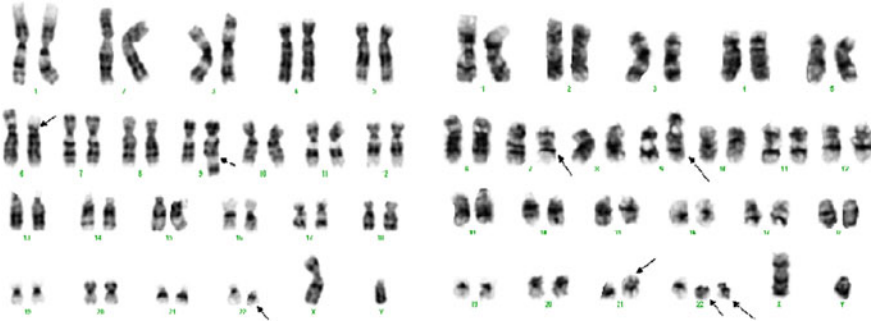

Fig. 14.16 *Left* Karyotype of a male with Chronic Myeloid Leukemia with Three-Way Translocation, 46, XY, t(6;9;22)(p21.1;q34;q11.2). *Right* Karyotype of a male with Chronic Myeloid Leukemia with three-way translocation, 46, XY, t(9;22;7)(q34;q11.2;q22), +der(22)t (9;22)(q34;q11.2). *Arrows* indicate abnormal chromosomes. The figures adopted from cytogenetic lab of hematology, oncology and stem cell transplantation research center, Tehran University of Medical sciences, Dr. Yaghmaie's Archives

The most of CML cases have classic Ph or its variant as the sole abnormality at time of diagnosis. However, additional chromosomal abnormalities (ACAs) such as +Ph, +8, +Y or i(17)(q10) in Ph+ cells may occur in about 5% of cases. The emergence of ACAs during the treatment is considered as a poor prognostic feature and generally known as clonal evolution (CE) and seems to play a key role in imatinib resistance (Heim et al. 1985).

14.4.5 Molecular Genetic

14.4.5.1 Drug Resistance

Despite a majority of CML patients initially respond to the tyrosine kinase inhibitor (TKI) imatinib mesylate (IM), approximately 30% of patients develop resistance to imatinib treatment. Biochemical and molecular analysis have shown that there are a number of mechanisms by which resistance to imatinib arises including overexpression of BCR-ABL1, and the emergence of mutations in the ABL-kinase domain as well as the development of BCR-ABL1-independent pathways of signal transduction. An initial study on mechanism of imatinib resistance in CML patients showed that there is a single amino acid substitution in a threonine residue (T351I) of the ABL kinase domain in 6 of 9 patients (Srivastava and Dutt 2013). In other patients BCR-ABL gene amplification is associated with imatinib resistance (Czyżewski et al. 2012). Sequencing of the BCR-ABL gene in patients who relapsed after imatinib chemotherapy revealed a limited set of kinase domain mutations that mediate drug resistance.

14.4.6 Epigenetic

14.4.6.1 DNA Methylation in CML

CML may display a biphasic or triphasic course and progresses through a series of stages. Patients who were initially diagnosed in the chronic phase (CP) frequently pass through an intermediate or accelerated phase (AP), and finally evolve into blast crisis (BC) in which transformation to an acute leukaemia occurs. In order to better understand the molecular mechanisms underlying disease progress, several studies have been conducted to investigate DNA methylation at each of the three stages of CML. Increasing levels of methylation of the various genes such as calcitonin (CALCA), HIC1, ER and ABL1 genes have been found during CP to BC progression.

The BCR-ABL chromosomal translocation has a central role in the pathogenesis of CML. An upstream promoter of ABL1 (Pa) with the coding region of the ABL1 are often translocated intact to the BCR locus. The Pa promoter appears to be silent in most CML cases. Recently, it is demonstrated that methylation of Pa promoter represents a likely marker of CML pathogenesis and hypermethylation of Pa is associated with advanced stages of the disease. HIC1 (Hypermethylated in Cancer 1) is a tumor suppressor gene located 17p13.3 that has been shown to be methylated in 50% of patients in CP-CML, and all patients in blast-crisis. In contrast to other myeloid malignancies, methylation of the p15 tumor suppressor gene promoter is not or only very rarely observed in CML and the significance of *p15* methylation in CML patients is not fully understood. The loss of imprinting of IGF2 by

demethylation and biallelic expression of IGF2 in CML was associated with disease progression and occurred in all patients with BC-CML but rarely in CP-CML.

RASSF1A, TFAP2A, EBF2, ATG16L2, DAPk1, OSCP1, PGRA and PGRB are another candidate genes that differentially methylated during CML progression (Boultwood and Wainscoat 2007).

14.4.7 Diagnosis

Among the classic MPNs, only CML is cytogenetically characterized by the reciprocal chromosomal translocation between chromosomes 9 and 22, t(9;22). This translocation is related with a shortened chromosome 22 (the Philadelphia chromosome) in 95% of the cases. In the remaining cases, t(9;22) can be established by either FISH or RT-PCR techniques for detection of BCR-ABL1.

Regarding to CML, BCR-ABL1+, most CML patients can be diagnosed from PB findings conjugated with identification of t(9;22) in chronic stage or,more specifically, BCR-ABL1 by molecular methods. However, a BM aspirate is essential to ensure adequate material for morphologic assessment and for a complete karyotype to confirm the disease phase (Jabbour and Kantarjian 2014; O'Brien et al. 2014). Newly diagnosed CML patients in the era of tyrosine-kinase inhibitor (TKI) therapy may have an almost normal lifespan, but regular MRD follow-up monitoring by RQ-PCR and for BCR-ABL1 burden and also for evidence of genetic alterations and the development of TKI therapy resistance assessment is essential to identify disease progression (Baccarani et al. 2013, Hehlmann 2015). Although in the TKI therapy era the accelerated phase (AP) of CML is becoming less frequent, there are no generally accepted criteria for its definition. The AP criteria in the 2016 revision to the WHO classification include hematologic, morphologic, and cytogenetic factors which are supplemented by additional factors frequently attributed to genetic evolution (Deininger 2015) and demonstrated by evidence of resistance to TKIs (Table 14.4).

Within the context of the MPNs, a raised red cell mass is specific for PV. Occasionally both CML and MDS may present with either isolated thrombocytosis suggesting ET (Bench et al. 2013), or related bone marrow fibrosis suggesting myelofibrosis (Bench et al. 2013; Singh et al. 1994). As a result, the diagnostic work up of patients with suspected MPN should always include cytogenetic analysis and careful morphologic assessment to rule out the presence of t(9;22) (CML) and dysmyelopoiesis (myelodysplastic syndrome), respectively.

Though most of the chronic myeloid disorders are classifiable as MDS, CML, PV, ET, or PMF, some are difficult to categorize and can be referred to as atypical MPNs (Singh et al. 1994). Rare patients expressing both BCR-ABL1 and a JAK2 mutation have been described (Singh et al. 1994).

Table 14.4 WHO diagnostic criteria for CML, accelerated phase

CML, accelerated phase criteria	
Any 1 or more of the following hematologic/cytogenetic criteria or response to TKI criteria	
Persistent or increasing WBC (More than 10×10^9/L), unresponsive to therapy	"Provisional" response-to-TKI criteria
Persistent or increasing splenomegaly, unresponsive to therapy	Hematologic resistance to the first TKI (or failure to achieve a complete hematologic response to the first TKI) or
Persistent thrombocytosis (more than 1000×10^9/L), unresponsive to therapy	Any hematological, cytogenetic, or molecular indications of resistance to 2 sequential TKIs or
Persistent thrombocytopenia (less than 100×10^9/L) unrelated to therapy	Occurrence of 2 or more mutations in BCR-ABL1 during TKI therapy
20% or more basophils in the PB	
10–19% blasts in the PB and/or BM	
Additional clonal chromosomal abnormalities in Ph1 cells at diagnosis that include "major route" abnormalities (second Ph, trisomy 8, isochromosome 17q, trisomy 19), complex karyotype, or abnormalities of 3q26.2	
Any new clonal chromosomal abnormality in Ph1 cells that occurs during therapy	

14.4.7.1 Flow Cytometry in MPD

In CML reported abnormalities include abnormal expression of CD56 on blasts and myeloid cells (Lanza et al. 1993) decreased CD16 on granulocytes (Carulli et al. 1992) decreased L-(CD62L) and P-selectin (CD62P) expression on CD34+ cells (Martín-Henao et al. 2000; Sullivan et al. 2011) and abnormal expression of lymphoid antigens such as CD2, CD5, and CD7 on the blasts in CML blast crisis (Khalidi et al. 1998). In non-CML MPN, the most common changes were abnormal expression of CD13, CD33, HLA-DR, and/or CD16 on maturing granulocyte precursors. BM eosinophils, found by expression of relatively bright CD11b, CD13, CD15, and CD45, without CD16, are expanded markedly in the patients with putative chronic eosinophilic leukemia. A higher incidence of basophils with aberrant immunophenotype was also identified in different MPNs (Kussick and Wood 2003). Increased CD56 expression and minor size of granulocytes as measured by Flow Cytometry were described in primary myelofibrosis (Singh et al. 1994; Feng et al. 2010). Increase of cells in the blast gate and emerging abnormal phenotypes in the blast population herald transformation to AML.

14.4.8 Epidemiology of CML

Each year, about 5000–7000 persons are identified with CML in the U.S. The annual occurrence is 1–2 cases per 100,000 individuals. CML accounts for about 15% of all cases of leukemia, or about 6500 new cases in the U.S. in 2015. The age-adjusted incidence rate in the U.S. is about 2.3 per 100,000 individuals for men and about 1.2 per 100,000 individuals for women. The incidence around the world varies by a factor of about twofold. The lowest incidence is in Sweden and China (about 0.7 per 100,000 individuals), and the highest incidence is in Switzerland and the U.S. (about 1.5 per 100,000 individuals) (Redaelli et al. 2004). CML comprises less than 5% of pediatric leukemia.

Treatment of MPN-associated acute leukemia-Treatment of Leukemic/blast phase transformation (LT) of an MPN is usually difficult. Current agreement is to induce such patients into complete remission using AML-like induction chemotherapy followed by allogeneic hematopoietic cell transplantation (allo-HCT). However, few studies propose the potential value of such an approach.

14.4.9 Targeted Therapy for MPDs

The detection of activating mutations in the JAK2 gene in MPDs disorder provides a potential opportunity to improve targeted therapies and lower side effects for patients with these genetic alteration. It is believed that JAK2 inhibition kinase activity will be valuable for *JAK2V617F*+ patients, particularly in light of the imatinib safety and efficacy and next generation inhibitors of ABL kinase for the treatment of CML (Druker et al. 2001; Shah et al. 2004; Kantarjian et al. 2006). This has led several teams to develop specific JAK2 inhibitors (Pardanani et al. 2007) and based on activity in MPD preclinical models (Wernig et al. 2008). Phase 1 clinical trials with inhibitors of JAK2 have been started in PMF and post-PV/ET myelofibrosis (Verstovsek et al. 2007a, b). However, there is significant optimism that inhibition of JAK2 will offer considerable benefit for MPD patient, there also may be important toxicities related with inhibition of JAK kinase. Potential side effects of candidate inhibition of JAK2 must be assessed carefully. Furthermore, Janus kinase family of enzymes (JAK1, JAK3), and TYK2 inhibitors should be minimized, particularly given the immune deficiencies associated with *JAK3* and *TYK2* inherited loss-of-function mutations (Minegishi et al. 2006; Russell et al. 1995; Macchi et al. 1995). Nonetheless inhibitors of JAK2 are currently being evaluated in PMF patients and Post–polycythemia vera myelofibrosis (post-PV MF), the indolent and benign clinical course of PV and ET and the safety and efficacy of current therapies (hydroxyurea, anagrelide, phlebotomy) for these disorders will necessitate that inhibitions of JAK2 have desirable side effect profiles before being evaluated in ET and PV. Moreover, the information from PV, ET, PMF and acute myeloblastic leukemia patients consistent with the

presence of a "pre-*JAK2*" malignant clone (Campbell et al. 2006; Theocharides et al. 2007) propose the possibility that inhibitors of JAK2 therapy might select for the emergence of *JAK2V617F*− clones for leukemic transformation. With improved understanding of the basic mechanisms underlying disease, therapies which target the type of molecular abnormalities in hematologic malignancy have increasingly been developed (Table 14.5).

As new disease alleles are detected, either alone or in conjunction with mutation of JAK2, extra therapies may develop that target these mutant alleles. Based on experimental response to treatment there is also still infancy for development of drugs. For instance, recently there are information of inhibitors activity of chromatin remodeling proteins in MPD such as histone deacetylase inhibitors (HDACi) and HSP90 inhibitors. (Guerini et al. 2008; Shi et al. 2007). If corroborated, these clinical observations may further inform molecular mechanisms of disease. Moreover, molecular responses have been detected in treated PV patients with pegylated interferon-α-2a (Shi et al. 2007), proposing present therapies might have targeted effects in PV, ET, and PMF.

14.5 Lymphoid Neoplasms

14.5.1 Overview

This section outlines the category of neoplastic lymphocyte and plasma cell disorders. It introduces a framework for assessing lymphoid neoplasms and plasma cell disorders, outlines clinical syndromes associated with such disorders, and guides the reader to the sections in the text that discuss each of these disorders with more detail.

14.5.2 Definition

Chronic lymphocytic leukemia (CLL) is a type of leukemia that starts from cells that become some WBC in the marrow. The leukemia cells initiate in the marrow but then go into the bloodstream (Fig. 14.17a, b).

Table 14.5 Targetable pathway activated in MPD

Myeloproliferative disorder	Genetic change/mutation	Function(s)
	BCR-ABL fusion, JAK2 muations, c-MPL and CALR	Kinase activation or ligand-independent signaling or hypersensitivity to lower levels of growth factor (Tefferi et al. 2009)

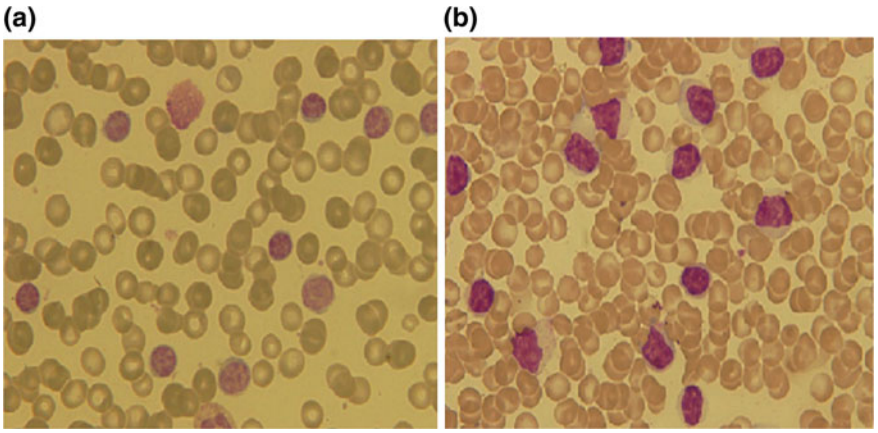

Fig. 14.17 Chronic lymphocytic leukemia: peripheral blood films showing lymphocytosis and smear cells. High power view (**a** and **b**) (Used with permission from A. Ghavamzadeh: A color atlas of morphologic Hematology)

In CLL, the leukemic cells frequently build up slowly over time, and some people don't have any signs and symptoms for at least a few years. In time, the leukemic cells may spread to other sites of the body, including the lymph nodes, liver, and spleen.

The categorization retains the goals of helping to recognize homogeneous groups of well-defined entities and facilitating the recognition of infrequent diseases that require further categorization (Campo et al. 2011). This text will review the key areas in lymphoid neoplasms where changes from the prior edition are foreknow as well as emphasize conceptual themes (Table 14.6).

Table 14.6 Prominent change in the 2016 WHO classification of chronic lymphoid neoplasms

Disorder	Alteration
CLL/SLL	(1) Cytopenias or disease-associated symptoms are now inadequate to make a diagnosis of CLL with less than 5×10^9/L PB CLL cells (2) Large/confluent and/or highly proliferative proliferation centers are bad prognostic markers (3) Mutations of potential clinical relevance, such as TP53, NOTCH1
Monoclonal B-cell lymphocytosis	(1) Should discriminate low-count from high-count MBL (2) A lymph node equivalent of MBL exists
Hairy cell leukemia	(1) BRAF V600E mutations in nearly of all cases with MAP2K1 mutations in most cases that use IGHV4-34 and lack BRAF mutation
Lymphoplasmacytic lymphoma (LPL)	(1) MYD88 L265P mutation in greater number of cases impacting diagnostic criteria even though finding is not specific for LPL (2) IgM MGUS is more closely correlated to LPL and other B-cell lymphomas than to myeloma

14.5.3 Classification

Mature B-cell lymphoid neoplasms.

A significant element that pervades several parts of the new monograph derives from an explosion of new clinical, molecular genetics and pathological information concerning the "small B-cell" lymphomas. The notion that there are lymphoid proliferations that we used to diagnose as overt lymphoid neoplasms but which are not considered as such in 2016 will be further emphasized. Between the aggressive B-cell lymphomas, there are main alterations that impact how these cases should be assessed and diagnosed that have vital therapeutic implications as well as being of biologic interest (Swerdlow et al. 2016).

14.5.4 CLL/SLL and Monoclonal B-Cell Lymphocytosis

The 2008 monograph identified monoclonal B-cell lymphocytosis (MBL) as the presence of B-cell monoclonal populations in the PB of up to 5×10^9/L either with the morphology and/or phenotype of CLL, atypical CLL, or non-CLL ($CD5^-$) B cells without other lymphomatous features. In up to 12% of normal individuals identified, in some it may be a very small population, but in others related with a lymphocytosis (Nieto et al. 2009). Whiles in 2008 it was unidentified whether monoclonal B-cell lymphocytosis was a precursor of CLL, now determined that monoclonal B-cell lymphocytosis precedes virtually all cases of CLL/small lymphocytic lymphoma (SLL) (Landgren et al. 2009). The revised WHO will keep the present criteria for monoclonal B-cell lymphocytosis, but will highlight that "low-count" MBL, well-defined as a PB CLL count of less than 0.5×10^9/L, should be discriminated against "high-count" MBL because MBL with low count has important differences from CLL, a very limited, if any, progression chance, and, until novel document is provided, outside of standard medical care does not require routine Follow-up. (Rawstron et al. 2010; Vardi et al. 2013). In contrast, high count MBL requires routine monitoring, and has extremely similar morphologic/phenotypic and molecular/genetic features as Rai stage 0 CLL, albeit mutation in immunoglobulin heavy chain variable region (IGHV) cases are more common in MBL (Morabito et al. 2013). Moreover impacting of 2016 revision of the WHO diagnostic criteria, the revision will remove the option to confirm of the diagnose of CLL with, 5×10^9/L PB CLL cells without extramedullary involvement even if there are cytopenias or disease-associated symptoms. Non-CLL phenotype (non-CLL-MBL), at least some of which may be closely associated to splenic marginal zone lymphoma, is also identified (Xochelli et al. 2014; Bruscaggin et al. 2014).

14.5.5 Epidemiology

CLL is one of the most frequent leukemia in adults in Western countries, accounting for approximately 25 to 30% of all leukemia in the U.S. (Siegel et al. 2016). The disorder is more common in men, with a male to female ratio of approximately 1.3:1 to 1.7:1 (Siegel et al. 2016; Hernández et al. 1995). The incidence rates among men and women in the U.S. are approximately 6.75 and 3.65 cases per 100,000 population per year, respectively (Yamamoto and Goodman 2008). In Europe, these incidence rates are 5.87 and 4.01 cases per 100,000 population per year, respectively (Sant et al. 2010).

14.5.6 Clinical Presentation

14.5.6.1 Symptoms and Signs

Patients with CLL may have a wide range of symptoms and physical and laboratory findings at the time of diagnosis. Some patients consult a physician because they have noted painless swelling of lymph nodes, often in the cervical area, which spontaneously wax and wane, but do not altogether disappear.

Lymphadenopathy

The most frequent abnormal finding on physical examination of the patient with CLL is lymphadenopathy, present in 50–90% of patients among various series (Rai et al. 1975; Binet et al. 1981). Lymphadenopathy may be generalized or localized, and individual lymph nodes can vary greatly in size. The most frequently affected sites are cervical, supraclavicular, and axillary.

Splenomegaly

The spleen is the second most commonly enlarged lymphoid organ, being palpably enlarged in 25–55% of patients (Rai et al. 1975; Binet et al. 1981). As is the case with enlarged lymph nodes, an enlarged spleen in CLL is usually painless and nontender to palpation, with a sharp edge and a smooth firm surface. Painful and infarcted splenic enlargement is an uncommon presenting feature.

Hepatomegaly

Enlargement of the liver may be noted at the time of initial diagnosis in 15–25% of patients (Rai et al. 1975; Binet et al. 1981). The liver is generally only mildly enlarged, ranging from 2 to 6 cm below the right costal margin, with a span of

dullness to percussion of approximately 10–16 cm. Upon palpation, the liver is usually nontender and firm with a smooth surface.

Up to 80% of patients with chronic lymphocytic leukemia (CLL) harbor Chromosomal abnormalities. Among these abnormalities deletions of 11q, 13q, 17p, and trisomy 12 have an important roles in CLL pathogenesis and are associated with patient's prognosis. As Standard methods both conventional G-banding cytogenetic (CGC) and fluorescence in situ hybridization (FISH) were routinely used identify these genomic aberrations. However, genomic arrays provide higher resolution allowing the detection of cryptic abnormalities. Although, certain cytogenetic abnormalities in CLL cases are associated with an adverse clinical outcome and have become important prognostic factors, these aberrations may not be responsible for the whole clinical heterogeneity of CLL. Recent studies using next generation sequencing techniques have allowed the identification of new genomic abnormalities which may explain part of the clinical heterogeneity of CLL. In this session main genetic abnormalities identified in CLL patients and their clinical importance were summarized.

14.5.7 Common Chromosomal Aberrations

14.5.7.1 13q14 Deletion

Deletion of 13q14 region (Fig. 14.18) is the most frequent structural abnormality in CLL that found in more than 50% of cases (Döhner et al. 2010). Monoallelic deletion occur in 76% and biallelic deletion occur in 24% of cases, and deletions occur most commonly in patients who have mutated IgVH disease. It is showed that according to percentage of cells with del(13q) CLL patients have clinical course.

Higher proportion (>80%) of del(13q) cells is associated with shorter OS and time to first therapy (Hernández et al. 2009). There are several candidate genes located at 13q that could be responsible for CLL pathogenesis. DLEU2 gene and microRNA (miR)-15a/16-1 are located in the minimal deleted region (MDR) (Ouillette et al. 2008). DLEU2 encodes a noncoding RNA with unknown function, whereas miR-15a/16-1 have been described to exhibit a tumor suppressor function in CLL. Recent studies show that apart from (miR)-15a/16-1, other genes located in 13q, such as DLEU7 and RB1 could cooperate in the tumor suppressor activity. It has been widely shown that large 13q losses involving RB1 gene are associated with shorter overall survival (OS) than those small deletions encompassing only miR-15a and miR16-1.

14.5.7.2 Trisomy 12

Trisomy 12 is the third most common cytogenetic change in CLL and is detected in 10–20% of cases. Trisomy 12 often appears as the sole cytogenetic alteration. It is

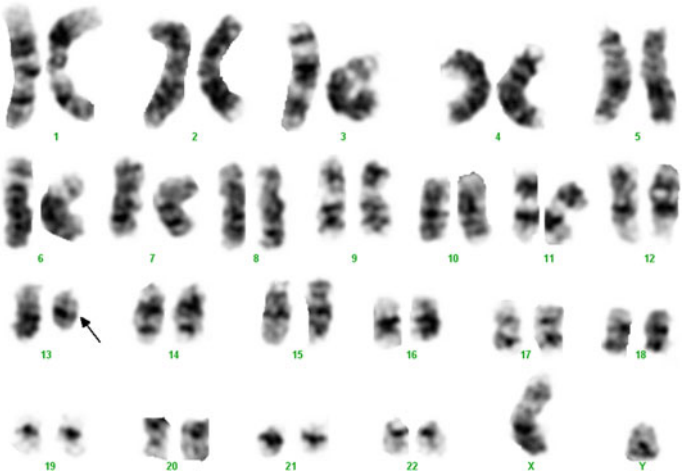

Fig. 14.18 Karyotyping results from a CLL case showing the characteristic cytogenetic lesion del (13q14) indicated by the *arrow*. The figures adopted from cytogenetic lab of hematology, oncology and stem cell transplantation research center, Tehran University of Medical sciences, Dr. Yaghmaie's Archives

associated with an intermediate prognosis when seen as the sole anomaly and is associated with shorter survival time compared to patients with 13q abnormalities and normal karyotypes when seen in conjunction with add(14q) abnormalities (Rossi et al. 2013).

14.5.7.3 11q23 Deletion

Deletion 11q22-23 (Fig. 14.19) is detected in 5–20% of CLL patients. It is seen in younger patients, and is associated with rapid disease progression, and poor survival. The critical region for the deletion is a 3-Mb segment at 11q22.3-q23.1 harboring ATM, RDX, FRDX1, RAB39, CUL5, ACAT, NPAT, KDELC2, EXPH2, MRE11, H2AX, and BIRC3. Deletion of ATM gene is occurred in almost all CLL cases. ATM is a DNA damage response protein that activated by DNA double-strand breaks and activation of TP53 by its phosphorylation. About 30% of patients with 11q22-23 deletion have a mutation on the remaining ATM allele and this subgroup has a poorer overall survival (OS) than the group of patients with a deletion 11q22-23 and wild type ATM on the other allele (Wierda et al. 2011; Tsimberidou et al. 2009). BIRC3 is another gene that located near to ATM gene, at 11q22. IRC3 mutations have been detected in 4% of newly diagnosed CLL patient and 24% of chemorefractory CLL patients.

14.5.7.4 17p13 Deletion

Deletion of 17p is occurs in about 3–8% of CLL patients at diagnosis and usually is associated with a mutation of TP53 on the other allele and these patients usually have aggressive and drug-resistant disease with poor survival (Delgado et al. 2012). Moreover, it is found in up to 30% of refractory CLL patients.

14.5.8 Molecular Genetic

The study of the CLL genome by the recent advent technologies such as NGS revealed several genetic mutations that are important for outcome prediction of CLL patient. Mutations detected in several genes such as NOTCH1, MYD88, SF3B1, TP53 and ATM provide new prognostic information and impact the future management of CLL patients.

14.5.8.1 P53 Mutations

It has been recognized that patients with CLL and p53 mutations have an aggressive clinical course and resistance to chemotherapy. About 15% of patient with previously untreated CLL harbor p53 mutations. However, CLL patients harboring

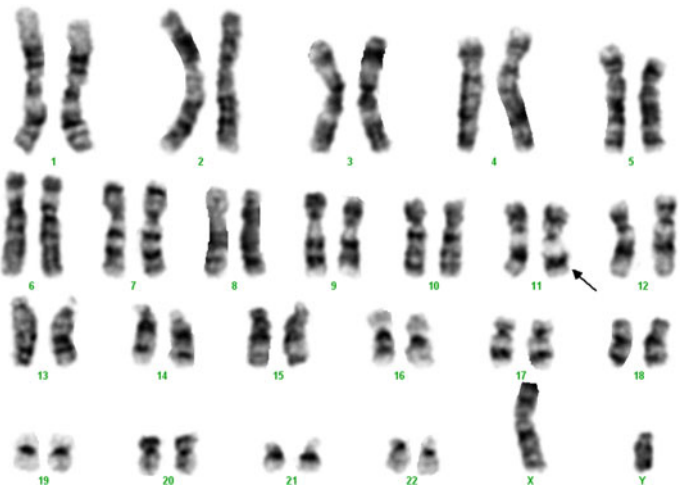

Fig. 14.19 Karyotyping results from a CLL case showing the characteristic cytogenetic lesion del (11q22q23) indicated by the *arrow*. The figures adopted from cytogenetic lab of hematology, oncology and stem cell transplantation research center, Tehran University of Medical sciences, Dr. Yaghmaie's Archives

deleted or mutated *TP53* have a considerable prognostic heterogeneity that may reflect the percentage of the mutated leukemic clone, mutation type and position and the functional properties of the mutated protein.

14.5.8.2 NOTCH1 Mutation

NOTCH1 gene located in the chromosome 9q34.3 encodes a trans-membrane protein involved in the regulation of hematopoietic cell development (Suresh and Irvine 2015). A frame shift deletions two nucleotide deletion (c.7541_7542delCT) at the C-terminal region is the most frequent mutation of this gene (Willander et al. 2013). This mutation produces a truncated, stable isoform of the NOTCH1 leading to constitutive activation of the NOTCH1 pathway. Mutation of NOTCH1 occurs in 4–10% of newly diagnosed CLL patients and 20% of refractory CLL disease. NOTCH1 mutations are an independent predictor of CLL overall survival (OS) and identify a subset of high-risk patients with poor prognosis (Willander et al. 2013). NOTCH1 mutations are strongly associated to other markers of poor prognosis in CLL patients such as un-mutated IGHV, ZAP-70 overexpression, CD38 positivity, and trisomy 12. Impact of NOTCH1 aggressiveness of CLL is similar *TP53* abnormalities.

14.5.8.3 SF3B1 Mutation

SF3B1 gene, located in long arm of chromosome (2q33.1), encodes subunit 1 of the splicing factor 3b that together with other ribonucleoproteins create the spliceosome to pre-mRNA splicing. The most frequent mutation of SF3B1 gene are missense mutations and in-frame deletions occur in codons Lys666, Lys700, and Gly742 (Rossi et al. 2011). Mutations of SF3B1 can be detected in about 10% of newly diagnosed CLL patients and 17% of patients with chemo-refractory disease.

SF3B1 mutations leads to abnormal mRNA splicing of transcripts involved in cancer-related processes, including cell-cycle control, angiogenesis, and apoptosis.

Some studies suggest that mutations of SF3B1 are an independent factor for adverse prognosis in CLL patients and CLL patients with SF3B1 mutations have a shorter PFS and TFS compared with CLL patients with wild type SF3B1(Rossi et al. 2013; Amaya-Chanaga and Rassenti 2016).

However, mutations of SF3B1 in CLL patients have not been correlated with decreased OS.

14.5.8.4 ATM Mutation

The ATM gene located on the long arm of chromosome 11(11q22-q23), encodes protein that activated by DNA double-strand breaks and by phosphorylation of

several proteins initiate DNA damage checkpoint signaling activation and leading to cell cycle arrest, DNA repair or apoptosis.

Wide spectrum of mutation such as nonsense or missense substitutions, in-frame or frame-shift insertions or deletions occurred in ATM gene were seen in CLL patients (Guarini et al. 2011). These mutations can cause lack of function of the ATM protein that affects the prognosis of CLL patients. Mutations of ATM are detected in 25% of CLL patients at diagnosis and are correlated with un-mutated IGHV, ZAP-70 overexpression, and del(11q). Early studies focusing on molecular genetics of CLL demonstrated that 11q22-q23 deletions are major determinants of chemo-refractoriness in CLL. In addition these deletions always include the ATM gene.

Although, ATM mutations are detected In 30–40% of CLL patients harboring del(11q), del(11q) in patients harboring ATM mutations varies from 22 to 75%.

Recent studies showed that the prognosis of patients with del(11q) and ATM mutations is inferior compared with patients of with del(11q) only, and also with decreased OS when compared with patients without del(11q) (Austen et al. 2007).

14.5.8.5 MYD88 Mutation

The MYD88 gene located on the short (p) arm of chromosome 3(3p22) encodes a cytosolic adapter protein that plays an important role in the innate and adaptive immune response. This protein is an essential signal transducer in the interleukin-1-Toll-like receptor (TLR) signaling pathways that regulate the activation of numerous proinflammatory genes (Rawlings et al. 2012).

MYD88 protein contains of an N-terminal death domain and a C-terminal.

Toll-interleukin1 receptor domain.

MYD88 gene mutations have been detected in 3–5% of CLL patients. Patient with Mutations in MYD88 in contrast to those with NOCTH1, SF3B1, TP53 and ATM mutations have favorable outcomes. MYD88 mutations in CLL patients are correlated with down-regulation of ZAP-70 and CD38. These mutation more frequently found in younger patients independently of their clinical stage. Moreover, MYD88 mutations identify a subgroup of young CLL patients with good outcome (Martínez-Trillos et al. 2014).

14.5.9 Epigenetic

14.5.9.1 DNA Methylation in CLL

The level of DNA methylation CLL patients is generally lower than healthy individuals. However, regional hypermethylation of gene promoters leads to gene silencing in these patients. DNA methyltransferases I and IIIa are predominant DNA methyltransferases expressed in CLL cells and down-regulation of DNA

methyltransferase IIIb was seen in CLL cells compared with normal B lymphocytes. Moreover, DNA methyltransferase I is not up-regulated in CLL. Several studies have attempted to identify disease causing genes in patients with CLL that are aberrantly methylated and multiple genes have been reported such as Twist2, CDH1, CDH13, DAPK, CRBP1, and RARb. However, DNA methylation appears to be only one component of epigenetic modulation and expression of other genes that have important roles in epigenetic alteration such as histone methyltransferases, methyl-CpG binding proteins and chromatin associated proteins may all contribute to the CLL pathogenesis (Raval et al. 2006; Yu 2006).

14.5.10 Diagnosis

CLL diagnosis is based on a CBC with differential count, flow cytometry of the peripheral blood to determine the immunophenotype of circulating lymphocytes, and examination of the PBS (Hallek et al. 2008).

To diagnose CLL, the 2008 International Workshop on Chronic Lymphocytic Leukemia (iwCLL) update of the National Cancer Institute guidelines on the diagnosis and treatment of CLL concluded that the following two criteria must be met (Hallek et al. 2008):

- Absolute B lymphocyte count in the peripheral blood $\geq 5000/\mu L$ [$5 \times 10^9/L$], with a preponderant population of morphologically mature-appearing small lymphocytes.
- Demonstration of clonality of the circulating B lymphocytes by flow cytometry of the peripheral blood. A majority of the population should express the following pattern of monoclonal B cell markers: extremely low levels of SmIg and either kappa or lambda (but not both) light chains; expression of B cell associated antigens (CD19, CD20, and CD23); and expression of the T cell associated antigen CD5.

14.5.11 Treatment

Treatment of CLL patients is initiated at the time of symptomatic progressive disease. The main criteria for initiating therapy have been detailed in the IwCLL-2008 guidelines (Hallek et al. 2008). This recommendation is mainly based on older works that failed to show a survival advantage in CLL patients treated early in the course of disease (Dighiero et al. 1998). These results were verified by a large study of fludarabine treatment in CLL persons with early stage disease conducted by the German CLL study group, which failed to show a survival advantage with early treatment using conventional chemotherapeutic agents (Busch et al. 2013). Trials are currently underway with kinase inhibitors to determine if early

intervention can change the natural history of the disease. Regardless of the prognostic markers, it is recommend initiating treatment when patients fulfill the IwCLL-2008 criteria for treatment.

14.6 Acute Lymphoblastic Leukemia

14.6.1 Definition

Acute lymphoblastic leukemia, also known as or acute lymphoid leukemia (ALL), is an severe form of leukemia, or malignancy of the white blood cells, characterized by the hyper proliferation and accumulation of cancerous cells, immature WBC, known as lymphoblast's in BM (Fig. 14.20) and peripheral blood. In patient with ALL, lymphoblast's are uncountable in the BM and continuously multiply, causing damage and death by inhibiting the normal hematopoiesis (such as red and WBCs and platelets) in the BM and by spreading (infiltrating) to other organs. ALL is most common in childhood, with a maximum incidence at 2–5 years of age and another peak in old age.

ALL occurs in both infant and adults but its sharp incidence between 2 and 3 years (>90 cases per 1 million per year). ALL cause is not clear but risk factors is multifactorial and endogenous or exogenous exposures, inherited genetic factor, and luck have roles. Survival in childhood ALL has enhanced to roughly 90% in trials with risk assignment by biological characters of leukemic cells and response to therapy, based on patients pharmacodynamics and pharmacogenomics treatment modification and enhanced supportive care were performed. Though, new approaches to further improve survival are needed while reducing side effects. Unfortunately, in infants and adults prognosis remains bad. Genome-wide profiling studies of germline and leukemic cell DNA has detected new submicroscopic

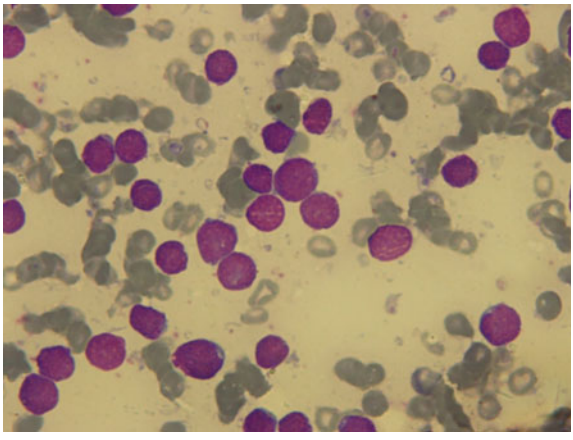

Fig. 14.20 Acute lymphoblastic leukemia, bone marrow smear showing many lymphoblasts. The cytoplasm is blue and scant. High power view (Used with permission from A. Ghavamzadeh: A color atlas of morphologic Hematology)

genetic alterations and mutations in DNA sequence that contribute to leukaemogenesis, describe new leukemia subtypes, affect responsiveness to treatment, and might provide new prognostic factors and therapeutic targets for individual medicine (Wang et al. 2016).

An estimated 6000 new cases (male: female incidence of roughly 1.3:1) of ALL are detected yearly in the U.S. (Siegel et al. 2012). Patients are mostly children; roughly 60% of cases occur in people aged younger than 20 years (Pui et al. 2008; Stanulla and Schrappe 2009; Hunger et al. 2012; Bassan and Hoelzer 2011). Survival in pediatric ALL is approaching 90% (Pui et al. 2009) but treatment in infants (i.e., infant less than 12 months) and adults needs enhancement (Pieters et al. 2007).

14.6.2 Classification

ALL Leukemic cells clearly reveal their belonging to a B- or T-cell lineage. As in AML, ALL specific immunophenotypes pattern have been associated with main groups of chromosomal abnormalities (Table 14.7).

B-lymphoblastic leukemia/lymphoma (called B-ALL) by expression of CD19, HLA-DR, and TdT together with many B-cell surface markers such as membrane and/or cytoplasmic CD22 and cytoplasmic CD79a is characterized. In several cases, CD45 is negative. Five immunologic subtypes, roughly corresponding to successive stages of B-cell differentiation have been identified. However, the presence of CD10 negative normal early B-cell progenitors is controversial. B-ALL can be immunologically categorized into five groups (Bene et al. 1995; Basso et al. 2001):

- B I/Pro-B/Early B: CD10- CD20-, cytoplasmic IgM-, Secretory immunoglobulin-
- B II/common/Early B: CD10+, CD20 ±, cytoplasmic. IgM-, Secretory immunoglobulin-
- B III/Pre-B: CD10 +, CD20 ±, cytoplasmic IgM +, Secretory immunoglobulin-
- B III/Pre-B/B (very rare): CD10+, CD20±, cytoplasmic. IgM+, Secretory immunoglobulin+ (κ or λ-)
- B IV/B-mature: TdT±, CD10±, CD20+, cytoplasmic IgM-, Secretory immunoglobulin+ (κ or λ-).

T-cell lineage in T-lymphoblastic leukemia/lymphoma (also known as T-ALL) is confirmed by expression of cytoplasmic CD3, TdT (Campana et al. 1987) and CD7, which is detected in most cases (Costa et al. 2010). Other T-cell-related indicators are variably expressed. In certain cases, cytoplasmic weak expression of CD79b has been described (Campana et al. 1987). There is no clear agreement concerning immunologic classification of T-ALL. The European Group for the Immunologic Classification of Leukemia (EGIL) classification included:

Table 14.7 Classification and association of immunophenotypic patterns of ALL with recurrent specific cytogenetic abnormality

Disease	Cytogenetic aberrations	Flow cytometry finding
BALL	t(4;11)(q21;q23) AF4-MLL	CD34+, CD19+, CD10−, CD20−, CD13 and/or CD33 could be positive, often CD15 and/or CD65+, 7.1+, cyt. IgM−
	t(9;22)(q34;q11.2) BCR-ABL1	CD34++, CD19+, CD10+, CD20∓, CD13, CD33, CD66c frequently positive, CD15−, CD65−, 7.1−, cyt.IgM−
	t(12;21)(p12;q22) TEL-AML1	CD34+ or −, CD19+, CD10+, CD20∓, CD13, and/or CD33 frequently positive, CD66c−, CD15−, CD65−, 7.1−, cyt.IgM−
	Hyperdiploid	CD34+ or subset, CD19+, CD10+++, CD123++, CD20∓, CD13−, CD33−, CD66c∓, CD15−, CD65−, 7.1−, cyt. IgM−
	t(1;19)(q23;p13.3) TCF3-PBX1	CD34− or subset, CD19+, CD10− or subset, CD20+, CD13−, CD33−, CD66c∓, CD15−, CD65−, 7.1−, cyt. IgM+
TALL	FLT3 activating mutation	Expression of CD117

- Pro-T (or T-I) positive for only CD7.
- Pre-T (or T-II) positive for CD2 and/or CD5 and/or CD8.
- Cortical T (or T-III) positive for CD1a (irrespective of other markers).
- Mature T (T-IV) positive for surface CD3 and negative for CD1a (irrespective of other markers)(130).

Early T-precursor subtype (ETPs), by an aggressive clinical course characterized and carrying immunophenotype related with ETPs has recently been recognized (Coustan-Smith et al. 2009). ETPs are a subtype of thymus cells that recently migrated from the bone marrow to the thymus; they keep multilineage differentiation potential, proposing their directly derivate from hematopoietic stem cells. The immunophenotype of ETP subset of T-ALL includes an absence of CD1a and CD8, very weak or negative CD5, and expression of one or more early precursor or myeloid-related cell surface markers: CD117, CD34, HLA-DR, CD13, CD33, CD11b, and/or CD65 (Coustan-Smith et al. 2009).

In more than 95% of both B- and T-ALL cases, leukemic blasts show abnormal immunophenotypes that allow us to discriminate them from normal B- and T cell precursors (Lucio et al. 2001). Minimum residual disease (MRD) detection by flow cytometry is well established and already included in some clinical trials (Campana 2012). Characteristic immunophenotypes should be detected at diagnosis for each patient by comparing the cell surface marker profile of leukemic blasts to that of normal and regenerating bone marrow samples. Transient alterations in immunophenotypes of residual leukemic cells have been described, but some

abnormal features are usually retained (Gaipa et al. 2005). Sensitivity of MRD detection at 0.01% can be achieved, provided that adequate numbers of cells are studied (5–10 $\times$ 10^5) in each antibody combination group.

14.6.3 Epidemiology

Generally, ALL similar cancer, probably arises from interactions between endogenous or exogenous agents, inherited susceptibility, and chance. These risk factors account for the roughly 1 in 2000 risk of the disease in infantile (0–15 years). The challenge is to recognize the relevant environmental factors and inherited mutation and detect these risk factors when and how contribute to the multistep natural history of ALL from initiation via the largely covert development to overt disease (Treviño et al. 2009). The infrequency of the disease and the presence of biologically distinct subgroups that might not share frequent causative devices complicates matters (Sherborne et al. 2010). For instance, in childhood ALL is usually associated with MLL rearrangement, and the strangely high concordance rate in monozygotic twins (approaching 100% in those with a single or monochorionic placenta) proposes that leukaemogenesis is largely complete at birth. By contrast, prevalence of non-MLL-rearranged B lymphoblastic leukemia peaks between 2 and 5 years and has a concordance rate of 10–15%, suggesting that, though initiation in utero usually occurs, other so-called promotional exposures are probably necessary for disease appearance.

Acute lymphoblastic leukemia (ALL) is a neoplastic disease characterized by clonal expansion, accumulation, and tissue infiltration of neoplastic cells. Although they are mainly regarded as childhood diseases, ALL has a bimodal distribution, with a first peak at 2–5 years of age and a second peak around age 50. ALL is regarded as genetic disease because most patients with ALL harbor acquired genetic alterations or somatic mutations that contribute to the increased proliferation and impaired differentiation of the lymphoid hematopoietic progenitors. In the majority of ALL patients, one or more of these genetic alterations are in the form of microscopically detectable nonrandom chromosome aberrations. Moreover application of modern genome-wide molecular analyses reveal many additional genetic rearrangements that cannot be detected by conventional cytogenetic analyses. Furthermore, the analysis of epigenetic alterations in ALL samples suggests that epigenetic alterations may also be important in ALL pathogenesis. Several of the ALL-specific chromosomal and molecular genetic abnormalities at diagnosis play an important role in the prognosis of the both childhood and adult ALL patients and have been included in World Health Organization (WHO) classification of ALL patients especially about B-lineage ALL (B-ALL) classification. Here, major cytogenetic, molecular and epigenetics findings and their clinical relevance in ALL patients were described briefly.

14.6.4 Common Chromosomal Aberrations

14.6.4.1 Numerical Chromosome Abnormalities

Hyperdiploidy

Hyperdiploid is one of the most frequent ploidy groups in ALL patients (Fig. 14.21). Hyperdiploid karyotypes, particularly those with >50 chromosomes, are more frequent in children than in adults as well as in B-ALL than T-ALL (Pui et al. 1990).

Various chromosomes are involved in hyperdiploidy but certain karyotypes seem to be more dominant. The distribution of specific chromosome gains involved in hyperdiploidy karyotype is nonrandom. In most of hyperdiploid ALL patients each of chromosomes 21 (often tetrasomy), X, 14, 6, 18, 4, 17 and 10 is gained in over half of hyperdiploid cases, followed by gain of chromosomes 8, 5, 11, and 12 which occur more often in high hyperdiploidy patients with 57 or more chromosomes (Ankathil et al. 1996).

Children with high hyperdiploidy karyotype have excellent prognosis with approximately 100% CR rates, 5-year overall survival (OS) rates of near 90% and 5-year event-free survival (EFS) rates between 71 and 83% in some reports (Faderl et al. 1998; Pui et al. 1990).

The good prognosis of ALL children with hyperdiploidy karyotype is thought to be related to leukemic cell sensitivity to a number of antileukemic drugs and the tendency of leukemic cells to respond to apoptosis.

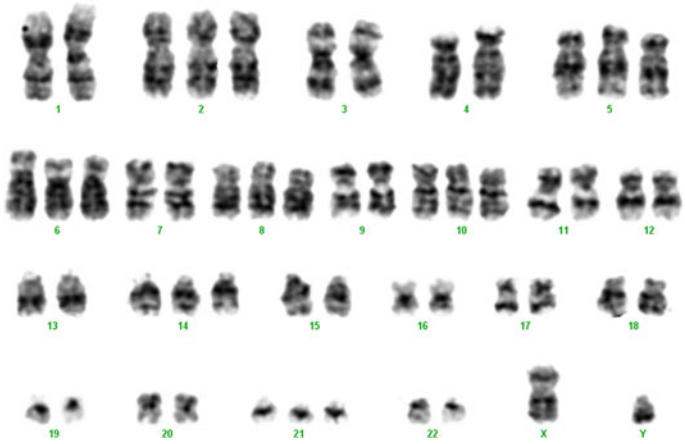

Fig. 14.21 An ALL case with hyperdiploid karyotype and extra copies of chromosomes 2, 5, 6, 8, 10, 14 and 21. The figures adopted from cytogenetic lab of hematology, oncology and stem cell transplantation research center, Tehran University of Medical sciences, Dr. Yaghmaie's Archives

Although, it is not comparable to the outcome of children with high hyperdiploidy, some, but not all, studies showed that outcome of ALL adults with high hyperdiploidy has been improved in relation to other cytogenetic groups. Furthermore, prognosis of adults ALL may also be influenced by specific cytogenetic features of the hyperdiploid karyotype. Hyperdiploid adult patients with concurrent trisomy of chromosomes 4, 10 and 17 or chromosomes 4 and 18 have especially favorable prognosis. On the other hand, the presence of other structural aberrations in the relatively rare patients with hyperdiploidy karyotype such as t(9;22)(q34;q11), t(1;19)(q23;p13) or translocations involving 11q23, influence the prognosis of patients. They excluded from the hyperdiploidy category and have outcomes similar to non-hyperdiploid patients with these aberrations.

Structural aberrations such as duplications and gains of 1q, del (6q) or i (17) (q10) were seen in approximately one-half of the high hyperdiploid patients but presence of these aberrations does not seem to influence prognosis.

Hypodiploidy

ALL patients with Chromosome numbers of 45 or less and haploid numbers of 24–36 are rare. About 2–8% of adult patients display hypodiploid chromosome numbers. Hypodiploid karyotype (Fig. 14.22) usually seen in cases with B-cell ALL and only 20% of hypodiploid cases have T-cell ALL. Near-haploid karyotypes are frequent in children or teenagers compared with older patients. There are not random pattern of chromosome loss in near-haploid cases with preferential retention of

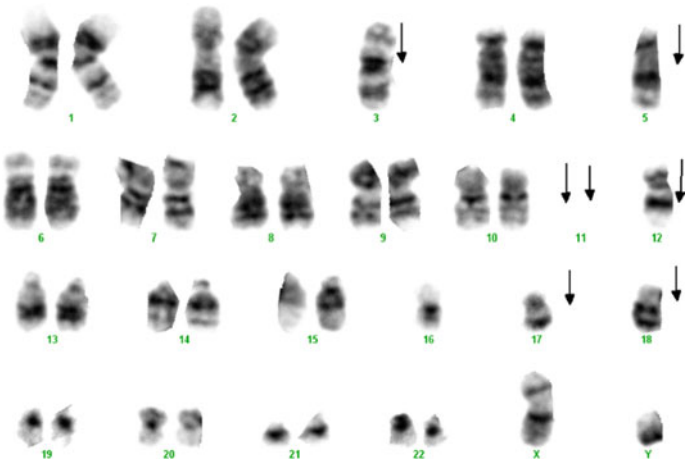

Fig. 14.22 An ALL case with hypodiploidy karyotype. Loss of chromosomes: 3, 5, 11 (two copies), 12, 17, 18. The figures adopted from cytogenetic lab of hematology, oncology and stem cell transplantation research center, Tehran University of Medical sciences, Dr. Yaghmaie's Archives

two copies of chromosomes 6, 8, 10, 14, 18, 21, and the sex chromosomes. Loss of chromosomes 1, 5, 10, 11, 19, 21, and 22 are the most common abnormalities in near-haploid karyotypes.

More cases with Near-haploid karyotype always have only numerical aberrations, however cases with 30–44 chromosomes show frequent structural aberrations, mainly translocations.

It is not clear how massive chromosome losses contribute to leukemogenic process. But it is proposed that a near-haploid karyotype allows expression of recessive genes that in diploid condition have been under the dominance of their allelic counterparts. It causes loss of regulatory control of growth and differentiation of lymphoid cells.

As an independent prognostic factor hypodiploid adult patients have the worst prognosis (Mullighan et al. 2015). It is thought that, like hyperdiploidy, hypodiploidy have more effect on outcome of children than in adults. However, in most studies of adult ALL hypodiploidy conferred poor prognosis compared with other poor-risk chromosomal abnormalities such as t(4;11) and t(1;19).

14.6.4.2 Single Chromosomal Gains or Losses

Although incidence of single chromosome aneuploidy is lower than in myeloid leukemias, gains or losses of single chromosome occur frequently in ALL patients and in rare cases they are the sole cytogenetic abnormality.

The precise mechanisms by which such abnormalities contribute to leukemogenesis are unclear but it is proposed that dosage effect of certain gene products resulting in abnormal proliferation or differentiation.

Only in 10–20% of childhood ALL single chromosome gains or losses are the sole karyotypic anomaly.

Trisomy 21 is the most frequent chromosomal gain observed in both adult and childhood ALL. Its overall incidence is approximately 15% of cases but mainly as a part of other cytogenetic changes such as hyperdiploidy. However as the sole acquired clonal abnormality, trisomy 21 accounts for 2% of pediatric and less than 1% of adult ALL cases. In childhood ALL with hyperdiploid, it is very common.

In childhood ALL, trisomy 21 is often associated with structural abnormalities such as t(12;21)(p13;q22), chromosome 6q abnormalities, t(1;19)(q23;p13), t(4;11)(q21;q23) and 14q abnormalities.

In adults cases, trisomy 21 is associated most frequently with t(9;22)(q34;q11.2).

Trisomy 21 generally associated with a good prognosis when it is seen as the sole abnormality.

About 1–2% of ALL patients show trisomy 8 as the only cytogenetic abnormality. But its prognostic value in either adults or children with ALL is not clear.

Another numerical chromosomal abnormalities such as isolated trisomy 4, 5 and monosomies of chromosomes 5, 7 and 20 are very rare in ALL cases and their clinical significance is unclear.

14.6.4.3 Structural Chromosome Abnormalities

More than 30 different nonrandom chromosomal rearrangements are presently known in patients with ALL. Structural abnormalities are found in 78% of cases. Chromosomal translocations are the most common structural changes in ALL that found in 30–37% of adult cases.

Translocation t(9;22)(q34;q11)

The t(9;22) translocation is a recurrent abnormality occurred in up to 95% of CML patients. However, it can be observed in about 1–2% of AML patients, as well as in up to 1–3% of children and 15–30% of adults with ALL (Secker-Walker and Craig 1993).

This reciprocal translocation between the long arms of chromosome 9 and 22 results in a shortened chromosome 22 known as the Philadelphia chromosome (Ph). The molecular consequences of this translocation is creating a chimeric and transcriptionally active BCR-ABL gene.

T(9;22) is found in B-lymphocytic progenitor cells and generally, but with rare exception, Ph+ patients are diagnosed with T-ALL. Both adults and children with Ph chromosome have very poor prognosis (Secker-Walker and Craig 1993). In overall less than 5% of adults with Ph + ALL being cured. Therefore, these patients with Ph + ALL are candidates for treated with innovative and intensified strategies. The only potentially curative therapy for Ph + ALL is allogeneic hematopoietic stem cell transplantation (HSCT).

Secondary chromosome aberrations such as an extra copy of der(22)t(9;22), −7 or loss of 7p arm, an abnormality of 9p, +21, +8, and +X were seen in approximately two-thirds of newly diagnosed ALL patients. As well as in approximately 15% of Ph + ALL patients high hyperdiploidy is detected.

The presence of aberrations in addition to t(9;22), regardless of type, when compared with a sole t(9;22), did not alter prognosis in pediatric or adult ALL patients.

Rearrangements of (9p)

Abnormalities of chromosome 9p including i(9q), balanced translocations with 9p breakpoints and dicentric chromosomes occur in approximately 10% of childhood and adult with ALL. These abnormalities are generally associated with a poor prognosis in B-cell ALL.

Deletion of 9p21 is observed in approximately 10–30% of adult with ALL and results in loss of the CDKN2A gene, also known as p16. CDKN2A gene deletion is associated with a poor prognosis in pediatric B-lineage, but not in adult or T-lineage ALL (Heerema et al. 1999). Chromosome 9p abnormalities most often occur with

other abnormalities, rather than as a sole anomaly, particularly including chromosome 12p rearrangements and del(6q).

Abnormalities Involving 11q23

Abnormalities of 11q23 involving MLL gene rearrangements with variable chromosome partners are among the most common cytogenetic abnormalities in a variety of adult hematopoietic malignancies. These abnormalities also occur in 60–70% infant acute leukemias. In older children and adults with

ALL their frequency is lower than 10%. Previous therapy with topoisomerase II inhibitors increase the frequencies of abnormalities involving 11q23.

MLL gene rearrangements are not typically seen in patient with hyperdiploidy, and in infants, it is considered a clinically distinct entity from that diagnosed in older children.

Infant ALL is strongly associated with a poor prognosis, and molecular studies demonstrated that 11q23 abnormalities in infants with ALL occur in utero (Harrison and Foroni 2002). The MLL gene is involved in normal hematopoietic growth and differentiation.

Because of MLL gene abnormalities can occur very early in hematopoietic stem cell development, in utero exposure to natural or synthetic substances topoisomerase II inhibirors such as genistein, catechins and flavonoids may result in acute leukemia.

Approximately, 60–80% of infants with ALL have 11q23/MLL abnormalities whereas in children older than 1 year the incidence of these abnormalities is from 4.5–5.7%. Depending on age of onset and the type of abnormality detected prognosis of patients is variable. However, infants with MLL translocations, especially those less than 6 months of age, have a particularly poor prognosis (Pui et al. 2003).

The most common reciprocal chromosomal loci that participate in 11q23 translocations are 4q21 [MLL-AFF1 (AF4)], 9p22 [MLL-MLLT3 (AF9)] and 19p13 [MLL-MLLT1 (ENL)]. Among them t(4;11)(q21;q23) is the most common translocation that is observed in more than 60% of infants with ALL, 2% of children with ALL, and 3–6% of adults with ALL. Both adults and children with the t(4;11)(q21;q23) have the poor clinical outcome.

Rearrangements of 19p13.3

The t(1;19)(q23;p13.3) (Fig. 14.23) is one of the most common chromosomal abnormalities in B-cell precursor acute lymphoblastic leukemia (B-ALL). It usually gives rise to the TCF3/E2A-PBX1 fusion gene and occurs in 1–3% of adult and 1–6% pediatric ALL. This translocation can be in either balanced or unbalanced form, as der(19)t(1;19) with two normal chromosomes 1. Most patients with the t(1;19)(q23;p13.3) have pseudodiploid karyotypes, and often are diagnosed with pre-B ALL. ALL patients with t(1;19) have controversial outcome.

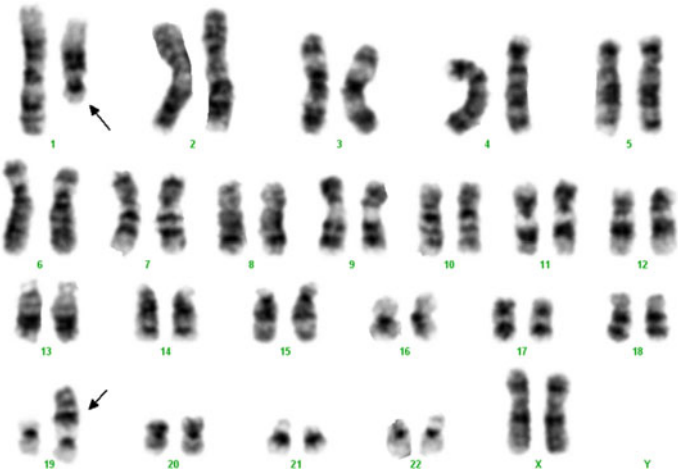

Fig. 14.23 Karyogram of chromosomes from G-banded bone marrow cells of an ALL case showing 46, XX, t(1;19)(q23;p13). The figures adopted from cytogenetic lab of hematology, oncology and stem cell transplantation research center, Tehran University of Medical sciences, Dr. Yaghmaie's Archives

Adult patients with t(1;19) have poor or relatively favorable prognosis. Children with ALL and t(1;19) have initially unfavorable prognosis that has been improved by the use of more effective therapies (Barber et al. 2007).

Abnormalities Involving the T-Cell Receptor (TCR) Genes

Translocations involving the TCR genes are among the most prevalent abnormalities in T-ALL patients however, these rearrangements are found at a lower frequency in patients with B-ALL. In general T-cell ALL is characteristic by special cytogenetic profile. Many of these patients do not harbor a cytogenetically detectable chromosomal abnormality, more patients have pseudodiploid karyotypes, and only few have hyperdiploidy. In childhood T-cell ALL translocations have overall frequency of 40–45% that half having breakpoints at chromosomal regions encoding for TCR genes including chromosome 14 q11 (TCR-α and -δ genes), 7q32-36 (TCR-β gene), as well as 7p15 (TCR-γgene) (Harrison and Foroni 2002).

The t(10;14)(q24;q11.2) is the most common of these translocations, it results in overexpression of the TLX1 (HOX11) gene, and is associated with a favorable outcome in T-cell ALL patients. The t(1;14)(p32;q11.2) is detected in 3–6% of childhood T-ALL and juxtaposes TCRD and TAL1 (SCL) resulting TAL1 gene up-regulation.

14.6.4.4 Complex Karyotype

Although, complex karyotype is a well-established adverse prognostic factor in AML, prognostic significance of a complex karyotype (more than five abnormalities) in ALL is suggested in few studies.

14.6.5 Molecular Genetic

Recently, using array-based comparative genomic hybridization or single nucleotide polymorphism (SNP) microarrays several the genomic landscape of DNA copy number alterations were described in ALL patients. The majority of these studies focused on childhood ALL and similarly studies are ongoing on adolescent and adult ALL cohorts. Compared with solid tumors, ALL genomes typically harbor fewer structural alterations. However more than 50 recurring deletions or amplifications have been identified which many of them involve a single gene or few genes. However, relatively few of these novel genetic alterations have been found to be associated with outcome of patients. Majority of these genes encode proteins with important roles in lymphoid development and signaling such as PAX5, IZKF1, EBF1, LMO2, BTLA, CD200, TOX, and the glucocorticoid receptor NR3C1, cell-cycle regulation and tumor suppression such as CDKN2A/CDKN2B, PTEN, and RB1 and transcriptional regulation such as TBL1XR1, ETV6, and ERG.

Since, several of these genes such as PAX5, WT1, and PTEN displayed multiple types of genetic alterations, including copy number alteration translocation and sequence mutation, therefore microarray profiling alone cannot detect all genetic alterations in ALL. Distribution of genetic lesions is dependent on subtype of ALL. Although, additional structural or sequence alterations are very rare in MLL-rearranged leukemias, ETV6-RUNX1 and BCR-ABL1 ALL harbor more alterations.

14.6.5.1 IKZF1 Alterations in B-ALL

The product of IKZF1 gene (IKAROS) is a transcription factor that belongs to the family of zinc-finger DNA-binding proteins associated with chromatin remodeling and has a key role in development of lymphocytes, B- and T-cells. Two-thirds of patients with B-ALL show alterations of this gene with deletion, sequence mutations, or rearrangements of the transcription factors PAX5, IKZF1, and EBF. Deletions and sequence mutations of IKZF1 are less common and being present in approximately 15% of childhood ALL cases (Tokunaga et al. 2013).Two types of sequence alterations of IKZF1 gene were observed in ALL cases. Broad deletions that result in loss of function and partial deletions of coding exons 4–7. The late

mutation remove the N-terminal DNA-binding zinc fingers and leads to expression of a dominant-negative isoform (IK6). Alterations of IKZF1 gene are present in more than 70% of BCR-ABL1 lymphoid leukemia. There is an association of IKZF1 alterations with poor outcome of ALL patients with BCR-ABL1 these alterations are associated with poor outcome, which are reported in multiple studies. Moreover, many of these patients have gene-expression profile similar to BCR-ABL1^{+} ALL and harbor novel kinase-activating mutations and rearrangements.

14.6.5.2 CRLF2 Rearrangements and JAK Mutations in ALL

Rearrangements of CRLF2 gene are reported in approximately 7% of childhood ALL cases using FISH and SNP array profiling. CRLF2 gene encodes cytokine receptor–like factor 2 and located in the pseudoautosomal region (PAR1) at Xp22.3/Yp11.3. It forms a heterodimeric receptor with IL7 receptor alpha. Two rearrangements of CRLF2 were identified including IGH-CRLF2 and P2RY8-CRLF2 (Harvey et al. 2010). IGH-CRLF2 is result of CRLF2 translocation into the immunoglobulin heavy chain locus and P2RY8-CRLF2 is result of a focal deletion upstream of CRLF2. Both rearrangements result in aberrant CRLF2 overexpression on the surface of leukemic lymphoblasts cell. In addition with low incidence a p.Phe232Cys CRLF2 mutation results in high expression of receptor. Alterations of CRLF2 are correlated with the presence of activating mutations in the JAK1 and JAK2 genes. Co-expression of CRLF2 and JAK1/2 mutations is transforming in vitro, suggesting that these two lesions have a key role in lymphoid transformation.

Recent studies have shown that CRLF2 and IKZF1 mutations are associated with adverse outcome in ALL children (Harvey et al. 2010).

14.6.6 Epigenetic

14.6.6.1 Aberrant DNA Methylation in ALL

Aberrant epigenetic lesions are common in acute lymphocytic leukemia (ALL) and hypermethylation of promoters is a frequent mechanism of gene silencing associated with the prognosis and the response to therapy. Recent data indicates that wide spectrum of genes involved in critical molecular pathways, are epigenetically suppressed in ALL. Aberrant epigenetic lesions in ALL participate in deregulation of three key cellular pathways including cell growth, apoptotic program; and the cell-cell adhesion. Since these lesions are potentially reversible, the reactivation of

these pathways by hypomethylating agents may be potential therapeutic approach in this disease. Initial epigenetic studies in ALL focused on aberrant methylation of single genes such as *calcitonin*, *p15*, *p73*, *E-cadherin*, *ER*, Dkk3, LATS2/KPM, Hck, DBC1 and BNIP3. Later, the methylation analysis of multiple genes including MDR1, THSBS2, THSBS1, MYF3, ER, P15, CD10, c-ABL, p16, p73 in adult ALL patients demonstrated that about 77–85% of ALL patients have methylation of at least 1 gene and 35–40% of them have methylation of 3 or more genes. Moreover, increased number of methylated genes was associated with an adverse outcome (Garcia-Manero et al. 2009; Takeuchi et al. 2011). Genome-wide analysis by Methylated CpG Island Amplification (MCA) technique showed that aberrant methylated genes were distributed through all autosomes and could be clustered in multiple functions molecular pathways.

14.6.7 Diagnosis

Morphological and immunophenotying of lymphoblasts-immature lymphocyte- by light microscopy and evaluation of lineage commitment cells and ontogeny stage using flow cytometry analysis for the diagnosis of ALL are essential (Pui et al. 2008). In the initial cytogenetic work-up chromosomal study still has an important role. Fluorescence in situ hybridization (FISH) or multiplex ligation-dependent probe amplification (MLPA), Reverse transcriptase PCR (RT-PCR and flow cytometry are used to detect submicroscopic chromosomal abnormalities, leukemia-specific translocations and cellular DNA content, respectively. When genome-wide study becomes time and cost effective, it might replace some diagnostic techniques.

14.6.8 Treatment

Treatment usually spans 2–2.5 years, and includes three stages: induction of remission, consolidation and maintenance (Pui et al. 2008). The majority of the drugs to treatment of ALL used were developed before 1970. Nevertheless, their dosages and combination chemotherapy schedule on the basis of leukemic-cell biological features, responsiveness have been adjusted, and pharmacogenomics and pharmacodynamic findings in patients, resulting in the high survival rate. Treatment of CNS prevents relapse due to trapped leukemia cells. For patients at very high risk group is a choice for patients at very high risk. Allogeneic hematopoietic stem cell transplantation is an option for patients with ALL after first relapse, and is also recommended for high-risk cases in first CR.

14.7 Psychological Aspects of Leukemia

14.7.1 Psycho-oncology

14.7.2 Introduction and Historical Perspective

For hundreds of years, a cancer diagnosis was considered as equal to death. The cause of cancer was not known and cure from it was viewed as a miracle. Most of the physician and family members did not reveal the diagnosis to a patient because they think the patient could not cope with this situation and would lose their hope. The Death of Ivan Ilyich, a Tolstoy's masterpiece, first published in 1886, is an excellent example for describing the status of cancer patients in the past centuries (Brungardt 2009). But, this situation gradually changed, as the cancer was known a public health problem. In North America and European countries, the medical and social atmosphere for cancer patients changed dramatically in the second half of 20th century. The changes included new developments in cancer diagnosis and treatment, the right of patients to know, and living wills.

Psycho-oncology is a relatively new discipline that has developed gradually during the last quarter of 20th century. Psycho-oncology which is also called psychosocial oncology deals with the human side of cancer. The main goal of most of cancer centers was treatment of cancer and increasing the survival of the patients. But, patients with cancer need something beyond physical treatments. They required support to adapt with cancer and to continue living in spite of having cancer. In most countries, the development of psychosocial care for cancer patients results from three movements (Uchitomi 1999; Holland 2002). In the first movement, a group of cancer survivors and their families have started activities that have shaped the small sidling which has gradually grown. The second movement was shaped by a small group of the health professionals including oncologist, psychiatrist, psychologist, social workers and oncology nurse that work with cancer patients who joined the supporting stream and in final movement, the hospitals and cancer treatment institute and health policy makers accept the importance of this services for patients with cancer.

The current mission of psycho-oncology goes beyond telling the diagnosis or treatment of mental health problems of cancer patients and their families. The quality of life of patients and cancer survivorship are two new areas of interest for research and service developments in this field (Holland 2002)

14.7.3 Crisis of Cancer Diagnosis

When a person is announced about the diagnosis of cancer, at least in the short term, the usual stream of his/her life would be changed. For this reason, this period

should be considered as a crisis. In the first step of the crisis, the patient will hear the diagnosis and in the second step, she/he should adapt with her/his new life with cancer.

14.7.4 Breaking Bad News

An operational definition of the bad news has been presented by Robert Buckman: "any news that adversely and seriously affects an individual's view of his or her future" (Buckman 2005). The way the bad news is disclosed to the patients could affect their perception of the disease (Fujimori et al. 2007), their psychological coping with illness (Meredith et al. 1996; Valizadeh et al. 2012; Yun et al. 2004), satisfaction with care (Parker et al. 2001; Butow et al. 2002) and the extent of hope (Rassin et al. 2006).Currently, it's vastly approved that being informed about the disease, is the patients' legal and ethical right (Schofield et al. 2003; Buckman 2005) and hiding the information about the disease may lead to distrust towards physicians (Mueller 2002).

A review of the literature showed that 50–90% of patients request for full diagnostic disclosure, but still a minority of patients does not want to be aware of bad news, as a result clinicians should assess their patients' needs on this issue (Baile et al. 2000). Attitude toward bad news disclosure could be different based on various cultures. In Western countries, most clinicians express the diagnosis clearly, but in Asia and the Middle East, due to dominated paternalistic view, some patients are excluded to receive information about their disease (Elger and Harding 2002; Ozdogan et al. 2006). Non-Western societies are more likely to have collectivist models of medical decision-making than the patient-autonomy model favored by most Western countries.

Several studies were accomplished to explore the patients' and clinicians' preferences and attitudes toward diagnostic disclosure in Iran. In one study, in Tehran, 93% of patients tended to know the diagnosis, 75.5% accepted that patient should be the first person to be informed and 87% of patients preferred to be informed by their own physician (Arbabi et al. 2014). Another study was done to evaluate the attitude of medical staff toward breaking bad news. The majority of the physicians (86%) and nurses (74%), mostly the older and more experienced, tended to reveal the diagnosis to patients (Arbabi et al. 2010).

Breaking bad news is a difficult task for clinicians, patients, and families because it is accompanied by distress for both sides of the communication. To overcome this complex task, clinicians should have enough mastery in advanced communication skills. One framework that clinician may find helpful is that developed by Baile and Buckman (Baile et al. 2000). This guideline included the major points to be considered when giving bad news to patients and/or their relatives (Table 14.8).

Table 14.8 SPIKES—the six-step protocol for delivering bad news, Baile and Buckman (2000)

Steps		Component	Measures
1	S	SETTING UP the interview	Maximize privacy, avoid interruption, respect confidentiality and provide support
2	P	Assessing the patient's PERCEPTION	Demonstrate how much the patient knows, how serious she/he thinks the illness is, and how much it will affect the future?
3	I	Obtaining the patient's INVITATION	Declare how much the patient wants to know
4	K	Giving KNOWLEDGE and information to the patient	Keep in mind objectives for the consultation: diagnosis, treatment plan, prognosis and support Listen to patient's agenda
5	E	Addressing the patient's EMOTIONS with empathic responses	Responding to the Patient's Feelings. Responses can vary from silence to distress, denial or anger. Observe the patient and give them time. Empathetic reflection
6	S	STRATEGY and summary	Make a Plan or Strategy and Explain it. Identify coping strategies of the patient and reinforce them Tell them what happens next

14.7.5 Adaptation to Cancer

Adjustment to cancer is facing up to the problems that arise from the disease which includes a change in life, family and work situations, pain, and disability due to cancer and its treatments as well as living as cancer survivors. Adaptation to cancer is a dynamic process. The initial response usually is denial or despair, followed by dysphoria that may be associated with anxiety, depression, insomnia, poor concentration and would usually terminate to acceptance and resumes usual activities over months. Patients vary widely in how they cope with cancer over time. Thus it is very important to recognize factors that predict poor/good adaptation to facilitate early recognition of vulnerable patients (Holland 2002).

Adaptation to cancer depends on three group of factors: (1) Community factors, which include society attitude and perception of cancer and its treatments. (2) The patient's factors that include the individual (psychological profile and abilities); interpersonal (relational issues and support from others); and socioeconomic (availability of resources for support). (3) Cancer factors, which include stage of cancer at diagnosis, type, and severity of symptoms including pain, extent of treatments and adverse effects, effect of cancer on body image and possibility of rehabilitation (Holland and Rowland 1989).

Predictors of poor adaptation are social isolation, low socioeconomic status, drug abuse, prior psychiatric history, prior experience with cancer, recent losses/bereavement, the rigidity of the coping, pessimistic philosophy of life and absence of value system (Spencer et al. 1998).

14.7.6 Psychiatric Syndromes

14.7.6.1 Adjustment Disorder/Demoralization

According to Diagnostic and Statistical Manual for Mental Disorders (DSM-5) criteria, when a person fails to cope with a stressor and show maladaptive response which does not meet the criteria of major mood or anxiety, the diagnosis of adjustment disorder could be considered for her/him (American Psychiatric Association 2013). Subjective nature of threshold for diagnosis of adjustment disorder made it difficult to apply in patients with medical illness.

Demoralization is a good diagnostic substitute for adjustment disorder in medical settings. It is more compatible with patients own words for expressing existential uncertainty. Fava et al. (1995) and Kissane et al. (2003) proposed diagnostic criteria for demoralization which should persist for at least 2 weeks and includes loss of hope and meaning, sense of being trapped, feel like giving up, unable to cope with the predicament, social isolation, and potential suicidal thinking (Fava et al. 1995; Kissane et al. 2003).

Treatment of adjustment disorder and demoralization is mainly brief psychotherapy because this disorder tends to be time-limited (Casey and Bailey 2011).

14.7.6.2 Anxiety Disorders

Cancer is associated with the threat, uncertainty, and lack of control that could cause anxiety. Anxiety is more common in crisis situations like starting a new treatment or the recognition of recurrence or progression of cancer. Simple phobia, panic disorder, generalized anxiety disorder and posttraumatic stress disorder (PTSD) are the most usually reported anxiety disorders in patients with cancer (Roy-Byrne et al. 2008). Some types of anxiety can interfere with patients' compliance to treatment. For example, patients with claustrophobia may have a problem in tolerating MRI, radiation therapy and isolation which is necessary for some instances of neutropenia. Posttraumatic stress disorder was noted in 4–6% of cancer patients, but more patients experience subsyndromal forms of PTSD.

Clinicians should consider conditions that mimic anxiety disorders (e.g. pulmonary emboli) and medication-induced anxiety (antiemetic induced akathisia) before making the diagnosis of anxiety disorders. Pain and anxiety have a direct bidirectional; pain is associated with anxiety and anxiety amplifies pain.

14.7.6.3 Depressive Disorders

Although depression by itself is not associated with increased risk of cancer, comorbidities like obesity, smoking, decrease physical activity and alcoholism which are accompanied with depression, can increase the risk of cancer (Wise et al. 2013).

Depression may occur in cancer patients as a result of biological, psychological and/or social consequences of cancer and its treatment. Massie (2004) in a systematic review of more than 150 studies found that prevalence of major depression and other depressive syndromes in patients with cancer was up to 38 and 58%, respectively (Massie 2004). Although prompt recognition and complete treatment of depression are very important for improving the cancer patient's quality of life, most of the cases remained unrecognized and undertreated. Implementation of screening tools and increasing the awareness of patients, their family, nurses, and physician is a useful way to raising the possibility of diagnosis and effective treatment.

Diagnosis of depression in patients with cancer is more complex than in physically healthy individuals. Because neurovegetative symptoms like decreased appetite, loss of energy, insomnia, loss of sexual drive, and psychomotor retardation are highly likely to be caused by cancer or its treatments. Hopelessness, worthlessness, excessive guilt, loss of self-esteem, and wishes to die are more diagnostically reliable symptoms of depression in cancer patients.

14.7.6.4 Cognitive Disorders

Cognitive disorders are one of the most puzzling adverse effects in patients with cancer, especially in children. The reported incidence of cognitive dysfunction range from 15 to 80% which depends on the methodology of studies and the type of neurocognitive tests used. In up to 35% of cases of cognitive disorder, this problem persists for months or years following treatment. The most frequently reported dysfunction include difficulty with memory, learning, attention, concentration, information-processing speed, organization, and executive function (van Dam et al. 1998; Brezden et al. 2000; Ahles et al. 2002).

The etiology of cognitive problems in cancer patients is multifactorial and include cancer, treatments (chemotherapy and/or radiation), inactivity, anemia, endocrine disturbance (menopause, hypo/hyperthyroidism), comorbid disease, pain, emotional distress, sleep disturbance, poor nutrition and other comorbid diseases. Some people are more susceptible to this condition (Wefel et al. 2004; Yamada et al. 2010).

14.7.6.5 Suicide

Previous research has suggested that patients with cancer have a higher rate of suicide than the general population (Hem et al. 2004; Robson et al. 2010). Suicide was higher in male, white race and older age at diagnosis (Misono et al. 2008). In addition, the time after diagnosis is often linked with risk of cancer suicide, which is higher in the first year after diagnosis (Yousaf et al. 2005; Tanaka et al. 1999). The more physical and social impairments associated with cancer the more suicide rate. As a result, the reported incidence of suicide was higher in patients head and neck, gastrointestinal, urogenital and lung cancer. Depression, anxiety, aggression, family history of suicide, poor social support, loss of independence, feeling of being a burden, pain, advanced disease and poor prognosis were among other observed risk factors for suicide in cancer patients in prior studies (Spoletini et al. 2011).

14.7.6.6 Role of Chemotherapeutic Agents

It is difficult to recognize which psychiatric problems are a direct side effect of chemotherapy as compared to the physical and psychological sequel of cancer diagnosis and other treatments. Various chemotherapeutic agents can cause different psychiatric syndromes (Table 14.9) (Levenson 2011).

Patients with CNS tumors, female gender, young age, cranial radiotherapy, learning difficulties, hearing loss and lower socioeconomic group are especially at

Table 14.9 Psychiatric side effects of chemotherapeutic agents Levenson (2011)

Agent	Side effects
Corticosteroids	Depression, mania, and psychosis
Tamoxifen	Insomnia, depression, rarely psychosis
Vincristine	Depression, psychosis, lethargy
L-asparaginase	Depression, somnolence, delirium
Procarbazine	Somnolence, psychosis, suicide, depression, act as a monoamine oxidase inhibitor
Ifosfamide	Lethargy, seizure, hallucinations
Cytarabine	Delirium, cerebellar sign, leukoencephalopathy
Methotrexate	Delirium, leukoencephalopathy
Interferon	Psychosis, suicide, depression, mania
Cisplatin	Neuropathy
Chlorambucil	Hallucination, lethargy, seizure
5-Fluorouracil	Fatigue, cerebellar syndrome, rarely seizure
Texanes	Sensory neuropathy, depression, fatigue
Thalidomide	Fatigue, reversible dementia
Bevacizumab, Sorafenib, Sunitinib	Posterior leukoencephalopathy

risk for developing psychiatric side effects of chemotherapy (Ahmad et al. 2016). The ways that cancer drugs can induce psychiatric problem are largely unknown. The following candidate mechanism may play roles in the effects of chemotherapy on central nervous system function: change in blood-brain barrier permeability, the efficiency of cellular efflux pumps, DNA damage, telomere shortening, alteration of cytokine regulation, defects in neural repair, and oxidative stress (Ahles and Saykin 2007).

14.7.6.7 Diagnosis of Psychiatric Problems

More than half of psycho-oncological problems remained under-recognized. Thus active case finding is very important in this setting. A known barrier for mental health assessment is fear of the label of having psychiatric problems. For his reason and for the practical issues, it is preferred that mental health service in the oncological setting would be collaborative in nature. Steps of providing service to patients include brief screening for distress by using appropriate instruments, primary assessment and interventions by cancer clinicians (oncologists, nurses) or social workers and finally the referral to psychiatrist or clinical psychologist.

Currently, three validated methods of screening are available: Hospital Anxiety and Depression Scale-total score (HADS-T) (Zigmond and Snaith 1983), Distress Thermometer (Roth et al. 1998) and Single verbal question (Lloyd-Williams 2004). The HADS is a 14-item self-report questionnaire which has good accuracy but may be considered too long for routine use. The DT is a simple measure, consisted of 0–10 scale, started at zero with "no distress" and finished at 10 with "extreme distress". Patients asked to rate their level of distress during past week on a scale of 0–10. In single verbal question method, clinicians ask the patient, "Are you depressed?" or "Do you have loss of interest?" The DT and single verbal question are feasible and acceptable, but the accuracy of them was not sufficient (Mitchell 2010).

Rapid psychometric assessment, alone, is not enough and cancer clinicians should have basic required skills to communicate with patients who have emotional problems and to do a simple intervention for symptom relief. In more complicated cases, while more specialized treatment needed, the patients should manage with the collaboration of a psychiatrist or a clinical psychologist.

14.7.7 Psychiatric Treatments

14.7.7.1 Psychopharmacological Treatments

The psychopharmacological treatment of patients with cancer needs a high level of expertise and carefulness. The clinician should think carefully about specific symptoms profile (pain, insomnia, hot flash), adverse effects, route of administration and interaction with oncologic treatment. Thus, guidelines for treatment of

psychiatric disorders in general population may not applicable directly to psycho-oncology setting.

Bondi and Pasquini have suggested a useful dimensional approach for recognition and treatment of psychiatric problems in cancer patients (Wise et al. 2013). Dimensional approach in comparison with the categorical approach is a more sensible description about psychopathology and have more clinical flexibility which is very useful in psycho-oncology. In spite of this, both approaches should co-exist and one is complementary to other. Bondi and Pasquini (2013), suggest that categorical diagnosis be determined first to provide a general framework of patient's clinical condition and in the second phase a dimensional diagnosis should be considered to establish a good plan for treatment (Wise et al. 2013).

One of the most useful instruments for the previously mentioned dimensional approach in psycho-oncology setting is La Scala di Valutazione Rapida Dimensionale (Rapid Dimensional Assessment Scale [SVARAD]) which is developed by Pancheri et al. (2001). The SVARAD is a 10-item measurement designed for rapid assessment of some important psychopathological dimensions (Table 14.10). Each item is rated on a 5-point scale ranging from 0 to 4. The SVARAD inquire the following dimensions: apprehension/fear, sadness/demoralization, anger/irritability, impulsiveness, activation, apathy, obsessionality, somatic preoccupation/somatization, reality distortion, thought disorganization.

In dimensional approach, psychopathology could be considered as a response to the stress of cancer that involve both mind and body. The arising distress syndromes are multidimensional in nature and depending on personality, coping skills and resiliency of patient in one hand and the degree of psychopathological component like anxiety, obsession, depression and so on. In addition, this approach emphasizes the effect of stress on the brain and its neurotransmitters. Biondi and Pasquini (2013), have suggested psychopharmacological interventions according to this dimensional approach (Table 14.10) (Wise et al. 2013). This approach that shows similarities with personalized medicine, requires more evidence in future.

14.7.7.2 Psychosocial Treatments

Nowadays, Psychotherapy becomes a necessary part of any psycho-oncology care for patients with cancer. Various psychotherapeutic methods available for helping these patients including education, Behavior training, hypnosis, biofeedback, guided imagery, cognitive-behavior therapy, mindfulness-based cognitive therapy, short-term psychodynamic therapy, existential psychotherapy, group therapy and self-help group. All of this method should be tailored to patients with cancer in terms of duration, techniques and targeted outcomes. The numerous outcome studies have been supported the efficacy of different kind of psychotherapies at least with modest effect size (Devine 1996; Compas et al. 1998; Barsevick et al. 2002; Aziz and Rowland 2003; Fawzy and Fawzy 2003; Grunfeld 2006; Osborn et al. 2006; Akechi et al. 2008).

Table 14.10 Dimensionally oriented drug treatments Biondi and Pasquini (2013)

Psychopathological dimension	Suggested Psychotropic drugs
Sadness/demoralization	Serotonergic and noradrenergic drugs Dual acting drugs (serotonergic and noradrenergic) MAOIs High-dose omega-3 supplement
Apprehension/fear	BDZs Serotonergic and noradrenergic drugs (SSRIs, TCA) Dual acting drugs (5-HT and NA)
Anger/irritability	Mood stabilizers (anticonvulsant) Low dose serotonergic drugs or TCAs or low dose SSRIs
Activation and impulsiveness	Mood stabilizers (anticonvulsant) D2 or D2/5-HT2 antagonists (low dose typical and atypical antipsychotics) BDZ or non-BDZ hypnoinducers Antihistaminic and antidepressant with high antihistaminic sedative properties
Apathy	Dopaminergic drugs Noradrenergic drugs Dual-acting agents Low-dose sulpiride or amisulpride MAOIs
Obsessionality and somatic preoccupation/somatization	Serotonergic drugs (SSRIs; clomipramine)
Thought disorganization and reality distortion	D1 and D2 antagonists; D2/5-HT2 antagonists

BDZ benzodiazepine; *D1* dopamine1 receptor; *D2* dopamine2 receptor; *5-HT* serotonine; *MAOI* monoamine oxidase inhibitor; *SSRI* selective serotonin reuptake inhibitor; *TCA* tricyclic antidepressant

Earlier studies suggested that psychosocial intervention could increase survival of patients with cancer (Fawzy and Fawzy 2003; Spiegel et al. 1989). However, later studies that have more precise methodology failed to show any effects on survival (Linn et al. 1982; Edelman et al. 1999; Goodwin et al. 2001; Kissane et al. 2003). It seems that psychosocial interventions effects on survival time are more noticeable when oncologic treatments have been less successful (Spiegel 2011).

One of the most popular psychosocial treatment in psycho-oncology settings is group therapy which facilitates sharing of information and mutual support. Group therapies usually used the following strategies: social support, emotional expression, detoxifying dying, recognizing life priorities, enhancing family support, improving communication with physicians and symptom control (Cohen 2001).

14.7.8 Family and Cancer

Cancer affects family function profoundly. Family adaptation to cancer is a dynamic process with many cycles of hope and desperation. Kissane et al. (1994) developed an empirical model of family functioning in bereavement care that could be extrapolated to the family of patients with cancer (Kissane 1994). In this model, family adaptation depends on three "C's" of family relationship: cohesion, communication, and conflict resolution. These three items differentiate families into well-functioning, intermediate and dysfunctional classes.

Well-functioning families are supportive, conflict resolvers and have a high level of resiliency and low level of psychosocial morbidity. About 50% of the family in cancer care are like this and do not require family therapy. Dysfunctional families are hostile, sulky, have poor communication and high level of psychosocial morbidity including depression. Between 15 to 30% of families in cancer care are dysfunctional. One-third of families are intermediately dysfunctional and tend to become worse under the strain of critical cycles of crises. These two later types of dysfunctional family need family therapy (Kissane et al. 2003).

14.7.9 Bone Marrow Transplantation (BMT)

The presence of a life-threatening disease and the possibility of a treatment that may cure but which also has potentially deadly side effects puts the patients in a hope versus loss situation with significant emotional constraints. As a result, a significant proportion of patients may develop psychiatric morbidities including adjustment disorder, depression, anxiety and acute stress disorder (Prieto et al. 2002).

Psychosocial assessment instruments such as the Transplant Evaluation Rating Scale (TERS) have been developed for assessing the psychosocial status of BMT recipients, which could then entail early intervention in the case of adaptation. The TRES have following 10 items: psychiatric disorders, health behavior, substance abuse, coping style as a whole, coping with the disease, compliance, family support, affect quality, mental state. Items rated by the clinician on 3 point scale which high scores represent more problems (Hoodin and Kalbfleisch 2003).

Restriction to leave a room, the unpredictability of future of transplant, the complication of neutropenia like mucositis and infections, graft versus host disease (GVHD) and lengthy hospital stay are among the known sources of distress in patients underwent BMT. Common psychiatric disorders in BMT setting are anxiety, depression, delirium, non-adherence to treatment and body image disorders (Andorsky et al. 2006).

14.7.10 Cancer Survivorship

The term "cancer survivor" was invented for the first time by Fitzhugh Mullen, Physician and cancer survivor. He defined cancer survivors as a person "living with and beyond cancer". His definition continues to be used today (Mullan 1985).

Although cancer treatment improves survival it is often accompanied by long-term physical adverse effects and increased level of distress. During the cancer survivorship subtle psychological distress like anxiety, prior visits to the doctor are common. The major psychiatric disorders are uncommon but anxiety about recurrence/ illness/ death could be seen. About 15–25% of patients may suffer from posttraumatic stress disorder symptoms.

Survivors have a greater sense of vulnerability, less control, and lower self-esteem. A reminder of chemotherapy may produce anxiety and nausea. Body image disturbances and the problem with sexuality was reported in breast cancer survivors and patients with hematologic malignancies who underwent BMT. Sexual functioning may also decline in men following treatment of prostate cancer. Career goal may alter negatively as some patients have less ability for strenuous activities (Ganz 2007).

In the recent decades, the awareness of the importance of patients reported outcomes (PROs) was increased in the field of psycho-oncology. The quality of life (QoL) is an important issue in cancer survivors research which has 6 domains: physical health (cancer and its treatment), functional (usual activities), psychological health (well-being), sexual, social and work. In addition to research, QoL as a PRO could be used in the clinical setting as an indicator for symptom management and prognosis.

14.8 Conclusion

Cancers of the blood and lymph or immune cells, such as leukemia is the focus of the hematologic malignancies and bone marrow transplant. Since the Cytogenetic and, increasingly, molecular genetic findings at diagnosis provide important and independent prognostic factors in practically all types of leukemia, our main focus in this chapter was in these two topics. In acute myeloid leukemia, the karyotype is important to post-remission treatment decisions, and molecular factors determine the treatment of cases with normal karyotypes. In chronic myeloid leukemia, clonal evolution is associated with progression to the blast crisis that can be revealed by additional cytogenetic abnormalities. Patients on imatinib who cease responding may have mutations on their ABL gene that can be detected by sequencing. CLL cases with P53 mutation or deletion are candidate for HSCT. Finally, in acute lymphoblastic leukemia, the high risk patients according to cytogenetic must be transplanted in first complete remission and if the suitable donor is not available transplantation from haploidentical or unrelated donors should be considered.

We can conclude that the use of cytogenetic analysis, as a component of the routine diagnostic work-up of leukemia, can provide a framework for risk stratification, to be used in conjunction with screening for an increasing range of molecular markers—required not only to predict risk of relapse but also to further dissect out groups of patients with differing prognoses to develop more appropriate risk-stratified treatment approaches for such patients.

Since The lack of access to psycho-oncology services across the cancer care service delivery field has been a reality for decades so one of the other focus was in this topic. Otherwise, current evidences are supporting the use of psychological screening, education and psychosocial interventions in cancer care setting. Patients with cancer in their "cancer trip" should adapt with sequence of crises including crisis of hearing diagnosis, crisis of intensive therapy whether it is surgery, chemotherapy, radiotherapy or combination of them and survivorship crisis. Psycho-oncology is a rapidly progressing subspecialty. Building knowledge and skills are the required steps for the health professionals to enroll as a psycho-oncology care provider and to participate in cancer care networks.

14.9 Summary

Leukemia is cancer of the blood forming cells, frequently affecting the white blood cells, which causes these cells to not work properly. There are four main types of leukemia includes mainly AML, CML, ALL and CLL. Leukemia can occur in either the lymphoid or myeloid white blood cells. The disease can be either acute (begins abruptly and is usually short lived) or chronic (persists for a long period of time). Acute leukemia involves new or immature cells, called blasts, which remain very immature and cannot perform their functions. The blasts increase in number rapidly, and the disease progresses quickly. In chronic leukemia, there are some blasts present, but they are more mature and can perform some of their functions. The cells grow more slowly so the disease progresses gradually. Current advances in diagnostic methods, our understanding of its molecular basis and therapeutics have made leukemia one of the most exciting and rapidly changing fields in oncology. Owing to the routine use of sensitive and innovative molecular and cytological techniques to diagnose or prognosticate leukemia and applying novel targeted agents for leukemia therapy, the study of leukemia has been always at a forefront of cancer research. Diagnostic karyotype provides the framework for risk-stratification schemes in leukemia and according to this we can determine which patients benefit from allogeneic transplantation, autologous transplantation or high dose chemotherapy alone. The molecular diagnostic tests are also necessary in all leukemia patients. For example in AML cases, NPM1 and FLT3-ITD mutation status provide independent prognostic information in patients who would otherwise have been considered intermediate risk on the basis of the karyotypic abnormalities.

Moreover, adaptation to different types of cancer such as leukemia is a dynamic and longitudinal process. Major psychological problems that may occur during this process include demoralization, depression, anxiety, suicide and cognitive dysfunction. Various psychotherapeutic methods available for helping these patients including education, Behavior training, hypnosis, biofeedback, guided imagery, cognitive-behavior therapy, mindfulness-based cognitive therapy, short-term psychodynamic therapy, existential psychotherapy, group therapy and Self-help group. In this chapter expert clinicians and researchers have expressed in details the cytogenetic, molecular and hematological aspects of different types of leukemia and have attempted to briefly cover the major topics in the field of psycho-oncology.

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Chapter 15
Cancer Stem Cell: From Conjecture to Reality

Vahid Ezzatizadeh

Abstract This chapter compiles various approaches of the currently advocated theory in development of most cancers through activation of the small sub-population of malignant cells with stemness characteristics, termed as cancer stem cells (CSCs). It briefly deliberates for some fundamental molecular mechanisms involved in tumorigenesis, and the potential impact of intrinsic or extrinsic factors on inducing epigenetic and/or genetic changes to improve the capacity of CSC development. Capability of CSCs to proliferate, invade, migrate, relapse and resist to several therapies has confronted cancer treatment with several obstacles. Proliferative feature of this cell type could symmetrically lead to self-renewal. Asymmetric proliferation of CSCs contributes hierarchically to not only self-renewal, but also the other types of cell, including malignant progenitor or mature cells; the procedure culminating potentially to heterogeneity of tumor bulk. In this chapter, some direct/indirect effects of microenvironmental factors and metabolism on preservation of CSC fate, invasion or even metastasis of these cells are demonstrated. Considering the molecular mechanisms promoting CSCs development, some potential therapeutic approaches are herein reviewed. Ultimately, current challenges on determination and treatment of CSCs are discussed.

Keywords Cancer stem cell · Microenvironmental factor · Metabolism · Molecular mechanism · Therapeutic approach · Genetics · Epigenetics

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Abbreviations

AML	Acute myeloid leukemia
BCSC	Breast cancer stem cell
BLBC	Basal-like breast cancer
CIC	Cancer initiating cell
CML	Chronic myeloid leukemia
CSC	Cancer stem cell
CTC	Circulating tumor cell
CXCR4	Chemokine C-X-C motif receptor-4
DLL4	Delta-like ligand 4
DNMT	DNA methyltransferase
EMT	Epithelial-mesenchymal transition
EpCAM	Epithelial cell adhesion molecule
FAK	Focal adhesion kinase
FPB1	Fructose-1,6-biphosphatase
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GSI	Y-secretase inhibitor
GSK-3β	Glycogen kinase-3 beta
HDAC	Histone deacetylase
HGF	Hepatocyte growth factor
HGSOC	High-grade serous ovarian cancer
HIF	Hypoxia inducible factor
HSC	Hematopoietic stem cell
IGF-1	Insulin-like growth factor-1
LCSC	Liver cancer stem cell
lnc-RNA	Long-noncoding RNA
LSC	Leukemic stem cell
MET	Mesenchymal-epithelial transition
MMP-7	Matrix metalloproteinase-7
mTOR	Mammalian target of rapamycin
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NK	Natural killer
nSC	Normal stem cell
NSCLC	Non-small cell lung cancer
OPN	Osteopontin
PGC1α	Peroxisome proliferator-activated receptor gamma co-activator 1
PPARα	Peroxisome proliferator-activated receptor alpha
PRC2	Polycomb-recessive complex 2
PTEN	Phosphatase and tensin homolog deleted on chromosome 10
ROS	Reactive oxygen species
SDF1	Stromal cell-derived factor-1α
Shh	Sonic hedgehog

Snail-1	Snail homologue 1
TCF	T-cell factor
TFA	Thomsen-Friedenreich antigen
VEGF	Vascular endothelial growth factor
ZEB-1	Zinc-finger E box-binding homeobox 1

15.1 Introduction: Cell Malignancy, Heterogeneity and Cancer Stem Cell

It has now been few decades after the first pathological report, representing the existence of cell sub-populations with tremendous diversity within a single neoplasm. The so-called tumor heterogeneity confronted this disorder with several difficulties. Recent advances have introduced this phenomenon as a crucial event within most of the malignancies, whereby the cells are phenotypically and functionally different. Thus, several cancer patients yet fail therapies, leading to progression, even recurrence of this disorder and consequently reduction of survival length. This diversity could be caused by multiple mechanisms, emanated from intrinsic or extrinsic sources. Sequential developments of the current technologies have facilitated rapid and precise analysis of individual tumors, shedding light on the crucial role of epigenetic (Biswas and Rao 2017) or spontaneous genetic alterations (Vogelstein et al. 2013), as two intrinsic routs involved in intratumoral heterogeneity formation. Tumor cell heterogeneity can also arise due to several extrinsic factors including environmental interactions (*e.g.* lifestyle, stress and diet), which they consequently induce somehow intrinsic mechanisms.

On the other hand, existence of cellular hierarchy is the other leading cause of tumor heterogeneity (Beck and Blanpain 2013). It has currently been concluded that dominant genetic or epigenetic alterations support clonal evolution theory, in which similar tumorigenesis could potentially be transmitted to offspring (Visvader and Lindeman 2012). In contrast, cellular hierarchical organization is generally observed in non-inherited malignancies, the phenomenon that leads to reduction of chemotherapy response potential, higher chance of recurrence, resistance to the treatment and metastasis. This organization constructs an important substance of "Cancer Stem Cell Theory". Thus, a sub-population of malignant stem cells initiates tumorigenesis symmetrically or asymmetrically. This small sub-population is generally responsible for generation of all other malignant cells in the tumor bulk, comprised of tumorigenic or non-tumorigenic cancer cells (Magee et al. 2012). This conversion facilitates a mechanism to generate phenotypically and functionally heterogeneous cells, consequently harboring fully treatment of the generated cancer.

15.2 Cancer Stem Cell Theory

Historically, the concept of cancer stem cell (CSC) is not a novel idea. In 1968, Robert Mcallister and George Reed implicated the capacity of particular human cancer cells to generate colony, as an essential feature of CSCs, in soft agar medium (McAllister and Reed 1968). Few years later, the theory of clonal evolution was proposed by Peter Nowell, when he indicated that most of neoplasms are originated from single cell, and tumor progression is caused by attainment of genetic variation in the generated clone, authorizing sequential selection of more aggressive sub-populations (Nowell 1976). Further literature reviews show that even before determining hematopoietic stem cell (Till and Mc 1961) and colony formation of tumors, existence of CSCs was suggested through findings obtained from Hewitt's investigations, in 1958. In this study, he transplanted different dosages of leukemia cell suspension into isogenic mice, using the limiting dilution assay (LDA), to determine the minimal quantity of malignant cells required to eradicate this disease. Curiously, findings revealed that existence of at least three lymphocytic leukemia cells could synergistically contribute to generation of a new tumor (Hewitt 1958; Trott 1994). Later, the concept of CSC model was further discussed by Dick and colleagues in 1990s. He demonstrated that tumorigenic feature of acute myeloid leukemia (AML) is attributed to a rare malignant initiating cells (1 in 250,000 malignant cells), distinguished from other cancer cells by expression of particular surface markers. These initiating cells signified several characteristics of human AML in vivo, while it was less mature than colony-forming cells (Lapidot et al. 1994). Simultaneously, several investigations reported the remarkable role of particular CSCs in the other distinctive types of malignancy emerged from brain, lung, breast, prostate and gynecological tissues (Cohen et al. 1994; Krystal et al. 1996; Tsai et al. 1996; Bonkhoff 1996; Bonkhoff and Remberger 1996; Inoue et al. 1994).

Although CSC theory had yet low face validity due to several controversies, further investigations revealed high correlation between this theory and neoplasm development hierarchical model (Bonnet and Dick 1997). So that, it has currently been accepted that abundance of these cells, in terms of quantity, contribute to the respective disease progression in human.

15.3 Stemness, a Crucial Feature of Cancer Stem Cells

In contrast to clonal evolution, whereby sub-clones could individually generate a new tumor, CSC theory emphasizes that a rare sub-population of intratumoral cells is responsible for possession of tumorigenesis. Similar to normal stem cells (nSCs), CSCs have low metabolic rate, low requirement of growth factor, long-life survival rate as well as slow cycling and unrestrained self-renewal ability (Ghaffari 2011; Li and Neaves 2006; Cabrera et al. 2015). Proliferation is also a crucial biological process in both nSCs and CSCs, leading to establishment of hierarchical cell

population with heterogeneous phenotype and genotypes. That means both of these cell types carry not only symmetric, but also asymmetric capacity for cell proliferation. Thus, several CSCs with retaining stemness property are established in symmetric cell division, while asymmetric cell division coordinate with generation of a rare cell population of multipotent CSCs as well as different sub-populations with less stemness properties, including progenitor and differentiated cells (Kreso and Dick 2014). In addition, findings demonstrated activity of several transcription factors in CSCs, contributing to the stemness property preservation in these types of malignant cell which will be discussed later.

15.4 Cancer Stem Cell and Markers

Remarkable efforts have been made in recent years to detect and characterize CSC markers. Thus, an overwhelming number of such molecular markers have been distinguished, among which there are several identical markers between nSCs and CSCs (Table 15.1). Hence, ectopic expression of these markers could be used neither to distinguish CSCs from nSCs, nor in therapeutic approaches. For instance, several studies have used Prominin-1 (CD133) as a CSC surface marker in different types of malignancy (Singh et al. 2004; Mizugaki et al. 2014; Nomura et al. 2015), while this marker is also expressed in some nSCs, including bone marrow and embryonic neural stem cells (Kania et al. 2005). Despite discovery of many more robust markers, like SSEA-1, CD15, CD26, a6-integrin, determination of CSCs is not feasible using single marker. To determine CSC using biological markers, three different approaches are currently recruited, included utilizing (I) combinational markers, (II) particular marker isoforms, (III) onco-fetal stem cell markers.

Combination of some markers, including CD44, CD133, ALDH1 and EpCAM, could potentially be useful to more precisely determine and isolate CSC enriched subsets in solid tumors. Co-expression of CD34, CD38 and IL-3Ra has been reported as an applicable method for isolation of leukemic CSCs, while none of them can individually be distinguished as an exclusive CSC marker (Visvader and Lindeman 2012).

Although not yet fully approved, it seems that different isoforms of nSC markers might also be specifically expressed in CSCs, in some cases. For instance, Herrlich and colleagues determined an isoform of glycopeptide CD44 attributed to increase metastatic potential in rat carcinoma cells (Karsten and Goletz 2013), when the normal isoform is observed in hematopoietic and pancreatic stem cells. As another example, Lee and colleagues demonstrated, in 2011, the isoform ALDH1A3 activity for aldehyde dehydrogenase in prediction of breast cancer metastasis (Marcato et al. 2011), while the normal isoform is generally expressed in the healthy mammary stem cell cytoplasm.

Table 15.1 Instance of the markers commonly expressed in different types of nSCs and CSCs

Cell marker	Cellular localization	nSC	CSC	References
CD24	Membrane	Breast	Pancreas, colorectal, liver, ovary	Han et al. (2014), Chiba et al. (2016)
CD29	Membrane	Breast, mesenchymal, neural	Breast, colon, head and neck	Karsten and Goletz (2013), Li et al. (2016), Romanyuk et al. (2015), Han et al. (2014)
CD34	Membrane	Hematopoietic, mesenchymal	Leukemia, skin	Karsten and Goletz (2013), Visvader and Lindeman (2008)
CD44	Membrane	Hematopoietic, pancreatic	Head and neck, breast, pancreas, liver, colon	Karsten and Goletz (2013), Visvader and Lindeman (2008), Chiba et al. (2016)
CD90	Membrane	Thymus, mesenchymal	Brain, breast	Karsten and Goletz (2013), Li et al. (2016)
CD117	Membrane	Mesenchymal	Brain, breast, ovary, lung	Karsten and Goletz (2013), Eterno et al. (2014)
CD133	Membrane	Hematopoietic, neural, colon	Brain, head and neck, lung, liver, pancreas, melanoma, colon	Karsten and Goletz (2013), Han et al. (2014), Visvader and Lindeman (2008), Chiba et al. (2016)
ALDH1	Cytoplasm	Breast	Breast, myeloma, colorectal, liver	Visvader and Lindeman (2008), Zhou et al. (2014b), Zhou et al. (2014a), Chiba et al. (2016)
EpCAM	Membrane	Liver	Pancreatic, liver, colon	Visvader and Lindeman (2008), Dolle et al. (2015), Chiba et al. (2016)

In addition, it has been proposed that the best option for detection of CSCs could be applying the onco-fetal stem cell markers. They are not expressed on normal adult stem cells and could be one of the clear-cut markers to distinguish CSCs from the normal types. In fact, a small quantity of discovered stem cell markers is composed of the glycan bound to proteins or lipids. These compounds are regulated through development and often changed in malignant cells. Findings demonstrated that ectopic CD176 (Thomsen-Friedenreich antigen; TFA), as a glycan bound onco-fetal stem cell marker, is co-expressed with CD133 stem cell marker in breast, lung and liver cell lines. Further investigations showed co-expression of TFA and CD44 in 5–30% of the indicated tumor tissues, implicating the existence of CSCs, as a sub-population in heterogeneous tumor tissue bulk (Karsten and Goletz 2013).

CSC concept essentially emphasizes that metastasis is a phenomenon in malignant cells, which is confined to these type of malignant stem cells. So that, higher rate of TFA-positive cells in several metastatic cases is determined (Sindrewicz et al. 2016; Zhan et al. 2015). As a case, Fiese and colleagues reported

that bone marrow disseminated tumor cells of breast cancer patients were TFA-positive in almost all cases (Schindlbeck et al. 2005). This is particularly consistent with the proposed role of CSCs in metastasis, while primary tumors are mosaic for TFA marker.

15.5 Impact of Microenvironment on Cancer Stem Cell Development

In addition to genetic diversity, microenvironment could have remarkable influence on tumor heterogeneity through induction of epigenetic factor modifications. Curiously, evidences show a bilateral interaction between niche (*i.e.* an intended environment to the particular sub-clonal cells or tissue) and intrinsic cell status. Thus, microenvironmental changes could itself be caused due to the activity of particular sub-clones in the same tumor. On the other hand, these environmental condition verifications could induce growth of dormant sub-population in the tumor (Marusyk et al. 2014; Visvader and Lindeman 2012). As a case, accumulating data obtained from several investigations show that VEGF secretion from glioblastoma CSCs into the respective niche promotes local vascularization. In this process, glioblastoma CSCs can conduct vascularization through transdifferentiation into endovascular cells. In contrast, nitric oxide is secreted from endothelial cells leading to glioma cells Notch signaling activity and consequently elevation of malignant cell survival rate and stemness capacity (Visvader and Lindeman 2012).

Despite detecting no evidence in some malignancies, crucial effect of microenvironment on development and progression of several CSCs has been determined. In this regard, it has been revealed that extrinsic interactions with stem cell niche could epigenetically transform the status of in site malignant cells into CSCs, leading consequently to self-renewal, angiogenesis and/or metastasis promotion (Plaks et al. 2015).

Recent findings implicate the pivotal role of endothelial cells and subsequently perivascular niche in the regulation of CSCs (Lu et al. 2013; Krishnamurthy et al. 2014). In fact, vascularization (angiogenesis) plays essential role in cancer initiation, development and homeostasis by regulating nutrient, oxygen, growth factor secretion as well as immune cell interactions. It has been demonstrated that perivascular niche and specifically vascular endothelial growth factor (VEGF) bear an impact on CSCs development in skin squamous cell carcinoma. Thus, inhibition of this growth factor activity causes regression of the respective stemness and symmetric cell division of CSCs localized in the perivascular niche (Beck et al. 2011). VEGF also serves similar effect on glioblastoma CSCs regulation (Hamerlik et al. 2012).

The influence of niche and respective constitutes is not limited to CSC of solid tumors. Even in non-solid malignancies, niche alteration could involve in initiation and development of CSCs. It has been demonstrated that bone-marrow (BM) niche

alteration, caused even due to the particular genetic mutation of the presented cells in microenvironment, could stimulate hematopoietic stem cell (HSC) niche remodeling toward the leukemic stem cell (LSC) niche. Although many aspects of this process have not been elucidated yet, it is clear now that the aforementioned microenvironment remodeling could consequently assist development of LSC and blood malignancies (Schepers et al. 2015).

15.6 Cancer Stem Cell Metabolism

In addition to the niche, metabolic mechanisms have been shown to play essential role in acquisition of CSC fate. Many decades ago, Otto Warburg determined that fermentation (glycolysis) of glucose is a desired procedure for metabolism of cancer cells, despite presence of sufficient oxygen (Warburg 1956). This phenomenon could presumably be described with regards to the capability of more rapid ATP production in glycolysis compared to oxidative phosphorylation in mitochondria. Nevertheless, current findings show more complication in tumor metabolism (inspired from different factors, such as hypoxia/normoxia, proliferation/quiescence) due to the cell heterogeneity. In has also been indicated that similar to cancer cells, several types of nSC (*e.g.* HSCs) recruit glycolysis, while the metabolism of differentiated counterparts rely more on mitochondrial respiration (Ito and Suda 2014). Thus, metabolic system switches from oxidative phosphorylation, whereby remarkably more ATP is produced, into glycolysis through conversion of normal somatic cell into induced pluripotent stem cells (Folmes et al. 2011). This suggests that glycolysis is required for survival and adaptation of the cells with stemness fate. Considering the singularity of embryonic stem cells and CSCs from many aspects, it appears in the first instance that the latter cell types should also apply glycolytic metabolism for survival and self-renewal. However, current evidences represent a distinct difference between the metabolic signature of CSCs and not only nSCs, but even tumor bulks (Dando et al. 2015).

Glucose is an essential nutrient, affluence of which in the malignant cell microenvironment further stimulates CSC survival and proliferation. This stimulation is mechanistically mediated through impairment of AMPK activity and recruiting activity of Akt signaling pathway, consequently increased expression of ATP-dependent efflux pump ABCG2 (Liu et al. 2014). Findings highlight the crucial role of glucose uptake level, glycolytic enzyme activity, lactate formation and ATP output in stimulation of glycolysis pathway, inhibition of mitochondrial respiration and development of several types of CSC, including brain, lung, breast, ovarian and colon cancers (Peiris-Pages et al. 2016). In these cell types the expression level of pyruvate dehydrogenase kinase is elevated, promoting conversion of pyruvate to acetyl-CoA or lactate. This mechanism inhibits metabolic flow into mitochondrial respiration, while promotes obtaining metabolic energy through glycolysis. Similar to nSCs, Akt pathway activity is triggered and AMPK is repressed in CSCs (Liu et al. 2014). It has been shown that activation of several

enzymes, including lactate dehydrogenase and glucose 6-phosphate dehydrogenase, switches BCSC metabolism from oxidative phosphorylation into glycolysis. Thus, BCSC proliferation is blocked by inhibition of glycolysis (Ciavardelli et al. 2014). In addition, silencing fructose-1,6-biphosphatase (FPB1), resulted from promoter methylation of the respective gene, induces glycolytic mechanism by prohibiting respiration in basal-like breast cancer (BLBC). In this metabolic flux, lack of FPB1 induces accumulation of pyruvate and glycogenesis, while the level of reactive oxygen species (ROS) is reduced. This mechanism consequently promotes β-catenin causing further susceptibility of BLBC to present CSC and epithelial-mesenchymal transition (EMT) phenotypes (Dong et al. 2013).

Contrarily, other evidences emphasis that CSCs uptake less glucose and they have less glycolytic enzyme activity, lactate formation and higher ATP output compared to their originated progenitors. In these cells, mass of mitochondria, potential of mitochondrial membrane (as a character of functional mitochondria) as well as ROS and oxygen consumption levels are increased in comparison with their differentiated counterparts. These features contribute to higher potential of CSC invasiveness and metastasis, through stimulation of peroxisome proliferator-activated receptor gamma co-activator 1 (PGC1α) and mitochondrial biogenesis (Peiris-Pages et al. 2016). Thus, loss of PGC1α has been attributed to block pancreatic and BCSC stemness features (De Luca et al. 2015; Sancho et al. 2015). Consistently, overexpression of oxidative phosphorylation associated genes, oxidation of fatty acids and higher ROS production level were observed in ovarian CSCs, implicating pyruvate direction capacity toward the mitochondrial respiration and Krebs cycle (Pasto et al. 2014). It has also been shown that oxidative phosphorylation is a crucial step in generation of sufficient ATP through sphere formation of glioblastoma CSCs (Janiszewska et al. 2012).

Although analysis of all findings is not feasible here, evidences indicate the controversial action mode of metabolic system in CSCs. There are currently several investigations undergoing to deeply comprehend CSC metabolism, as a critical factor involved in development of CSCs. In this regard, review (Dando et al. 2015; Peiris-Pages et al. 2016; Hsu and Sabatini 2008; Menendez et al. 2013) for more information.

15.7 Cancer Stem Cell Metastasis

Evidences emphasis that malignant cell invasion-metastasis process is the principal cause of death in more than 90% of cancer patients; a process through which malignant cell initially invade into the encompassing tissue, leading ultimately to migration and propagation of the malignant cells into the secondary organs (Diepenbruck and Christofori 2016). Meanwhile, CSC fate plays crucial role in the furtherance of invasion and metastasis of tumor. Several studies implicate that CSC state has a considerable plasticity, boosting capability of these cells to change the fate, invade and migrate. Niche principally equips an appropriate microenvironment

to facilitate CSC development and plasticity. This plasticity could generally be led due to the fluctuations in the particular niche or differences in various microenvironments. Thus, it facilitates conditions for CSC to get involved in the multistage invasion-metastasis process. In the first step, some malignant cells, including CSCs, aberrantly undergo a fundamentally conserved process, whereby the fate of these cells is transited from polar intercellular adhesive epithelial cells to mesenchymal cell states, calling as EMT process type 3 (Sato et al. 2016). Despite the fact that EMT in a natural process recruited in embryogenesis (EMT type 1) or wound healing and organ fibrosis (EMT type 2), aberrant activation of this procedure could lead to elevation of malignant cell invasion and metastasis (EMT type 3). Once CSCs loss epithelial cell-cell adhesion as well as apical-basal polarity, they are remodeled and turned into low proliferative cell with spindle shape, higher survival and migration capabilities, pervading the encompassing tissue. In the next step, these malignant cells migrate from stroma and basal membrane to blood or lymphatic circulatory system, a process so-called intravasation. In this procedure, the intravasated malignant cells are called circulating tumor cells (CTCs). CTCs are generally confronted a new niche and they have to resist against hemodynamic forces, anoikis (induction of apoptosis caused by loss of cell interaction with extracellular matrix) as well as fluid shear effects. In addition, leukocyte immunological signaling insults and erythrocyte collisions are recruited to help platelet and macrophages combat against CTCs. Thus, only small fractions of CTCs, including CSCs, are able to extravasate into the encompassing stroma and migrate toward secondary host organ parenchyma, under a reverse procedure termed as mesenchymal-epithelial transition (MET), upon attachment to the blood vessel wall (Rejniak 2016). Findings show that only 0.01% CTCs is able to generate secondary tumors, while the rest do not necessarily represent CSC fates during circulation. However, the extravasated CTCs mimic CSC features as soon as approaching to the metastatic niche. By that mean, they turn into long-lasting malignant cell with compatible proliferation, self-renewal and colony sphere formation potentials to the new microenvironment, as well as further invasion and metastatic abilities (Diepenbruck and Christofori 2016).

Considering the distinctive capability of CSCs on proliferation, long-lasting self-renewal and more importantly potential of differentiation to the other cell types, including secondary malignant cells, these cells could be responsible for metastasis. There is yet a non-fully answered question whether all malignant cell invasion and metastasis are caused by CSCs. Evidences emphasis from three aspects that CSC could be, at least, one of the fundamental malignant cells, contributing to metastasis of several cancer types. Firstly, several cell intrinsic molecules have been determined contributing to CSC metastasis. As a case, the chemokine C-X-C motif receptor-4 (CXCR4) is a metastatic marker expressed in different malignant cell types, including breast, prostate, pancreatic and melanoma cancers. It has been revealed that CXCR4 protein is expressed in a metastatic subset of pancreatic adenocarcinoma and colorectal cancer with $CD133^{+}$-CSC features (Yang et al. 2015). DCLK1 is another crucial marker contributing to CSC metastasis. It has

recently been demonstrated that up-regulation of DCLK1 stimulates pancreatic CSCs invasiveness and migration to the secondary organs, likely through modification of histones (Ito et al. 2016).

Secondly, findings present the critical impact of cell extrinsic factors (including microenvironmental hypoxic and inflammatory modulators) on promoting EMT process, and consequently metastasis, in CSCs. In addition to the malignant cells, tumor stroma cells (including cancer-associated fibroblasts, tumor-associated macrophages and tumor-cell platelets) might secret extracellular stimuli mediating EMT procedure. There are several growth factors (*i.e.* TGF-β, FGF, EGF, HGF, PDGF and VEGF) as well as the other bio-molecular stimulators extrinsically regulate EMT, including BMP, Wnt, Notch and sonic hedgehog (Shh) signals. Finding shows that cytokines osteopontin (OPN) and hepatocyte growth factor (HGF), as well as chemokine stromal cell-derived factor-1α (SDF1, also known as CXCL12), secreted from tumor-associated cells, induce Wnt/β-catenin signaling pathway. Activity of this pathway promotes CD44v6 expression, as an important protein required for CSC invasion and metastasis (Todaro et al. 2014). It has been revealed that chemical agents (such as UV-light, viral infection, alcohol and nicotine) could also regulate the microenvironments and indirectly stimulate EMT process (Diepenbruck and Christofori 2016; Nieto and Cano 2012).

Thirdly, evidences show the fundamental role of the cell adhesion molecules, composed of E-cadherin and β-catenin, in intermediating stem cells localization in the relevant niche. So that, α-catenin/β-catenin heterodimer forms a complex with E-cadherin leading to interaction of cell-cell junction as well as cell-niche and residing malignant cells. While interaction of β-catenin monomer with T-cell factor (TCF) in nuclei phosphorylates COOH terminus of this protein, promoting EMT procedure (Li and Neaves 2006) possibly through "cadherin switch" whereby E-cadherin is inhibited and N-cadherin is stimulated (Sato et al. 2016).

15.8 Mechanisms Underlying Cancer Stem Cell Development

With no doubt, elucidation of mechanisms underlying the survival and maintenance of CSCs is a fundamental step to contrive novel approaches on treatment of malignancies. In fact, cancer cell heterogeneity and CSC development are caused by acquisition of genetic mutations and epigenetic alterations, as double edges of the sward, culminating in changes of molecular networks and pathways.

Considering the resemblance to nSCs, it is not surprising to determine activity of several common developmental mechanisms in CSCs, including Hedgehog, Wnt and Notch pathways. This phenomenon could sometimes lead to conversion of nSC fate into CSC. Thus, growing body of evidences show that activity of particular

self-renewal pathways could stimulate development of particular CSCs from progenitor cell. Hedgehog signaling pathway is evolutionally a conserved molecular mechanism recruited at the late of embryogenesis in normal development. This pathway generally participates in the regulation of stem cell population, proliferation, differentiation and tissue polarity. For more information review (Jia et al. 2015). Dysregulation of this pathway could also lead to tumorigenesis. It has been demonstrated that reactivation of hedgehog signaling pathway could promote development of LSCs and resistance to chemotherapy in chronic myeloid leukemia (CML), while interruption of this pathway activity culminated in LSC depletion and survival lengthening rate in vivo (Siveen et al. 2017; Zhao et al. 2009). It has also been determined that Shh signaling pathway promotes maintenance and self-renewal of anaplastic thyroid CSCs, by up-regulating Gli-1 and Snail proteins. So that inhibition of Shh and Gli-1 leads to depletion of thyroid CSC self-renewal (Heiden et al. 2014). Similarly, the crucial effect of hedgehog pathway was demonstrated in the maintenance and proliferation of several types of CSCs presented in brain, pancreatic, gastric, breast, ovarian and prostate malignancies. Evidences show that inhibition of Shh could efficiently sensitize these malignancies to chemotherapy (Abdullah and Chow 2013; Kurebayashi et al. 2017).

Wnt signaling pathways are the other crucial mechanism involved in embryogenesis, while they regulate cell proliferation, migration and specification as well as body axis patterning. They also involve in balancing of adult stem cell self-renewal and differentiation. For more details, review (Bhavanasi and Klein 2016). Aberration of these mechanisms could generally contribute to CSC development in several malignancies. It has also been emphasized that Wnt signaling pathways coordinate in malignancies arising from digestive system and relative accessory organs. In this regard, mutations of *APC* or *CTNNB1* (encoding respectively APC or β-catenin protein), as two principle proteins committed in Wnt signaling, lead to up-regulation of the latter pathway in colon CSCs. Interestingly, findings demonstrated that this activity is preferentially observed in the cancer cells close to stromal myofibroblasts, proposing that stemness properties might be regulated due to the microenvironment features. So that factors secreted from these cells change microenvironments and stimulate stemness properties. This condition could also preserve CSC phenotype within differentiated cells in vitro and in vivo (Vermeulen et al. 2010). Moreover, mutation of *CTNNB1* activates Wnt/β-catenin pathway leading to preservation and proliferation of liver cancer stem cells (LCSCs) in hepatocarcinoma cell lines. Blockade of β-catenin and relative down-stream network proteins, Cyclin D1 and Survivin, inhibits LCSC proliferation by dysregulation of S-phase cell cycle (Gedaly et al. 2014). Furthermore, overexpression of Wnt1 protein activates Wnt signaling pathway, causing stimulation of gastric CSC proliferation and spheroid formation as well as expression of OCT4 and CD44 surface markers in these cells. Thus inactivation of Wnt1 and *β-catenin* could prohibit this type of CSC proliferation and growth (Mao et al. 2014). In addition to digestive organs, evidences implicate the decisive role of Wnt signaling pathways in reproductive system. Investigations implicate the resistance of breast cancer stem cells (BCSCs) to conventional chemotherapy and radiotherapy, likely causing

relapse of the respective tumor. In this type of malignancies, overexpression of β-catenin stimulates Wnt/β-catenin activity, consequently culminated in not only elevation of BCSC proliferation, survival and spheroid formation but also increased chance of relapse and resistance to several therapies. In comparison with bulk tumor cells, findings show that BCSCs express greater insulin-like growth factor-1 (IGF-1) and β-catenin (Fu et al. 2014; Jang et al. 2015). Similar to BCSCs, Wnt/β-catenin signaling activity promote CSC frequency, spheroid formation and platinum-based chemoresistance in high-grade serous ovarian cancer (HGSOC). In addition, the impact of this pathway has already been demonstrated in the stimulation of cancer initiating cells (CICs) through development of HGSOC (Nagaraj et al. 2015).

Notch signaling is another mechanism commonly observed in both normal cell development and carcinogenesis. In normal condition, this highly conserved signaling pathway complicatedly contributes to different stages of embryogenesis, such as neurogenesis and angiogenesis, and post-embryogenesis by regulating particular cell communication events, proliferation, cell fate decision and differentiation. For more information review (Bray 2016). Despite controversial effects of Notch signaling pathway on cancer progression, several investigations demonstrated the crucial role of this pathway activity on development of CSC properties. It has been shown that Notch signaling pathway is up-regulated in pancreatic CSCs. This hyperactivity leads to generation of greater CSC frequency and spheroid cells in pancreatic cancer (Abel et al. 2014). Further to these features, it has been shown that Notch signaling has important effect on invasiveness and metastasis of haptocarcinoma CSCs. So that impairment of this pathway activity could prohibit self-renewal, invasion and metastasis of the respective CSCs (Luo et al. 2016). In addition, overexpression of Notch generates more malignant lung adenocarcinoma cell spheres with greater resistance to chemotherapy in xenograft model. Inhibiting this pathway regulatory factors expression, in contrast, blockades regeneration of the related tumor (Hassan et al. 2013).

In addition to the indicated canonical pathways, there are other signaling pathways less frequently contributing to CSC development. With this regard, Hippo is an evolutionally conserved signaling pathway, comprised of the MST/Lasts kinase cascade negatively regulating the respective down-stream co-activators, YAP and TAZ. In normal condition, Hippo signaling pathway activity associates with nSC development by regulating cell proliferation, differentiation and tissue growth. In malignancies, this pathway could bilaterally influence tumorigenesis by prohibiting cell proliferation, promoting apoptosis and regulating nSC expansion, or stimulating tumorigenesis. Evidences show that phosphorylated YAP/TAZ is transported into cytosol and function against tumor growth, while unphosphorylated YAP/TAZ is present in nucleus, inducing cell and tumor growth. So that activity of these unphosphorylated proteins associate with several types of malignancy. For more information review (Mo et al. 2014). Further investigations implicated the crucial effect of the proteins (*i.e.* TAZ, YAP and TEAD) involved in this pathway on breast and brain CSC development and invasion (Cordenonsi et al. 2011; Fernandez et al. 2009).

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is the other crucial pathway correlated with some CSC features. NF-κB protein family is composed of five members (class 1: NF-κB1, NF-κB2 and class 2: RelA, RelB, c-Rel) associating with the different processes within the extensive variety of cells, including regulation of DNA transcription, pro-inflammatory pathways, immune responses and cytokines (Rinkenbaugh and Baldwin 2016). Stimulating NF-κB binding to DNA was initially observed in AML stem cells. In addition to AML, the role of this pathway has been determined in many other types of CSC. Evidences show high level of phosphorylated RelA and subsequently activity of the respective signaling cascade in CSCs obtained from prostate as well as glioblastoma, in vitro (Rajasekhar et al. 2011; Garner et al. 2013). Moreover, NF-κB pathway activity plays critical role in transcriptionally stimulation of inflammatory signatures in breast and ovarian CSCs (Rinkenbaugh and Baldwin 2016). This pathway also regulates several cytokines in development and preservation of CSCs. Through activation of NF-κB pathway, induction of TNF-α to breast cancer cell line can increase capacity of mammo-sphere generation (Storci et al. 2010). Investigations on animal model and human patients showed a feedback loop interaction between NF-κB pathway and TNF-α in AML stem cells (Kagoya et al. 2014). By that mean, in parallel, NF-κB pathway further stimulates TNF-α expression in CML stem cell, compared to normal hematopoietic stem cells. In addition, NF-κB pathway promotes survival and proliferation rates of these CSCs, by regulating common β-chain receptor of granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-3 (Gallipoli et al. 2013). Findings reveal that overexpression of SDF1 promotes generation of SDF1/CXCR4 ligand/receptor complex and up-regulation of NF-κB. Activity of NF-κB, subsequently stimulates Shh/Gli-1 pathway, leading consequently to overexpression of stemness genes (*i.e. NANOG*, *OCT4* and *SOX2*) and promotion of metastasis through EMT procedure (Kong et al. 2016; Es-Haghi et al. 2016).

Epithelial cell adhesion molecule (EpCAM) is a protein, generally expressed in stem and progenitor cells as well as different types of epithelial tissue in normal development. Expression of this pathway is also observed in adenocarcinoma. Evidences show that EpCAM could actively contribute to CSC development through a relative short amino-acid intracellular domain, EpICD, providing Wnt-like signaling profile in these malignant cells. This feature promotes further proliferation, self-renewal and invasiveness in CSCs. Expression of this protein, and therefore relative pathway, is observed in hepatic CSCs with epithelial origin. Activity of EpCAM pathway promotes spheroid formation and prevents CSC differentiation in hepatocarcinoma (Chiba et al. 2016). In addition to hepatocarcinoma, recruiting EpCAM pathway was detected in many other types of malignant stem cell, including breast, pancreatic and colorectal CSCs (Lawson et al. 2009; Badve and Nakshatri 2012; Vaiopoulos et al. 2012; Li et al. 2013). Findings introduced *EpCAM* as a target gene for Wnt/β-catenin pathway in CSCs. Although, expression of β-catenin could individually up-regulate EpCAM protein, this effect is not significant. In has been demonstrated that nuclear augment of β-catenin up-regulates formation of Tcf/β-catenin complex in hepatocarcinoma, leading to Tcf binding

with two sites to the *EpCAM* promoter and significantly overexpression of the latter gene (Yamashita et al. 2007). This achievement suggests the up-stream regulatory effect of Wnt/β-catenin pathway on the activity of EpCAM signaling pathway in many types of malignancy, whereby the former signaling pathway plays hallmark role in CSC development.

Findings highlight several signaling mechanisms involved in promoting metastasis fate in CSCs, by targeting EMT procedure. In this regard, defect of oxygen and presence of factors emanated from malignant cell microenvironment, like TGF-β, intrinsically induce various signaling pathways, such as hypoxia inducible factors (HIFs), contributing to promote EMT procedure and metastasis through activation of Snail homologue 1 (Snail-1), SNAI-2, TWIST-1, TWIST-2, Zinc-finger E box-binding homeobox 1 (ZEB-1), ZEB-2 transcription factors and suppressing E-cadherin (Beck and Blanpain 2013). It has been shown that signaling of protein kinase B (AKT) up-regulates expression of β-catenin and subsequently stimulates Snail-1 protein expression. Overexpression of Snail-1 could subsequently promote EMT procedure in the cisplatin-chemoresistent non-small cell lung cancer (NSCLC) line, A549 cells, promoting invasion and metastasis in the related CSCs (Wang et al. 2014).

In contrast to the presented proteins or pathways promoting CSC features, phosphatase and tensin homolog deleted on chromosome 10 (*PTEN*) is a tumor suppressor gene, negatively regulate development of these cell types. In normal condition, the respective protein encoded from this gene inhibits AKT and the related down-stream protein, mammalian target of rapamycin (mTOR), leading to inhibition of malignant tumor progression. Findings suggest the repressive effect of PTEN/AKT/mTOR signaling pathway on recurrence of LCSCs. Thus, lack of PTEN function leads to activation of the phosphorylated AKT/mTOR cascade, subsequent expression of EpCAM and recurrence of hepatocarcinoma CSCs (Su et al. 2016).

In addition to the indicated well distinguished pathways, there are proteins associated with particular CSCs proliferation, maintenance and invasiveness. Curiously, it has been reported that NESTIN has a crucial correlation with Wnt/β-catenin activity and invasiveness of triple negative BCSCs. In this report, it was shown that NESTIN could promote cell cycle, mammo-sphere formation and invasiveness in vitro, while it reduced apoptosis level. This protein could also stimulate vascular endothelial growth factor (VEGF), β-catenin, c-Myc, matrix metalloproteinase-7 (MMP-7) and cyclin D. In contrast, up-regulation of this protein inhibits expression of several critical proteins, including glycogen kinase-3 beta (GSK3β), APC, peroxisome proliferator-activated receptor alpha (PPARα), E-cadherin and N-cadherin (Zhao et al. 2014). This finding suggests the up-stream effect of NESTIN up-regulation, as a crucial stimulator of BCSC activity, on the activity of Wnt/β-catenin signaling pathway.

DNA methyltransferases (DNMTs) are the other protein family playing vital role in different features of cell development, through stimulation of crucial epigenetic mechanism, DNA methylation. This family of proteins generally induces stem cell differentiation, while they are not expressed in embryonic stem cells. Among this

family, DNMT1 has been reported as an essential protein for the hematopoietic stem cell self-renewal as well as maintenance of epidermal stem cell (Trowbridge et al. 2009; Sen et al. 2010). Expression of this protein has also been reported in maintenance of different CSC types. Findings showed that loss of Dnmt1 expression obstructed leukemia stem cells development in animal model. In contrast, expression of this protein preserved the respective cancer stem cell self-renewal (Trowbridge et al. 2012). Similarly, Dnmt1 expression stimulates self-renewal and maintenance of BCSCs, through hypermethylation and consequently down-regulation of several important genes (Pathania et al. 2015). Curiously, it has been demonstrated that IL-6 could promote DNMT1 expression, culminated in hypermethylation of the promoter region in the cell cycle gene regulators (including p53 and p21), down-regulation of these genes and consequently lung cancer stem cell proliferation as well as sphere formation (Liu et al. 2015). As a leading consequence of DNMT dysregulation, alteration of DNA methylation in several regulatory genes could also cause cancer initiation and CSC development.

Although genetic mechanism underlying CSC development has fairly been investigated thus far, epigenetic landscape of this phenomenon needs to be further elucidated. Indeed, gene expression also relies somehow on the modification of different epigenetic factors, one of which is DNA methylation. Despite that DNA methylation is generally determined as a "silencing epigenetic mark", it can variously regulate gene expression depending on different genomic contexts (Jones 2012). It has been demonstrated that hypomethylation of *NANOG* promoter overexpressed this gene, leading to preservation of proliferation and metastasis features for LCSCs (Wang et al. 2013). It is worthy to note that hypermethylation is generally induced in the promoter region of tumor suppressor genes, while promoter hypomethylation is arisen in oncogenes, in CSC.

In spite of playing remarkable role among the epigenetic factors, it is believed that modification of DNA methylation could not necessarily be the determinant feature in the regulation of, at least, some gene expressions through tumorigenesis. So that different modifications of histone could predominantly associate with CSC development. We recently demonstrated the superior effect of some histone modification factors on the stimulation of prostate CSC capacity, in comparison with DNA methylation. In this study, we determined down-regulation of *CDH-1* (encoding E-cadherin) in the prostate spheroid cells with metastatic capability, in vitro. Promoter analysis of this gene showed hypomethylation of DNA as well as reduction of H3K9ac and H3K4me3 levels, in parallel with enrichment of H3K27me3. Although findings obtained from DNA methylation was contrary to the *CDH-1* transcription status, we determined the decisive effect of these histone modification factors on down-regulation of *CDH-1* in the prostate CSC (Unpublished data). Moreover, hyper-stimulation of H3K4me3 and hypo-stimulation of H3K79me2 maintain CSC features in *MLL*-associated leukemia (Wong et al. 2015). Sometimes, gene expression could be regulated due to the cooperation of different epigenetics factors. In line with this, findings demonstrated expression of DNMT and histone deacetylase (HDAC) proteins in BCSCs, resulting in activation of growth-promoting signaling molecule (Pathania et al. 2016).

In addition to DNA methylation and histone modifications, there are several evidences implicating that noncoding-RNAs (*e.g.* microRNAs and long-noncoding RNAs) could considerably involve in the regulation of critical genes, contributing to the development, maintenance and invasion of different CSC types. Evidences show that p53 protein can effectively inhibit CD44 expression, as a surface marker utilized individually or in combination with the other markers, in prostate CSC, through induction of miR-34a. Thus, expression of this microRNA can prohibit clonogenic expansion, regeneration of tumor as well as metastasis (Liu et al. 2011). It has also been determined that miR-34a could act as a tumor suppressor molecule, decreasing stemness capacity in BCSCs. Expression of this molecule suppresses Notch1 expression and down-regulates the respective signaling pathway, consequently increasing sensitivity of BCSCs to natural killer (NK) cells (Zhang et al. 2016a). Further to the loss of p53 activity, hypermethylation of CpG islands in the related promoter leads to down-regulation of miR-34a in several types of cancer (Wang et al. 2016a).

Other findings showed at least 37 microRNAs differentially regulated in BCSCs, compared to the other cancer cell populations. Among these microRNAs, expression of miR-200c could partially repress expression of BMI-1 protein, as a stem cell self-renewal regulator, leading to reduction of BCSC proliferation rate. In addition, expression of this epigenetic factor restrains normal mammary stem cell transformation into BCSCs (Shimono et al. 2009). Curiously, it was later demonstrated that BMI-1 activity is suppressed in glioma, by induction of miR-218 expression, leading to prohibition of the related CSC proliferation, growth, invasion and metastasis (Tu et al. 2013).

In addition to the microRNAs, evidences implicate the remarkable role of long-noncoding RNAs (lnc-RNAs) in the regulation of CSC development. Recent findings show targeting of miR-34a expression through activity of a novel lnc-RNA, called lnc-34a. This lnc-RNA engages DNMT3a activity, through up-regulation of HDAC1 and prohibitin-2 proteins, leading to down-regulation of the latter microRNA due to hyper-methylation of the respective promoter in colorectal CSCs (Wang et al. 2016a).

Emerging evidences have introduced another novel lnc-RNA, called lnc-ROR, contributing to proliferation and invasion of gastric CSC. It has been determined that lnc-ROR activity stimulates expression of several stemness markers in these cells, including OCT4, SOX2, NANOG and CD133, leading to further invasiveness of gastric CSCs (Wang et al. 2016b).

In contrast to several lnc-RNA contributing to CSC stimulations, it has been demonstrated that some lnc-RNA act as tumor suppressor, prohibiting development of these cells. As a case, lnc-DILC expression has been attributed to hinder LCSC initiation and expansion in hepatocarcinoma cells. This molecule functionally binds to *IL-6* promoter and prevents the related gene transcription. Activity of lnc-DILC could also prohibit *IL-6* transcription through suppressing TNF-α or IL-1β expression. In addition, it impairs JAK2/STAT3 pathway leading to suppression of LCSC self-renewal (Wang et al. 2016c).

15.9 Potential CSC Therapies

Accumulating data has deduced that one of the principle causes of cancer therapy failures could be due to the undeniable role of CSCs in resistance to the conventional therapies, progression of more aggressive malignancies, metastasis or even recurrence of cancer. Thus, it is currently postulated that combination of conventional therapies with CSC targeting might further help fully treat aggressive malignancies. In this regard, targeting common key mechanisms regulating development of CSC populations could effectively facilitate obtaining this approach. Investigations on several therapeutic agents have currently been extended to the clinical trial phases (Table 15.2). Tarextumab is a human monoclonal antibody developed to treat breast, ovarian, pancreatic and small cell lung cancers. Study on the animal model and clinical trial have thus far shown that Tarextumab could efficiently suppress Notch signaling pathway through inhibition of *Notch2* and *Notch3*. This consequently represses CSC self-renewal and growth capacities, while it promotes differentiation in these cells (Yen et al. 2015).

In addition to this agent, scientists are currently appealing to negatively target γ-secretase, as an enzyme coordinating in cleavage and development of Notch active form. Application of MK-0752, as a γ-secretase inhibitor (GSI), could successfully impair Notch signaling pathway, in combination with gemcitabine hydrochloride (a chemotherapy drug) or individually, through treatment of the late stages (stage III or IV) of pancreatic cancer (Beatty et al. 2013). It has also been reported that this GSI agent could successfully reduce expression of *Notch* in phase I clinical trial investigation on the children suffering recurrence of CNS cancers (Hoffman et al. 2015). Recruiting Notch ligand is another approach to prohibit activity of this pathway. Delta-like ligand 4 (DLL4) is a potential target, contributed to Notch signaling pathway. Combinational administration of Demcizumab, an inhibitor agent of DLL4, and Gemcitabine not only modulated Notch signaling pathway and CSC growth in pancreatic cancer patients, but also it caused some clinical outcomes with no significant cardiopulmonary cytotoxicity (Hidalgo et al. 2016).

Focal adhesion kinase (FAK) is the other molecule regulated by the upstream proteins, KRAS and RHOA. Mutations of *KRAS*, as of the most common aberration among NSCLC oncogenes, contribute to the activity of FAK. This causes CSC survival and resistance to anoikis, by regulating ADRB2/Src signaling pathway, promoting invasion and metastasis of malignant cell. Pilot studies demonstrated inhibition of FAK phosphorylation, using Defactinib small molecule, and consequently suppression of malignant cell growth both in vitro and in vivo. Clinical trial investigations demonstrated inhibition of tumor growth and stability in different types of malignancy, with reversible mild to moderate treatment adverse effects in the phase 1 (Jones et al. 2015; Shimizu et al. 2016).

A plant alkaloid, named as Berberine, is a traditional therapeutic agent administrated for many types of disorder, including metabolic, neurodegenerative and cardiovascular diseases as well as cancers. Recent investigations demonstrated that

Table 15.2 Study of some novel compounds in clinical trials to treat CSCs

Compound	Targeting mode	CSC type	Research status	References
BB1603	STAT3 inhibition	Breast	Phase III	Ahmed et al. (2017)
BB1608	STAT3, β-catenin, Nanog inhibitions	Esophagus, gastric, colon	Phases II and III	Kaiser (2015)
Berberin	Wnt/β-catenin pathway inhibition	Breast, colorectal	Phases II and III	Ahmed et al. (2017)
Catumaxomab	EpCAM and CD3 inhibitions	Ovary, gastric, pancreas	Phases I and III	Ahmed et al. (2017)
Lapatinib Ditosylate	HER2 inhibition	Breast	Phase II	Chiotaki et al. (2015)
Teraxtumab	Notch receptor inhibition	Lung, pancreas	Phase II	Kaiser (2015)
Demcizumab	DLL4 (Notch ligand) antagonist	Ovary	Phase II	Kaiser (2015)
Reparixin	CXCR inhibition	Breast	Phase II	Chiotaki et al. (2015)
VS-6063	FAK inhibition	Lung, mesothelioma	Phase II	Kaiser (2015)
Bevacizumab	Angiogenesis	Breast	Phase II	Chiotaki et al. (2015)
Genistein	Wnt inhibition	Colon	Phase I	Ahmed et al. (2017)
PRI-724	CBP/catenin antagonist	Colorectal	Phase I	Ahmed et al. (2017)
RO4929097 and *Vismodegib*	γ-secretase, Notch, Hedgehog pathway impairments	Breast	Phase I	Chiotaki et al. (2015)
OMP-21M18	DLL4 (Notch ligand) antagonist	Lung, breast, colon	Phase I	Ahmed et al. (2017)
Cyclopamine	Smo antagonist, hedgehog pathway inhibitor	Brain, blood, gastric, breast, pancreas, prostate	Phase I	Ahmed et al. (2017)

Berberine could efficiently differentiate brain CSCs, through down-regulation of cancer stemness genes *CD133*, *β-catenin*, *n-Myc*, *Sox2*, *Nestin* and *Notch2* in mouse cell lines. In addition, this agent prohibits CSC proliferation and induces apoptosis through regulation of the respective cyclins and cyclin dependent kinase activities. Besides, Berberine prevents metastasis by dysregulation of PI3/Akt and Ras-Raf-ERK signaling pathways, consequently leading to impairment of EMT process (Naveen et al. 2016). Further investigations presented the prominent effect of Berberine adjuvant drug with Lapatinib in treatment of HER2-positive breast cancers. Findings show that administration of Berberine could not only induce reactive oxygen species (ROS) and mitochondrial-related apoptotic pathways, but also could overcome the Lapatinib-resistance effect in these malignant cells. In this combinational therapy, Laptinib is a small molecule inhibiting HER2 and EGFR activities in BCSCs, by blocking tyrosine kinase intracellular ATP binding site, leading to destruction of down-stream signaling pathways, Akt and MAPK (Zhang et al. 2016b; Farnie et al. 2014). Effect of Berberine administration on breast and colorectal cancers is currently investigated in the phase II/III clinical trial (Ahmed et al. 2017).

BBI608 agent, a CSC first-in-class small molecule inhibitor, has been determined to repress survival rate, spheroid formation and self-renewal capacity of numerous types of CSC, including glioblastoma, head and neck, lung, pancreatic, renal, ovarian and colorectal malignant cells. Besides, it prohibits metastasis of these cells, through inhibition of Stat3 and impairment of the respective signaling pathway(s) in animal models. It has been demonstrated that suppression of Stat3 generally contributes to down-regulation of some self-renewal genes, including *c-Myc* and *β-catenin*, as well as stemness genes, such as *Nanog* and *Sox2* (Li et al. 2015). There are several investigations on clinical trial, reported in cutting edge science seminars, to validate the potential efficacy of this agent against CSC development, side-effects and appropriate dosage; for more information review abstracts (Jonker et al. 2014; Langleben et al. 2013; Becerra et al. 2015). Interestingly, the effect of this agent is currently evaluated on colorectal CSCs, in phase III clinical trial (Ahmed et al. 2017).

Reparixin is another small molecule which is currently investigated in clinical trial, and summarized in (Goldstein et al. 2016; Schott et al. 2015a, b) original research abstracts. Findings obtained from animal model studies demonstrated the prohibitory effect of Reparixin against BCSC metastasis and growth, by inhibiting IL-8 receptor, CXCR1. Loss of CXCR1 activity also impairs FAK/AKT/FOXO3A pathway and subsequently FASL expression, leading consequently to induction of apoptosis in BCSCs (Ginestier et al. 2010).

In addition to the indicated agents, there are several components which are undergoing pre-clinical studies and might potentially contribute to CSC development prohibition, by regulating different genetic-based mechanisms (Table 15.3).

Findings obtained from administration of a novel small molecule antagonist, CWP232228, shows impairment of β-catenin transcription factor binding to TCF, leading to disruption of Wnt/β-catenin signaling pathway. In addition, this small molecule could hinder expression of IGF-1. Subsequent to these functions,

Table 15.3 Pre-clinical study of novel drugs against CSC activity

Compound	Targeting mode	CSC type	Model	References
Imetelstat	Telomerase antagonist	Breast	In vitro, In vivo	Koziel and Herbert (2015)
Salinomycin	Autophagy and metastasis inhibition	Lung, breast, colon	In vitro, In vivo	Pellegrini et al. (2016), Kopp et al. (2014)
Nutlin-3	MDM2 antagonist	Breast, colon	In vivo	Tosoni et al. (2017), Puca et al. (2014)
CWP232228	Wnt, β-catenin antagonist	Breast, liver	In vitro, In vivo	Jang et al. (2015), Kim et al. (2016)
Tazemetostat	EZH2 inhibition	Blood, brain	In vitro, In vivo	Knutson et al. (2014), Wiese et al. (2016)
DZNep	EZH2 inhibition	Biliary tract	In vitro	Mayr et al. (2015)
LF3	TCF4 and β-catenin interaction inhibition	Head and neck, colon	In vitro, In vivo	Fang et al. (2016)
Curcumin	AhR/ERK/SK1 signaling inhibition	Liver	In vitro, In vivo	Tsai et al. (2015)
VS-5584	PI3 K/mTOR inhibition	Lung, breast, ovary	In vitro, In vivo, Ex vivo	Kolev et al. (2015)
Salinomycin	Stemness	Ovary	In vitro	Lee et al. (2017)
Resveratrol	Wnt/β-catenin pathway inhibition	Breast	In vitro, In vivo	Fu et al. (2014)

CWP232228 could block BCSC growth (Jang et al. 2015). Application of resveratrol, as an herbal polyphenolic compound, has also been shown to reduce activity of Wnt/β-catenin and block the stimulatory effect of this pathway on CSC existence (Fu et al. 2014). With regards to the crucial role of this pathway in the CSC survival and chemoresistance, it has been demonstrated that inhibition of β-catenin, as a combination therapy, sensitized cells to cisplatin administration and reduced CIC sphere formation (Nagaraj et al. 2015).

In the case of hepatocellular carcinoma, a dose-dependent administration of diabetes drug, metformin, impairs EpCAM signaling activity, causing loss of self-renewal and spheroid formation capabilities, in addition to stimulating CSC differentiation into mature fate (Saito et al. 2013).

In addition, therapeutic compounds could impede CSC development and survival by regulating epigenetic mechanisms. As a critical polycomb-recessive complex 2 (PRC2) member, EZH2 is a methyltransferase contributing to tri-methylation of H3K27 and suppressing expression of the related gene. In differentiation procedure, the expression of stemness genes is down-regulated due to the activity of PRC2 complex. This mechanism could potentially navigate the approach of prohibiting CSC development and activity though promoting differentiation in these cells. Several EZH2 inhibitors have thus far been submitted with an anti-cancer activity. Tazemetostat (EPZ-6438), as an EZH2 inhibitor, dose- and time-dependently could hinder H3K27me3 formation in preclinical models of the non-Hodgkin lymphoma, caused due to the catalytic domain-mutation of EZH2. This process associates with malignant cell death in vitro and tumor growth inhibition in vivo (Knutson et al. 2014). Phase I clinical trial demonstrates the effective impact of this drug against the indicated malignant cells, and it is currently extended to the phase II (Ribrag et al. 2015). DZNep is another methyltransferase inhibitor, globally prohibiting tri-methylation of H3K27, likely through inactivation of EZH2. Findings show the anti-growth effect of this agent on several types of cancers. It has been proposed that co-administration of Tazemetostat or DZNep with 5-aza-2′-deoxycytidine, as an inhibitor of DNA methylation, could potentially further target CSC development (Momparler and Cote 2015).

15.10 Challenges and Future Directions

Tumors are generally comprised of heterogeneous population of malignant cells with distinguishable clonogenic, invasion and metastasis potentials. Over the last decade, substantial evidences indicated the crucial role of small sub-population of malignant cells in tumor initiation, proliferation, heterogeneity, invasion and metastasis of several cancers, named as CSCs. However, it is remained to be elucidated whether every types of tumor contain this small sub-population or it is limited to the particular types of cancer. CSCs are asymmetrically able to renew themselves as well as generating other malignant cells presented in the tumor bulk. These types of cell are simultaneously able to represent some nSC and malignant

cell features. Thus far, no definite marker has been discovered, independently discriminating CSC population from the other cell types in tumor and nSCs. This is particularly more challenging while it has been determined that niche and microenvironment could indirectly change the CSC networking through mediating genetic-or epigenetic-based alterations. So that a single marker could not be utilized to precisely trace whole process of CSC development, from tumor initiation to metastasis. Further investigation on the factors involved in normal cell stemness and differentiation as well as malignant cell invasion and metastasis could lead us to determine a compilation of markers commonly applied to distinguish particular CSC status. This event not only could facilitate diagnosis of cancer stage, but it could be helpful to find an appropriate approach for threating CSCs.

Findings of the recent years highlighted the role of metabolism not as a mere, but as an essential player in development of CSCs. however, the action mode of metabolic pathways in CSCs still remain controversial. Any change in metabolism could alter fate of CSC from stemness to differentiated status. In addition, metabolic activity of cells could not only prohibit invasion and metastasis, but also induce apoptosis and senescence. Undoubtedly, in addition to better understanding tumorigenesis and metastasis, precise navigation of metabolism process could help control CSC fate and development.

Despite effectiveness in several conditions, conventional therapeutic approaches are generally able to hinder development of tumor bulk cell proliferation with less influence on the CSC sub-populations. This could cause chemoresistance and recurrence of malignancy in many cases. Combination of conventional therapy with agents targeting CSC development could further contribute to fully eradicate the malignant cells. Ultimately, further exploration of microenvironment effect on the CSC metabolism and intrinsic signaling pathway activity could facilitate finding the most appropriate strategy for treatment of different cancers, in accordance with the field of personalized medicine.

15.11 Summary

Cancer tumors are generally composed of heterogeneous population of malignant cells with distinguishable clonogenic, invasion and metastasis potentials. Over the last decade, substantial evidences confirmed the reality of cancer stem cell (CSC) theory, asserting the crucial role of small sub-population of malignant cells in tumor initiation, proliferation and heterogeneity of several cancers. These functions are generally accredited to the bilateral capability of CSCs in symmetric and asymmetric proliferation. So that CSCs are indeed able to renew themselves as well as generate other malignant cells presented in the tumor bulk, through asymmetric proliferation.

In addition, CSC activity has been attributed to crucially associate with invasion and metastasis of malignancy. Several factors have been determined to coordinate in this procedure, including microenvironment and metabolic activity. Thus,

alteration of these factors could change genetic and epigenetic status in CSCs. This event consequently regulates activity of particular molecular pathways and subsequently they regulate intrinsic molecular networks of CSCs toward symmetric or asymmetric division. These types of cell are simultaneously able to represent some nSC and malignant cell features. Thus far, no definite marker has been discovered, independently discriminating CSC population from the other cell types in tumor and nSCs. However, utilizing particular isoform of these markers, combinational markers or onco-fetal stem cell markers could facilitate discriminating CSCs from other cancer cell population.

In this chapter, crucial role of CSCs on the stimulation of tumor heterogeneity, invasiveness, metastasis, chemoresistance and recurrence are briefly highlighted, through emphasizing some alterations of niche, metabolism, genetic and epigenetic, as well as molecular mechanisms and networking required for CSC survival and proliferation. Ultimately, currently utilized approaches of CSC treatment are appraised.

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Chapter 16
Cancer Immunotherapy: Friend or Foe of Mental Health?

Amene Saghazadeh and Nima Rezaei

Abstract Cancer morbidity with mental health problems is of utmost importance due to its considerable impact on mortality. Clinical trials demonstrate that immunotherapy could achieve clinical benefit in patients with cancer. However immunotherapy, like other treatments, has its potential side-effects such as autoimmunity, inflammatory responses, and immunosuppressive conditions-related side effects. As such evidence indicates that cancer immunotherapy might alter the patients' mental health status, particularly depressive symptomatology, fatigue, anorexia, anxiety, and life quality, according to the immunotherapy-related factors, e.g. type, dosage, and regimen. The present chapter corroborates a number of conflicts concerning the possible link between cancer immunotherapy and mental health.

Keywords Cancer · Immunotherapy · Mental health · Depression · Fatigue · Anxiety · Quality of life

Abbreviations

APA	American Psychiatric Association
CIK	Cytokine-induced killer

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CTLs	Cytotoxic T lymphocytes
DSM	Diagnostic and Statistical Manual
IFN-γ	Interferon gamma
IL-2	Interleukin 2
MADRS	Montgomery-Asberg Depression Scale
MDD	Major depressive disorder
NCS-R	National Comorbidity Survey Replication
PSK	Polysaccharide K

16.1 Introduction

The Diagnostic and Statistical Manual (DSM) of Mental Disorders, designed by the American Psychiatric Association (APA), is an invaluable reference tool for assessment and diagnosis of mental disorders. Since 1952 that the original version of DSM, named DSM-1, came out, the APA has unveiled several up-to-date and amended versions of the tool. According to the DSM-V, the latest version of DSM while writing this chapter, mental health disorders can be classified into the following distinct, broad categories; neurodevelopmental disorders, schizophrenia spectrum and other psychotic disorders, bipolar and related disorders, depressive disorders, anxiety disorders, obsessive-compulsive and related disorders, trauma- and stressor-related disorders, dissociative disorders, somatic symptom and related disorders, feeding and eating disorders, elimination disorders, sleep-wake disorders, sexual dysfunctions, gender dysphoria, disruptive, impulse-control, and conduct disorders, substance-related and addictive disorders, neurocognitive disorders, personality disorders, paraphilic disorders, and other mental disorders (American Psychiatric 2013). National surveys indicate that approximately one in two people in the US is likely to be affected by mental disorders in his lifetime (Kessler et al. 2005). The four most common mental disorders are anxiety disorders, impulse control disorders, mood disorders, and substance use disorders with the approximate prevalence of 30, 25, 20, and 15% respectively (Kessler et al. 2005) (Fig. 16.1). The high prevalence of mental disorders reflects the fact that they have a huge impact on human health, quality of life, and global economy. Thus it would not be astonishing that mental health is nominated as one of the top ten global health issues.

Cancer is a chronic condition that causes considerable morbidity and mortality worldwide. The American Cancer Society reported that about 170 persons per 100,000 people died in 2011 from cancer in the United States (Siegel et al. 2015a). According to the last published report by the American Cancer Society, cancer is the leading cause of death in more than 40% of states of the Unites States of America (Siegel 2015b). The report also said that the incidence of prostate cancer has been decreased whereas deaths related to some types of cancers, such as hepatic cancer, pancreatic cancer, and cancer of the corpus uteri, are increasing (Siegel et al. 2015b).

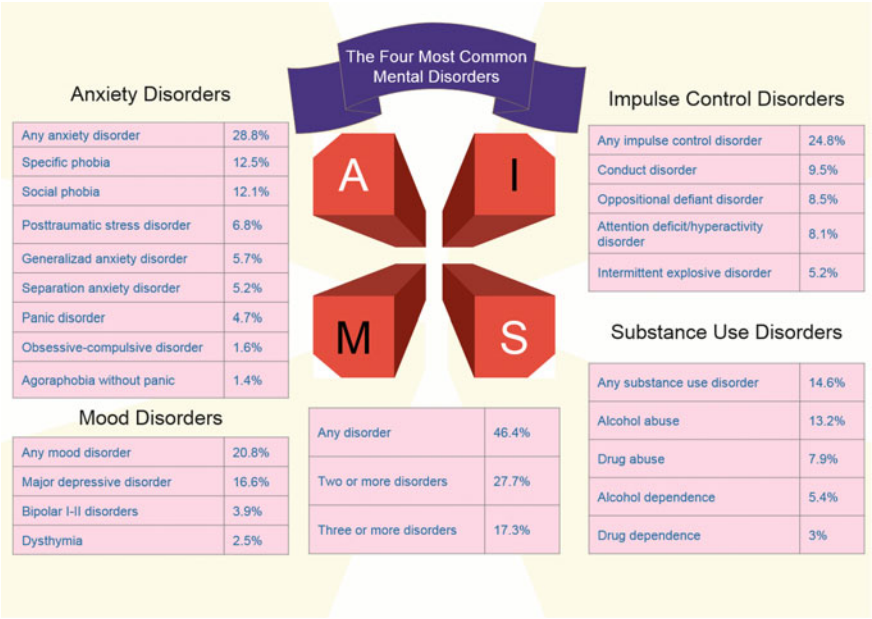

Fig. 16.1 The four most common mental disorders. This figure represents the life-time prevalence of anxiety disorders, impulse control disorders, mood disorders, and substance use disorders, which are thought to be the most common mental disorders Aaronson et al. (1993)

Mental health problems are common among cancer people. More than 10% of cancer patients suffer from major psychiatric disorders. Moreover 90% of cancer patients comply with therapy for emotional problems (Kadan-Lottick et al. 2005). Cancer morbidity with mental health problems is of utmost importance due to its impact on mortality. For example, a follow-up study identified depressive symptoms as a risk factor for death from cancer within the next 15 years (Zonderman et al. 1989). In addition to mental health, physical functioning and well-being get worse shortly after cancer diagnosis is made (Vinokur et al. 1990).

Cancer immunotherapy has been the subject of intensive and extensive research during the last five decades. Clinical trials indicated that immunotherapy could achieve clinical benefit in patients with cancer, particularly melanoma (Saghazadeh et al. 2015). However immunotherapy, like other treatments, has its potential side-effects. Among them the most important one are presumably autoimmune side-effects which are the consequence of halting immune checkpoints (Pardoll 2012). Additionally combination of immunotherapy and targeted cancer therapies, such as chemotherapy, can have serious side-effects associated with inflammatory and immunosuppressive conditions (Vanneman and Dranoff 2012). The aim of the present chapter is to address an open question; whether cancer immunotherapy might affect mental health of patients.

16.2 Depression, Cancer, and Immunotherapy

The category of depressive disorders comprises many disorders which all of them are characterized by "the presence of sad, empty, or irritable mood, accompanied by somatic and cognitive changes that significantly affect the individual's capacity to function" (American Psychiatric 2013). In general, the prevalence of depressive symptoms and disorders varies widely depending on many factors, such as age, race/ethnicity, marital status, and socioeconomic status (Riolo et al. 2005; Lehtinen and Joukamaa 1994). In addition, in study population with defined characteristics there exist wide differences across studies. For example, systematic review of twenty-four studies provided the range of 10–85% for the prevalence of depression among university students (Ibrahim et al. 2013). Major depressive disorder (MDD) is considered the prototype of the category and diagnosed by "discrete episodes of at least 2 weeks' duration (although most episodes last considerably longer) involving clear-cut changes in affect, cognition, and neurovegetative functions and inter-episode remissions" (American Psychiatric 2013). The life-time prevalence of MDD was estimated by the US National Comorbidity Survey Replication (NCS-R) at approximately 16% (Kessler et al. 2003). It is of importance that MDD mostly occurs secondary to other mental disorders, particularly anxiety disorders (Kessler et al. 1996).

Some professions underestimate the impact of depression on human lives, whereas depression must be seen as a functional obstacle to functioning and well-being. Studies indicate that depression threatens functioning and well-being of people as well as chronic medical illnesses such as diabetes and arthritis do. Depressive disorders are also comorbid with other mental illnesses, such as anxiety disorders (Rector et al. 2007), panic disorders (Roy-Byrne et al. 2000), personality disorders (Hirschfeld 1999), and eating disorders (Musante et al. 1998). Additionally, many physical disorders, especially chronic diseases, have been associated with increased risk of developing depressive symptoms (Chapman et al. 2005; Katon et al. 2007). They include asthma, arthritis, diabetes, heart disease, pulmonary disease, cancer, and obesity.

A comprehensive study revealed that approximately 30% of cancer people had either clinical or subclinical symptoms of depression (Linden et al. 2012). About 12% of cancer people fulfilled depression criteria similar to that of control subjects ($\sim$10%) (Linden et al. 2012; Mitchell et al. 2013). However meta-analysis study reported a range of 8–24% for the prevalence of depression among patients who suffered from cancer (Krebber et al. 2014). They demonstrated that the prevalence of depression in cancer patients might vary depending on a number of factors, such as gender, type of cancer, type of measurement, and treatment phase. A randomized trial of supportive-expressive group therapy in women with metastatic breast cancer reported that the median survival time increased more than twofold for women with decreasing depressive symptoms compared to women with increasing depressive

symptoms over one year (Giese-Davis et al. 2011). Also a meta-analysis of more than seventy prospective studies strongly support the link between depression and increased death rate among cancer patients (Pinquart and Duberstein 2010). Altogether it can be concluded that there exists, however, no significant difference in the prevalence of depression among cancer patients compared to control subjects, its impact on cancer mortality is clearly discernible.

Cancer immunotherapy can aggravate depressive symptoms as measured at different time points (three days, five days, one week, one month, and three months) following immunotherapy [Capuron et al. 2002, 2001, 2000, 2004; Maes et al. 2001; Lissoni et al. 1990; Pizzi et al. 2002; Tarhini et al. 2012) (Table 16.1)]. However when the study population was stratified based on the cytokine (i.e. IL-2 and IFN) used as therapy, the exacerbation of depressive symptoms was observed only among patients who received IL-2 alone or in combination with IFN, but not among those patients who received IFN alone. The intensity of depressive symptoms, assessed by the Montgomery-Asberg Depression Scale (MADRS) (Montgomery and Asberg 1979), in cancer patients treated with cytokine(s) was frequently between mild to marked and severe or very severe depression was not reported. The MADRS contains three main domains and ten items; the emotional/affective domain (apparent sadness, reported sadness, inner tension, and inability to feel), the neurovegetative domain (reduced sleep, reduced appetite, and lassitude), and the cognitive domain (concentration difficulties, pessimistic thoughts, and suicidal thoughts). Depressive symptoms that developed following immunotherapy were not restricted to domain-specific symptoms, but included all the three domains of depressive symptoms. Possible mechanisms that might underlie the exacerbation of depression following cancer immunotherapy include the decreased function of serotonin, decreased activity of dipeptidyl peptidase IV, and increased activity of cytokines (e.g. sIL-2R, IL-6, and IL-10) (Capuron et al. 2002, 2001; Maes et al. 2001). Neuroimmunotherapy might offer a solution to ameliorate the negative effects of immunotherapy alone. Depressive symptoms were less likely to develop following treatment with IL-2 combined with Melatonin compared to when IL-2 was used as a standalone treatment (Lissoni et al. 1990).

Altogether, these lines of evidence indicate that IL-2-based immunotherapy brings about mild to marked depressive symptoms in all likelihood. If cancer patients are well informed about potential side effects of IL-2 therapy pretreatment, then they may be more able to cope with post-treatment depressive symptoms.

16.3 Fatigue, Cancer, and Immunotherapy

Fatigue is considered a complex phenomenon and consequently could not be clearly defined yet. Roughly speaking fatigue refers to any state of extreme tiredness that affects normal cognitive and/or physical performance. To more accurately

Table 16.1 Summary of studies that evaluated depressive symptoms during and/or following immunotherapy in cancer patients

Immunotherapy	Study sample	Depression measurement	Change in depressive symptoms	Authors' conclusion	Reference
IL-2 alone	Patients with metastatic renal cancer	NA	Exacerbation	Depressive symptomatology occurred more frequently during IL-2 alone compared to IL-2 plus MLT	Lissoni et al. (1990)
IL-2 plus MLT		NA	Exacerbation		
IL-2 alone (SC)	Patients with renal cell carcinoma or melanoma (n = 20)	MADRS, day 5	Exacerbation	Patients treated with IL-2 alone or in association with INFα-2b had significantly higher MADRS scores after 5 days of cytokine therapy, and patients who received both cytokines had increased scores on day 3. In contrast, patients treated with INFα-2b alone did not have varying MADRS scores during the course of treatment	Capuron et al. (2000)
IL-2 in combination with INF-α-2b (SC)	Patients with renal cell carcinoma or melanoma (n = 6)	MADRS, day 3 and 5	Exacerbation		
Low dose INF-α-2b alone (SC)	Patients with renal cell carcinoma or melanoma (n = 8)	MADRS, day 5	No change		
High dose INF-α-2b alone (IV)	Patients with renal cell carcinoma or melanoma (n = 14)	MADRS, day 5	No change		
IL-2 alone (SC)	Patients with metastatic cell carcinoma (n = 10)	MADRS, day 3 and 5	Exacerbation	The MADRS scores were significantly elevated by treatment with IL-2 with or without IFN-α, but not IFN-α alone	Maes et al. (2001)
IFN-α alone (IV or SC)	Patients with metastatic cell carcinoma or melanoma (n = 12)	MADRS, day 3 and 5	No change		
IL-2 plus IFN-α (SC)	Patients with metastatic cell carcinoma (n = 4)	MADRS, day 3 and 5	Exacerbation		
IL-2 alone (SC)	Patients with metastatic renal cell carcinoma (n = 13)	MADRS, day 5	Exacerbation	Patients treated with IL-2 or IL-2 + IFN-α displayed concomitant mood symptoms and increased serum cytokine levels during treatment	Capuron et al. (2001)
IL-2 in combination with INF-α-2b (SC)	Patients with metastatic renal cell carcinoma (n = 5)	MADRS, day 5	Exacerbation		

(continued)

Table 16.1 (continued)

Immunotherapy	Study sample	Depression measurement	Change in depressive symptoms	Authors' conclusion	Reference
Low dose INF-α-2b alone (SC)	Patients with metastatic renal cell carcinoma (n = 5)	MADRS, day 5	No change		
High dose INF-α-2b alone (IV)	Patients with high-risk metastatic melanoma (n = 10)	MADRS, day 5	No change		
Low-dose recombinant IL-2	Patients with renal cell carcinoma with marginal activity in malignant melanoma and colorectal cancer (n = 10)	MMPI, 3 months	Exacerbation	80% of patients had a significantly increased score on the clinical scale of depression	Pizzi et al. (2002)
IFN-α alone (IV/SC)	Patients with renal cell carcinoma or melanoma (n = 9)	MADRS, 1 week, 1 month	Exacerbation	Depression scores on the MADRS scale significantly increased during the first month of cytokine therapy in the whole population under study	Capuron et al. (2002)
IL-2 alone	Patients with renal cell carcinoma or melanoma (n = 5)				
IL-2 plus IFN-α	Patients with renal cell carcinoma or melanoma (n = 2)				
IL-2 alone (SC)	Patients with renal cell carcinoma (n = 11)	MADRS, 1 month	Exacerbation	50% of the patients developed mild depressive symptomatology, 22% developed moderate to marked depressive symptomatology, and 28% of patients did not display any significant depressive symptomatology at endpoint	Capuron et al. (2004)
IL-2 plus IFN-α (SC)	Patients with renal cell carcinoma (n = 6)	MADRS, 1 month	Exacerbation		
IFN-α alone (IV)	Patients with advanced melanoma (n = 15)	MADRS, 1 month	Exacerbation		
IFN-α-2b (IV/SC)	Patients with stage IV melanoma (n = 35)	NR, 3 months	Adverse events included anxiety/depression un five patients (14%)		Tarhini et al. (2012)

deal with it, it must be determined if fatigue is acute or chronic, physiological or psychological, and central or peripheral (Shen et al. 2006). National surveys reported the prevalence of substantial fatigue lasting six months or longer ranging from ~11 to 19% in the general population (Loge et al. 1998; Pawlikowska et al. 1994). Fatigue has, however, been reported by more than 30% of US workers. There was approximately three-fold increase in report of health-related lost productive time by fatigued workers compared to non-fatigued workers (Ricci et al. 2007). Chronic fatigue is a frequent complaint of patients who suffer from physical disease such as multiple sclerosis, Parkinson's disease, stroke, and primary biliary cirrhosis (Fisk et al. 1994; Goldblatt et al. 2002; Martinez-Martin et al. 2006; Ingles et al. 1999). The impact of fatigue on daily life of patients has been very important since fatigue was directly associated with depression, perception of own mental health, and disability whereas it was inversely correlated with life quality (Martinez-Martin et al.; Amato et al. 2001; Bakshi 2003; Huet et al. 2000).

Cancer is presumably the most important clinical condition associated with fatigue so that the term cancer-related fatigue is used to represent this condition. Approximately half of patients who had cancer reported fatigue at diagnosis and that 60–96% of patients developed symptoms of fatigue following anti-cancer treatments (Wagner and Cella 2004; Hofman et al. 2007). Fatigue in these patients might be daily basis for no reason in particular or be associated with the recent chemotherapy or radiotherapy session (Curt et al. 2000). Fatigue in cancer patients is in general tended to be chronic, explaining how it can exert disastrous impact on many aspects of daily life, such as the return to work, physical functioning, and emotional, psychologic, and social life (Spelten et al. 2003; Curt 2000). Also cancer-related fatigue has been directly correlated with pain, dypnoea, depression, and anxiety (Brown and Kroenke 2009; Stone et al. 1999). In spite of its high prevalence and its huge impact on life quality of individuals, less than 20% of cases have received treatment and cancer-related fatigue is often neglected (Stone et al. 2000). There are, however, both pharmalogical and non-pharmalogical interventions that can help to control cancer-related fatigue (Wagner and Cella 2004).

Fatigue has been often among the most common adverse events during or following immunotherapy (Schmeel et al. 2015; Di et al. 2012; van den Heuvel et al. 2015; Zhang et al. 2015; Lammers et al. 2012; Dijkgraaf et al. 2015; Garcia et al. 2014; Kerst et al. 2005; Neri et al. 2002; van den Eertwegh et al. 2012; Jansen et al. 1992b; Quoix et al. 2011). A report of the international registry indicated fatigue as the second most common side effect of immunotherapy with cytokine-induced killer (CIK) cells with the relative frequency of 32% (Schmeel et al. 2015). Despite its high frequency, CIK-related fatigue that often had a short duration could be properly controlled with pharmalogical treatments (Schmeel et al. 2015). The effect of immunotherapy on symptoms of fatigue seems to be dose-dependent. A cohort study revealed that fatigue was not observed among patients who were treated with infusion of cytotoxic T lymphocytes (CTLs) with the mean number of 2.0–8.0 $\times 10^8$ whereas almost 20% of patients who received infusion of CTLs with the number above 8.0 $\times 10^8$ developed symptoms of fatigue (Di et al. 2012). This is

consistent with the finding of a phase I clinical trial reporting that almost 7, 10, and 12.5% of patients experienced symptoms of fatigue following treatment with single dose, multiple dose, and extension phase of MGN1703 (toll-like receptor 9 agonist) (Weihrauch et al. 2015). Surprisingly all patients who were treated with low dose of immunotherapy with NHS-IL2 had a complaint about fatigue whereas almost 29% of patients who received high dose of NHS-IL2 had symptoms of fatigue (van den Heuvel et al. 2015). A placebo-controlled trial on patients with stage IV non-small-cell lung cancer demonstrated that there was no significant difference in fatigue frequency among patients who received TG4010 immunotherapy and chemotherapy compared to patients who received placebo and chemotherapy (Quoix et al. 2015). On the contrary, a phase III trial on patients with non–muscle-invasive bladder cancer indicated significant increased relative frequency of fatigue among patients who received intravesical immunotherapy with Keyhole Limpet Hemocyanin compared to patients who received intravesical Mitomycin (Lammers et al. 2012). Altogether these lines of evidence indicate enormous difference between studies regarding the issue. As summarized in Table 16.2, studies (Atzpodien et al. 2003; Baer et al. 2008; Beer et al. 2011; Bergmann et al. 1993; Burch et al. 2000; Di et al. 2012; Dijkgraaf et al. 2015; Garcia et al. 2014; Gardner et al. 2012; Kerst et al. 2005; Kimura et al. 2008; Lammers et al. 2012; Lara et al. 2003; Nimura et al. 2003; Quoix et al. 2015, 2011; Ryan et al. 2007; Schmeel et al. 2015; Small et al. 2007; Stadler et al. 1995; Tarhini et al. 2012; van den Eertwegh et al. 2012; van den Heuvel et al. 2015; Weihrauch et al. 2015; Yano et al. 1991; Zhang et al. 2015; Zhong et al. 2011; Zustovich et al. 2007) provided fatigue frequency ranging from 0 to 100% during or following immunotherapy.

16.4 Quality of Life, Cancer, and Immunotherapy

Quality of life represents an overall assessment of individual well-being in the various life domains, including physical well-being, material well-being, social well-being, emotional well-being, and development and activity (Felce and Perry 1995). Hundreds measures have been developed to evaluate quality of life of people in the community. Moreover specific measured were designed for the assessment of quality of life in clinical conditions, such as skin diseases (Chren et al. 1997), diabetes (Jacobson et al. 1994), sciatica (Patrick et al. 1995), multiple sclerosis (Vickrey et al. 1995), and cancer (Spitzer et al. 1981; Aaronson et al. 1993).

An ample evidence emrged supporting that quality of life of cancer survivors differ from that of community people (Ferrell et al. 1995). FDA confirmed quality of life as one of patient-reported outcomes which are considered for approval of anti-cancer drugs (Hales et al. 2010). For example, combination of Mitoxantrone and prednisone has been shown to improve both clinical outcome and quality of life of patients with castration-resistant prostate cancer (Hales et al. 2010). It is very promising that a number of other medications and treatments, such as Epoetin alfa and chemotherapy, that cancer patients may receive help to improve their quality of

Table 16.2 Summary of studies that evaluated fatuge frequency among cancer patients during and/or following immunotherapy

Immunotherapy	Study design	Study sample (numbers of patients)	Fatigue prevalence during or following immunotherapy	Reference
Cytokine induced killer cells	Report of the international registry	The international registry (n = 2729)	32%	Schmeel et al. (2015)
Cytotoxic T lymphocytes infusion (with the mean amount of 2.0–8.0 $\times 10^8$)	Cohort	Cancer patients (n = 6)	0%	Di et al. (2012)
Cytotoxic T lymphocytes infusion (with number above 8.0 $\times 10^8$)		Cancer patients (n = 21)	~19%	
MGN1703 (toll-like receptor 9 agonist), single dose	Phase I clinical study	Patients with metastatic solid tumours (n = 15)	~7%	Weihrauch et al. (2015)
MGN1703 (toll-like receptor 9 agonist), multiple dose		Patients with metastatic solid tumours (n = 21)	~10%	
MGN1703 (toll-like receptor 9 agonist), extension phase		Patients with metastatic solid tumours (n = 8)	12.5%	
NHS-IL2 (which selectively activates the high-affinity IL-2 receptor), 0.15 mg/kg	Phase Ib trial	Patients with advanced-stage non-small cell lung cancer (n = 3)	100%	van den Heuvel et al. (2015)
NHS-IL2, 0.30 mg/kg		Patients with advanced-stage non-small cell lung cancer (n = 3)	~67%	
NHS-IL2, 0.45 mg/kg		Patients with advanced-stage non-small cell lung cancer (n = 7)	~29%	
TG4010 (a modified vaccinia Ankara expressing MUC1 and interleukin 2) and chemotherapy	Phase 2b part of a randomised, double-blind, placebo-controlled, phase 2b/3 trial	Patients with stage IV non-small-cell lung cancer (n = 110)	63%	Quoix et al. (2015)
Placebo and chemotherapy		Patients with stage IV non-small-cell lung cancer (n = 107)	59%	

(continued)

Table 16.2 (continued)

Immunotherapy	Study design	Study sample (numbers of patients)	Fatigue prevalence during or following immunotherapy	Reference
Cytokine-induced killer cells	Large-sample adverse events research	Patients with malignant tumour (n = 893)	0.49%	Zhang et al. (2015)
Intravesical Keyhole Limpet Hemocyanin	Prospective randomized phase III trial	Patients with non–muscle-invasive bladder cancer (n = 283)	~18%	Lammers et al. (2012)
Intravesical Mitomycin		Patients with non–muscle-invasive bladder cancer (n = 270)	~10%	
Gemcitabine	Phase 1/2 study	Patients with platinum-resistant ovarian cancer (n = 3)	100%	Dijkgraaf et al. (2015)
Gemcitabine + Pegintron (IFN-α)		Patients with platinum-resistant ovarian cancer (n = 9)	~78%	
Gemcitabine + Pegintron + p53 SLP		Patients with platinum-resistant ovarian cancer (n = 6)	~67%	
Sargramostim (GMCSF) and lenalidomide	Phase I-II trial	Patients with castration-resistant prostate cancer (n = 32)	69%	Garcia et al. (2014)
Sipuleucel-T (Provenge) autologous vaccine	Phase III trial	Men with asymptomatic or minimally symptomatic castrate-resistant metastatic prostate cancer (n = 512)	~41%	Gardner et al. (2012)
Placebo		Men with asymptomatic or minimally symptomatic castrate-resistant metastatic prostate cancer	~35%	
TS1, a FT derivative, and LNT (chemo-immunotherapy)	Multiinstitutional pilot study	Patients with unresectable or recurrent advanced gastric Cancer (n = 22)	~5%	Nimura et al. (2003)

(continued)

Table 16.2 (continued)

Immunotherapy	Study design	Study sample (numbers of patients)	Fatigue prevalence during or following immunotherapy	Reference
Prolonged low dose IL-2 and thalidomide	Phase II study	Patients with progressive metastatic renal cell carcinoma (n = 22)	~77%	Kerst et al. (2005)
Conventional NP chemotherapy followed by vaccinated with CEA (605–613) peptide pulsed autologous dendritic cells and CIK cells (chemo-immunotherapy)	Open-label phase I/II clinical trial	Patients with late-stage non-small cell lung cancer (n = 14)	~7%	Zhong et al. (2011)
Conventional NP chemotherapy		Patients with late-stage non-small cell lung cancer (n = 14)	~57%	
IL-2 and IFN-α	Prospective quality-of-life analysis	Patients with progressive metastatic renal cell carcinoma (n = 22)	Mean fatigue scores were significantly increased (from 33 to 56)	Atzpodien et al. (2003)
Combined immunotherapy with granulocyte-macrophage colony-stimulating factor-transduced allogeneic prostate cancer cells and ipilimumab	Phase 1 dose-escalation trial	Patients with metastatic castration-resistant prostate cancer (n = 28)	~79%	van den Eertwegh et al. (2012)
Adjuvant chemo-immunotherapy using autologous dendritic cells and activated killer cells from tissue culture of tumor-draining lymph nodes	Prospective phase II study	Patients with primary lung cancer (n = 28)	23%	Kimura et al. (2008)
Gemcitabine	Phase II Study	Patients with advanced renal cancer (n = 11)	~9%	Zustovich et al. (2007)
Gemcitabine and immunotherapy (IL-2 or IFN-α)		Patients with advanced renal cancer (n = 16)	37.5%	

(continued)

Table 16.2 (continued)

Immunotherapy	Study design	Study sample (numbers of patients)	Fatigue prevalence during or following immunotherapy	Reference
Low-dose recombinant IL-2	Phase III trial	Patients age 60 years and older with acute myeloid leukemia in first complete remission (n = 81)	15% (grade III–IV)	Baer et al. (2008)
High-dose IFN-α-2b and IL-2 bolus infusion	Phase II Study	Patients with metastatic renal cell cancer (n = 36)	33% (grade I–IV)	Bergmann et al. (1993)
Subcutaneous recombinant human IL-4	Phase II Study	Patients with metastatic renal cell cancer (n = 18)	~89%	Stadler et al. (1995)
Adoptive immunotherapy with lymphokine-activated killer cells and recombinant interleukin 2	NR	Patients with primary lung cancer (n = 5)	100%	Yano et al. (1991)
Autologous dendritic cells	Phase I trial	Patients with progressive hormone-refractory metastatic prostate carcinoma (n = 13)	~54% (grade I–IV)	Burch et al. (2000)
SU5416 and IFN-α	Phase II study	Patients with advanced renal cell carcinoma (n = 30)	~47%	Lara et al. (2003)
GM-CSF-secreting, allogeneic cellular immunotherapy, high dose	Phase III trial	Patients with hormone-refractory prostate cancer with radiologic metastases (n = 10)	20%	Small et al. (2007)
GM-CSF-secreting, allogeneic cellular immunotherapy, low dose		Patients with hormone-refractory prostate cancer with radiologic metastases (n = 24) or rising PSA only (n = 21)	37%	
GM-CSF-secreting, allogeneic cellular immunotherapy		Patients with hormone-refractory prostate cancer (n = 55)	26% (grade I–IV)	

(continued)

Table 16.2 (continued)

Immunotherapy	Study design	Study sample (numbers of patients)	Fatigue prevalence during or following immunotherapy	Reference
Sipuleucel-T (an autologous cellular immunotherapy)	Randomized, double-blind, controlled trial	Patients with prostate cancer (n = 116)	~45%	Beer et al. (2011)
Control		Patients with prostate cancer (n = 59)	~30%	
Ketoconazole plus granulocyte-macrophage colony-stimulating factor	Phase II study	Patients with progressive castration resistant prostate cancer (n = 49)	14% (grade III)	Ryan et al. (2007)
TG4010 plus cisplatin and gemcitabine (Immuno-chemotherapy)	Controlled phase 2B trial	Patients with advanced non-small-cell lung cancer (n = 74)	~60% (any grade)	Quoix et al. (2011)
Chemotherapy alone		Patients with advanced non-small-cell lung cancer (n = 74)	~51% (any grade)	
IFN-α-2b and tremelimumab	Phase II study	Patients with stage IV melanoma (n = 37)	100% (any grade)	Tarhini et al. (2012)

life and their long-term survival as well (Glimelius et al. 1996; Littlewood et al. 2001; Coates et al. 1987).

A systematic study on sixteen randomized controlled trials indicated that chemotherapy with temsirolimus, sunitinib and sorafenib was more beneficial to health-related quality of life of patients with advanced/metastatic renal cell carcinoma than immunotherapy with IFN-α (Takyar et al. 2016). This is consistent with the findings from a phase III trial (Chiarion-Sileni et al. 2003) on patients with advanced melanoma demonstrating that combination of immunotherapy and chemotherapy decreased scores in all the health related quality of life-related domains (for more information see Table 16.3). By contrast chemotherapy alone reduced scorese in only two domains, i.e. activity level and the physical symptom distress. However patients with gastric cancer who received immunotherapy after complementation of chemotherapy had better quality of life, in spite of no significant influence of immunotherapy alone on the global health status (Cui et al. 2015). Regarding patients with breast cancer, some certain domains of well-being but not overall quality of life were appeared to be amended by immunotherapy, whereas overall quality of life could be made better by radiotherapy (Lu et al. 2009).

A comprehensive review revealed that T-cell directed immunotherapies did not only seem to improve quality of life of patients, but also it might help to impair the patients' quality of life (Kobold et al. 2015). However individuals studies have demonstrated that immunotherapy using activated T lymphocytes tended to maintain the quality of life of patients with advanced lung cancer until the time of death (Iwai et al. 2012).

Immunotherapy using dendritic cell vaccine and cytokine-induced killer (CIK) cell could considerably improve the patients' quality of life post-treatment (Wang et al. 2015; Zhang et al. 2016; Zhu et al. 2014; Guo et al. 2014; Nencioni et al. 2008). As well, comprehensive and systematic reviews of clinical studies on cytokine-induced killer cells qualitatively confirmed that immunotherapy using these cells could make better the quality of life of patients with malignancies (Schmeel et al. 2015, 2014; Chen et al. 2014; Hontscha et al. 2010). However there was a reprot suggesting that immunotherapy with CIK cells worsened the quality of life of patients with gemcitabine-refractory advanced pancreatic cancer (Chung et al. 2014).

Gene-mediated cytotoxic immunotherapy which was used for patients with pancreatic adenocarcinoma and malignant glioma tended to maintain or even enhance the patients' quality of life (Aguilar et al. 2015; Chiocca et al. 2011).

Taken together current evidence proposes that immunotherapy alone is not likely to make profits for patients' life quality and long-term survival. However application of immunotherapy along a pivot treatment might fulfill this purpose.

Table 16.3 Summary of studies that evaluated change in quality of life in cancer patients during and/or following immunotherapy

Treatment	Study design	Study sample (numbers of patients)	Change in quality of life or authors' conclusion	Reference
Dendritic cell vaccine and cytokine induced killer cell	Open label, parallel group, single institution, non randomized study	Patients with hepatobiliary and pancreatic cancer (n = 72)	65% of the patients exhibited an improvement in quality of life	Zhang et al. (2016)
Dendritic cell vaccine and cytokine induced killer cell	Cohort	Patients with malignant tumors (n = 60)	~42% of the patients exhibited an obvious improvement in quality of life	Wang et al. (2015)
Cellular immunotherapy after the last chemotherapy	Open-label pilot cohort study	Patients with gastric carcinoma (n = 13)	The global health status was significantly better at 6 months after chemotherapy compared with that at 1 month after chemotherapy ($P = 0.036$)	Cui et al. (2015)
Cellular immunotherapy simultaneous with chemotherapy		Patients with gastric carcinoma (n = 17)	There was no significant difference in global health status between two periods (before and during treatment) ($P > 0.05$)	
Chemotherapy alone		Patients with gastric carcinoma (n = 28)	The global health status during treatment was significantly worse than that before treatment ($P = 0.032$)	
Gene-mediated cytotoxic immunotherapy	Dose escalation study	Patients with pancreatic adenocarcinoma (n = 24)	Health-related quality of life was stable after treatment compared with baseline	Aguilar et al. (2015)
Dendritic cell vaccine and cytokine induced killer cell	Open-label, single-institution, parallel-group, nonrandomized, retrospective study	Patients with advanced colorectal cancer (n = 97)	75.2% of patients showed a positive improvement in their physical strength, 74.2% had improved appetite, 72.1% were able to sleep better, and 70.1% had an increase in body weight	Zhu et al. (2014)

(continued)

Table 16.3 (continued)

Treatment	Study design	Study sample (numbers of patients)	Change in quality of life or authors' conclusion	Reference
Cytokine-induced killer cells	Phase II trial	Patients with gemcitabine-refractory advanced pancreatic cancer (n = 20)	Global health status scores worsened after therapy but was not statistically significant	Chung et al. (2014)
Dendritic cell-cytokine induced killer cell immunotherapy (DC-CIK) combined with transcatheter arterial chemoembolization (TACE)	Comparative study	Patients with hepatocellular carcinoma (n = 30)	Compared with TACE alone, DC-CIK immunotherapy combined with TACE can improve the quality of life of the patients	Guo et al. (2014)
Transcatheter arterial chemoembolization (TACE)		Patients with hepatocellular carcinoma (n = 38)		
Immunotherapy	Comparative study	Patients with cancer (n = 39)	Patients treated with immunotherapy exhibited a higher physical conditions score than did patients receiving chemotherapy	Nagao et al. (2012)
Adoptive activated T lymphocyte immunotherapy	Multicenter historical cohort study	Patients with advanced lung cancer (n = 540)	Immunotherapy can maintain good quality of life of the patients until near the time of death	Iwai et al. (2012)
Gene-mediated cytotoxic immunotherapy	Phase IB study	Patients with malignant glioma (n = 12)	Health-related quality of life was stable or improved after treatment	Chiocca et al. (2011)
Conventional treatment plus IMMUNEPOTENT CRP (bovine dialyzable leukocyte extract) adjuvant immunotherapy	Phase I study	Patients with non-small cell lung cancer	The administration of IMMUNEPOTENT CRP increased the quality of the patients' lives	Franco-Molina et al. (2008)
Conventional treatment (external radiotherapy and chemotherapy) alone		Patients with non-small cell lung cancer		

(continued)

Table 16.3 (continued)

Treatment	Study design	Study sample (numbers of patients)	Change in quality of life or authors' conclusion	Reference
Bio-chemotherapy (cisplatin, dacarbazine, IL-2 and IFNα-2b)	Phase III study	Patients with advanced melanoma (n = 88)	During the treatment, the mean values decreased significantly in all domains (overall quality of life, activity level, physical symptom distress, psychological distress)	Chiarion-Sileni et al. (2003)
Chemotherapy (cisplatin and dacarbazine) alone		Patients with advanced melanoma (n = 88)	The decrease was significant only for the activity level and the physical symptom distress	
Immunotherapy with a heat-killed suspension of *Mycobacterium vaccae* (SRL172) added to chemotherapy	Phase III study	Patients with advanced non-small-cell-lung cancer (n = 45)	Quality of life was improved in those receiving SRL172	Stanford et al. (2008)
Chemotherapy alone		Patients with advanced non-small-cell-lung cancer (n = 21)		
Combination of epoetin beta, IFN-α-2a, and IL-2	Phase II study	Patients with metastatic renal cell carcinoma (n = 21)	The quality of life increased in ten patients, nine of whom exhibited an increase in their haemoglobin during therapy. Five of the nine patients with decreased quality of life also experienced a decrease in their haemoglobin	Schenck et al. (2007)
Inhaled immunotherapy with IL-2	Clinical trial	Patients with pulmonary metastases of renal cell carcinoma (n = 21)	The patients' quality of life did not change significantly at any time during therapy	Lummen et al. (2004)

(continued)

Table 16.3 (continued)

Treatment	Study design	Study sample (numbers of patients)	Change in quality of life or authors' conclusion	Reference
TS1 and lentinan combination immunotherapy	Pilot study aiming at a randomized trial	Patients with advanced or recurrent gastric cancer (n = 5)	Quality of life scores for appetite, nausea/vomiting, and abdominal pain/diarrhea showed improvement, although not in statistically significant values	Kimura et al. (2003)
Subcutaneous IFN-α-2a and subcutaneous IL-2	Clinical trial	Patients with progressive metastatic renal cell carcinoma (n = 22)	Mean quality of life deteriorated significantly during the first 3 weeks after treatment	Atzpodien et al. (2003)
Heat-shock protein peptide complex 96 (HSPPC-96) vaccine	Phase I trial	Patients with advanced melanoma (n = 30)	Melanoma patients reported worse quality of life scores on the physical dimensions and similar quality of life scores on the psychosocial and emotional dimensions compared with the general population	Cohen et al. (2002)
Intralymphatic administration of non-recombinant IL-2 and LAK cells, IFN and TF	Retrospective study	Patients with metastatic renal-cell cancer (n = 122)	Quality of life was not affected	Pizza et al. (2001)
Inhalational IL-2	Prospective long-term analysis	Patients with metastatic renal cell carcinoma (n = 15)	The mean quality of life score deteriorated modestly but significantly 1 month after treatment initiation but did not differ significantly from pretreatment scores after 3, 6, 9, and 12 months of treatment	Heinzer et al. (1999)
Intravenous IL-2		Patients with metastatic renal cell carcinoma (n = 10)	Patients who received intravenous IL-2 showed a more marked deterioration in mean quality of life score during treatment	

(continued)

Table 16.3 (continued)

Treatment	Study design	Study sample (numbers of patients)	Change in quality of life or authors' conclusion	Reference
Immunotherapy with a polyvalent melanoma cell vaccine	Clinical trial	Patients with in-transit melanoma metastases (n = 54)	The quality of life was not significantly change	Hsueh et al. (1999)
Combination immunotherapy with tumor infiltrating lymphocytes and IL-2	Retrospective, cross-sectional study	Patients with advanced renal cell carcinoma (n = 20)	Patients reported better health related quality of life than those with other malignancies and better physical function than patients with congestive heart failure	Litwin et al. (1997)
Sequential chemotherapy and immunotherapy	Clinical study	Patients with advanced stage malignancies (eight head and neck squamous cell carcinomas, two melanomas) (n = 10)	The association of chemotherapy and immunotherapy did not significantly worsen the quality of life of patients as compared to chemotherapy alone	Curreli et al. (1996)
Topical immunotherapy with bacille Calmette-Guérin	Clinical study	Patients with superficial bladder cancer (n = 85)	The overall quality of life was mostly only moderate, were poor during initial therapy and better during 3monthly maintenance therapy	Mack and Frick (1996)
Intravesical bacillus Calmette-Guerin immunotherapy	Pilot survey	Patients with superficial stages pTa to pT1, grades 1 to 3 urothelial bladder carcinoma (n = 30)	Satisfaction with life in the patients studied was high and was not impaired during the treatment	Bohle et al. (1996)
LAK immunotherapy	Comparative study	Patients with advanced gastric or colon cancer (n = 17)	LAK immunotherapy is better than EAP chemotherapy from the standpoint of the quality of life	Ochiai et al. (1990)
EAP chemotherapy		Patients with advanced gastric or colon cancer (n = 10)		

(continued)

Table 16.3 (continued)

Treatment	Study design	Study sample (numbers of patients)	Change in quality of life or authors' conclusion	Reference
Bacillus Calmette-Guerin immunotherapy	Randomized controlled trial	Patients with advanced cancer of the prostate (n = 21)	The quality of life was significantly less in BCG immunotherapy-treated group	Guinan et al. (1982)
Conventional treatment		Patients with advanced cancer of the prostate (n = 21)		

16.5 Anorexia, Cancer, and Immunotherapy

Anorexia nervosa is of feeding and eating disorders that share the common feature of "disturbance of eating or eating-related behavior that results in the altered consumption or absorption of food and that significantly impairs physical health or psychosocial functioning" (American Psychiatric 2013). The condition "when the eating of nonnutritive, nonfood substances is primarily used as a means of weight control" is diagnosed with Anorexia nervosa (American Psychiatric 2013). Based on the findings of meta-analysis studies, anorexia nervosa affects 370 persons per 100,000 young females in the community (Hoek and Van Hoeken 2003), with the relatively high mortality rate of 5 (Arcelus et al. 2011). Moreover approximately one-fifth of dead patients committed suicide (Arcelus et al. 2011). These facts warrant opportune diagnosis and treatment of anorexia nervosa.

Anorexia has been associated with a number of medical conditions, including infection, inflammatory and immunological diseases, and injury (Plata-Salamán 1996). Anorexia caused by clinical condition can be acute or chronic. Acute anorexia seems be beneficial to health because pathogens need energy and as a result growth of pathogens, e.g. bacteria, is controlled in low-calorie conditions. On the contrary chronic anorexia appears to be detrimental to health because of its negative influence on the body's major organs. Chronic anorexia may eventually lead to cachexia, "a complex metabolic syndrome associated with underlying illness and characterized by loss of muscle with or without loss of fat mass" (Evans et al. 2008). Cachexia is believed to have a huge impact on the prognosis of underlying clinical diseases such as kidney, cardiac and pulmonary diseases (Tan and Fearon 2008). Cancer anorexia-cachexia syndrome that is characterized by sequential series of events from anorexia to cachexia, including weight loss, tissue wasting, limited functioning, and death, is considered as one of common serious complications caused by malignancies, particularly gastrointestinal and lung cancers (Inui 2002).

Anorexia has been identified as one of the most common adverse events of different immunotherapeutic protocols, including dendritic cell vaccine and cytokine-induced killer cell for patients with hepatobiliary and pancreatic cancer (Zhang et al. 2016) and patients with colorectal cancer (Niu et al. 2014), recombinant IFN-α-2a for patients with metastatic melanoma (Schuchter et al. 1992), IL-2 for patients with non-small cell lung carcinoma (Lissoni et al. 1993), IL-2 plus IFN-α for patients with renal cell carcinoma (Maroto et al. 2013; Neri et al. 2002) and for patients with advanced non-small-cell lung cancer (Jansen et al. 1992a), IL-4 for with metastatic renal cell cancer (Stadler et al. 1995), TG4010 for patients with advanced stages of non-small-cell lung cancer (Quoix et al. 2011), and NHSIL2 for patients with metastatic non-small cell lung carcinoma (van den Heuvel et al. 2015). However, a systematic review of studies evaluating effectiveness of Polysaccharide K (PSK), which is used as an adjuvant immunotherapy, indicated that PSK might improve anorexia (Fritz et al. 2015). A comparative study demonstrated that adding IL-3 to IL-2 might be a possible strategy to ameliorate negative affect of IL-2 on anorexia. More than 70% of patients with non-small cell

Table 16.4 Summary of studies that evaluated anorexia frequency among cancer patients during and/or following immunotherapy

Immunotherapy	Study design	Study sample (numbers of patients)	Anorexia-cachexia prevalence during or following immunotherapy	Reference
Dendritic cell vaccine and cytokine induced killer cell	Open label, parallel group, single institution, non randomized study	Patients with hepatobiliary and pancreatic cancer (n = 72)	17%	Zhang et al. (2016)
NHSIL2, 0.15 mg/kg	Phase Ib trial	Patients with advanced-stage metastatic non-small cell lung carcinoma (n = 3)	100%	van den Heuvel et al. (2015)
NHSIL2, 0.30 mg/kg		Patients with advanced-stage metastatic non-small cell lung carcinoma (n = 3)	0	
NHSIL2, 0.45 mg/kg		Patients with advanced-stage metastatic non-small cell lung carcinoma (n = 7)	~29%	
Dendritic cell vaccine and cytokine induced killer cell immunotherapy plus chemotherapy	Retrospective comparative study	Patients with colorectal cancer (n = 70)	~39%	Niu et al. (2014)
Dendritic cell vaccine and cytokine induced killer cell	Open-label, single-institution, parallel-group, nonrandomized, retrospective study	Patients with advanced colorectal cancer (n = 100)	~9%	Zhu et al. (2014)
Subcutaneous IL-2 plus IFN-α followed by sorafenib	Phase II trial	Patients with non-resectable, clear cell renal cell carcinoma (n = 41)	~46%	Maroto et al. (2013)

(continued)

Table 16.4 (continued)

Immunotherapy	Study design	Study sample (numbers of patients)	Anorexia-cachexia prevalence during or following immunotherapy	Reference
Vaccination with TG4010 and first-line chemotherapy	Controlled phase 2B trial	Patients with advanced stages of non-small-cell lung cancer (n = 73)	~45%	Quoix et al. (2011)
First-line chemotherapy alone		Patients with advanced stages of non-small-cell lung cancer (n = 72)	~43%	
Human recombinant IL-4	Phase II study	Patients with metastatic renal cell cancer (n = 18)	~61%	Stadler et al. (1995)
OK-432, a Streptococcal preparation	Multi-institutional randomized trial	Patients with gastric cancer (n = 145)	~23%	Tanaka et al. (1994)
IL-2 alone	Comparative study	Patients with metastatic non-small cell lung carcinoma (n = 12)	~73%	Lissoni et al. (1993)
IL-2 plus IL-3		Patients with metastatic non-small cell lung carcinoma (n = 6)	~8%	

lung carcinoma who received IL-2 alone developed anorexia whereas one among patients who received IL-2 plus IL-3 experienced anorexia (Lissoni et al. 1993).

In Table 16.4, we have summarized findings of studies that evaluated anorexia during or following immunotherapy in cancer people. These studies indicate that immunotherapy with IL-2 or IL-4 results in the highest rate of anorexia post-treatment, whereas the lowest frequency of anorexia was observed among patients who received dendritic cell vaccine and cytokine-induced killer cell.

16.6 Anxiety, Cancer, and Immunotherapy

The category of anxiety disorders comprises a number of disorders which are characterized by the presence of "excessive fear and anxiety and related behavioral disturbances" (American Psychiatric 2013). Generally, it has been estimated that approximately 7% of the population worldwide is affected by anxiety disorders (Baxter et al. 2013). However epidemiological studies provided a broad range (0.9–28.3%) of current prevalence rates of anxiety disorders (Baxter et al. 2013). A systematic study of more than eighty studies demonstrated that prevalence of anxiety disorders vary considerably among studies depending on several factors e.g. gender, age, culture, economic status, and methodology-related factors (Baxter et al. 2013). Results of a US national survey revealed that women are not only more likely to develop anxiety disorders than men during their life-time (44.8% vs. 34.2%), but they are so more likely to be disabled with anxiety disorders (McLean et al. 2011). Moreover findings of a meta-analysis study indicated the importance of genetic factors in familial aggregation of some anxiety disorders e.g. panic disorder, generalized anxiety disorder, phobias, and obsessive compulsive disorder (Hettema et al. 2001).

Some anxiety disorders, particularly social and simple phobias, are likely to occur early in life (Regier et al. 1998) and that in general anxiety disorders tend to be persistent (Lépine 2002). Besides, comorbidity of anxiety disorders and other mental disorders, such as substance use disorders (Grant et al. 2004), bipolar disorders (Freeman et al. 2002), and major depressive disorder (Fava et al. 2000), has been frequently been the subject of research.

As well, anxiety disorders have been associated with many physical conditions, e.g. allergy, arthritis, gastrointestinal disease, respiratory disease, thyroid disease (Sareen et al. 2006). Co-occurrence of anxiety disorders and physical disease contribute substantially to physical disability and thereby reducing life quality (Sareen et al. 2006). Therefore it would be understandable that health care professions have adduced the onerous social and economic burden of anxiety disorders (Lépine 2002).

Similar to that of observed in the general population, the prevalence of anxiety disorders ranging from 4 to 18% in patients with cancer varies widely across studies (Derogatis et al. 1983; Stark et al. 2002; Watson et al. 1991; Aass et al. 1997; Lueboonthavatchai 2007; Brintzenhofe-Szoc et al. 2009). This variability might be

Table 16.5 Summary of studies that evaluated anxiety among cancer patients during and/or following immunotherapy

Immunotherapy	Study sample	Anxiety measurement	Change in anxiety symptoms	Authors' conclusion	Reference
IL-2 alone (SC)	Patients with renal cell carcinoma or melanoma (n = 20)	CAS, 3 and 5 days	No change	Cytokine therapy had no effect on anxiety, except in patients treated with IL-2 in combination with INFalpha-2b. In these patients, the enhancement in anxiety scores that was observed on day 5 was mainly attributable to increased somatic complaints	Capuron et al. (2000)
IL-2 in combination with INF-α-2b (SC)	Patients with renal cell carcinoma or melanoma (n = 6)	CAS, 3 and 5 days	Exacerbation		
Low dose INF-α-2b alone (SC)	Patients with renal cell carcinoma or melanoma (n = 8)	CAS, 3 and 5 days	No change		
High dose INF-α-2b alone (IV)	Patients with renal cell carcinoma or melanoma (n = 14)	CAS, 3 and 5 days	No change		
IFN-alpha	Patients with metastatic renal cell carcinoma (n = 24)	NA, 4 and 8 weeks	Improvement	The anxiety scores were somewhat lower at 4 and 8 weeks compared with baseline	Van Gool et al. (2008)
IFN-alpha	Patients with metastatic malignant melanoma (n = 75)	STAI, 1, 3, 6, and 12 months	Exacerbation	There was a greater increase in anxiety in the IFN group on both trait and state anxiety	Caraceni et al. (1998)
IFN-alpha	Patients with high-risk melanoma (n = 75)	NR, 4 weeks	Four patients were clinically diagnosed with an IFN-α induced anxiety disorder and/or irritability, all during the induction phase		Van Gool et al. (2003)

owing to methodology-related factors, such as the inventory used for anxiety assessment. Some factors, including female gender, a history of previous psychiatric problems, impaired social life, and impaired physical activity, might allow us to predict occurrence of anxiety disorders in cancer people (Aass et al. 1997). It is important to note that cancer site is of the key elements that might contribute to development and progression of anxiety disorders (Zabora et al. 2001; Brintzenhofe-Szoc et al. 2009). The most severe forms of anxiety disorders have been observed among patients with pancreatic cancer. Cancer types that showed the highest prevalence of pure anxiety symptoms included lymphoma and breast cancer.

There are few studies evaluating changes in anxiety scores following immunotherapy in cancer people (Capuron et al. 2000; Caraceni et al. 1998; Van Gool et al. 2003, 2008) (Table 16.5) and these very few studies have provided very different results. One study examined changes in anxiety scores following immunotherapy with IL-2 with or without IFN-α (Capuron et al. 2000). The authors could find significantly higher anxiety scores following immunotherapy with IL-2 plus IFN-α-2b, but no change was observed following immunotherapy with IL-2 alone or IFN-α alone (Capuron et al. 2000). However two other studies found an exacerbation of anxiety symptoms following IFN-α therapy in a number of patients (Caraceni et al. 1998; Van Gool et al. 2003). Even one study reported the improvement of anxiety symptoms following IFN-α therapy (Van Gool et al. 2008).

16.7 Future Perspectives and Concluding Remarks

The present chapter examined clinical studies about whether immunotherapy might affect mental health of cancer patients. Current evidence indicates that cancer immunotherapy might alter the patients' mental health status, particularly depressive symptoms, fatigue, anorexia, anxiety, and quality of life. However this alteration varies dependent on the immunotherapy-related factors, i.e. type, dosage, and regimen. For example, immunotherapy using IL-2, but not IFN, brings about mild to marked depressive symptoms in all likelihood. Fatigue has been often among the most common adverse events during or following immunotherapy. However clinical studies provided fatigue frequency ranging from 0 to 100% during or following immunotherapy. T-cell directed immunotherapies might help to impair the patients' quality of life whereas immunotherapy using dendritic cell vaccine and cytokine-induced killer cell could considerably improve the patients' quality of life. Immunotherapy with IL-2 or IL-4 resulted in the highest rate of anorexia post-treatment, whereas the lowest frequency of anorexia was observed among patients receiving dendritic cells. Few studies evaluating changes in anxiety scores following immunotherapy in cancer people have provided very different results. Altogether, this chapter corroborated a number of conflicts concerning the possible link between cancer immunotherapy and mental health.

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Chapter 17
Challenges of Endocrine Therapy in Breast Cancer

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Abstract This chapter provides evidences on the magnitude of endocrine therapy in the treatment of breast cancer patients and also discusses challenges in endocrine therapy as well as reviewing recent literature on psychotherapy in the management of adverse effect of endocrine therapy. At first, there is a quick review on the role of estrogen in the breast cancer development which was followed by a brief data on the significance of endocrine therapy. Secondly, the major challenges in endocrine therapy such as non-adherent patients during the course of endocrine therapy, endocrine therapy failure especially Tamoxifen and Letrozole resistance and finally adverse effects of endocrine therapy will be discussed in the rest of chapter. Resistance to the endocrine therapy will be explained in details to elucidate the molecular mechanisms underlying this phenomenon. Finally, the recent evidences on the administration of psychotherapy to modulate the adverse effect of endocrine therapy briefly mentioned.

Keywords Adherence · Adverse effect · Letrozole resistance · Molecular mechanisms · Psychotherapy · Tamoxifen resistance

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Abbreviations

SERM	Selective estrogen receptor modulator
AI	Aromatase inhibitor
ERα	Estrogen receptor α
HER2	Human epidermal growth factor receptor 2
EGFR	Epidermal growth factor receptor
RTK	Receptor tyrosine kinases
PI3K	Phosphoinositide 3-kinase
MAPK	Mitogen-activated protein kinases
ERK	Extracellular signal-regulated kinase-1
PDK	Phosphoinositide-dependent kinase-1
mTOR	Mammalian target of rapamycin
AIB1/SRC-3	Steroid receptor coactivator-3/Amplified in breast 1
ERE	Estrogen receptor element

17.1 Introduction

17.1.1 The Role of Estrogen in Breast Cancer Development

It has long been observed that many risk factors are involved in development of breast cancer each with different disease inducing potencies. For instance early menarche, late menopause, nulliparity, Hormone Replacement Therapy (HRT), alcohol consumption and postmenopausal obesity are some of the low-grade breast cancer pre-disposing risk factors, pre-maternal age of first birth more than 35 years and dense breast tissue observation in mammographic as intermediate predisposing risk factors and finally mutations in BRCA1 and BRCA2, Lobular carcinoma in situ (LCIS), atypical hyperplasia and radiation exposure before thirties as high-grade pre-disposing risk factors (Amir et al. 2010; McPherson et al. 2000). As most of these factors affect serum estrogen levels, many estrogen modulators were turned into the most important therapeutical against in breast cancer therapy. Considering the fact that 17β estradiol is the most common form of estrogen found in body, two main endocrine therapies consisting of Selective Estrogen Receptor Modulators (SERMs) and Aromatase Inhibitors (AIs) have been established. Estrogen Receptor (ER) is located in cell's nucleus, which upon activation results in up-regulation of a variety of genes taking part in breast cancer development (Bjornstrom and Sjoberg 2005; Mansouri et al. 2017a). Tamoxifen, as the most common member of SERM family, is an ER competitive molecule widely administered in both pre- and post- menopause patients (Karn et al. 2010). Aromatase is a specific enzyme enrolled in a key step in estrogen biosynthesis, and is mostly found in ovary, fat, muscles, liver and breast. Inhibitors of this enzyme, so-called "Aromatase inhibitors", are newly developed therapeutic agents for preventing breast cancer from further progression with higher

prominence in post-menopausal women (Miller 2003). Normally, in a post-menopausal female, the activity of ovarian Aromatase is ceased and peripheral tissues take part in estrogen synthesis. In another words, in a menopause female, adrenal gland secrete androstenedione, the precursor of estradiol molecule and Aromatase enzyme in peripheral tissues, predominantly in adipose tissue, convert this precursor and produces body required estrogen (Schweikert et al. 1976). This amount of produced estrogen is not equal to the ones synthesized by ovaries in a sexually active woman and may be the answer to the question why physical and behavioral changes occur in post-menopausal females, nevertheless, this mesenchymal adipose source of estrogen does not have systemic activity and its effects are site-limited, also referred as paracrine effects. By the time which amounts of adipose tissue are high, synthesized estrogen may find a way to the circulation and demonstrated systemic effects. Consequently, higher amount of adipose tissues and obesity in menopaused female, is together with higher risk of breast cancer development. The Fig. 17.1, the role of estrogen in the breast cancer cell growth was shown.

17.1.2 Endocrine Therapy in Breast Cancer

Multiple cytosolic growth pathways hyperactivity has been proposed to be involved in cancer cells proliferation. Among these pathways, estrogen and estrogen receptor seems to be the main cascade to induce the growth. The Estrogen Receptors is positive in around 80% of Ductal Carcinoma (DCIS), and about 70% of Invasive Ductal Carcinoma (IDC) in situ. According to American Society of Clinical Oncology (ASCO) Clinical Practice Guideline, 5 year administration of Tamoxifen for hormone receptor positive pre- or post-menopausal patients is together with beneficial outcomes (Burstein et al. 2016). Regarding to an update published by The National Guideline Clearinghouse (NGC) in 2014, After 5 years of treatment with Tamoxifen, therapy should continue for second 5 year period both in pre- and post- menopausal patients. However, considering the fact that Aromatase is an alternative estrogen production pathway in post-menopausal patients, Aromatase inhibitors should be administered as an alternative therapy for continuing Tamoxifen regimen. Post-menopausal patients have three recommendations: (1) Tamoxifen for 10 years, (2) Aromatase inhibitors for up to 5 years and (3) Tamoxifen therapy for 5 years following by concurrent administration of Aromatase inhibitors up to 5 year (Burstein et al. 2010). According to ATLAS trial, a randomized clinical trial on benefit of continuing administration of Tamoxifen for more than 5 years, usage of Tamoxifen for more than 5 year reduces recurrence, breast cancer specific mortality and overall mortality (Davies et al. 2013). A meta-analysis on comparing the outcome of hormone therapy with Tamoxifen or Aromatase inhibitors showed that either their administration as monotherapy for 5 years or initiating administration of Aromatase inhibitors after 2–3 years of Tamoxifen, around 1–3% lesser recurrence and mortality rates compared to Tamoxifen monotherapy was observed (Dowsett et al. 2010). Although hormone therapy in management of breast cancer patients is beneficial, some of

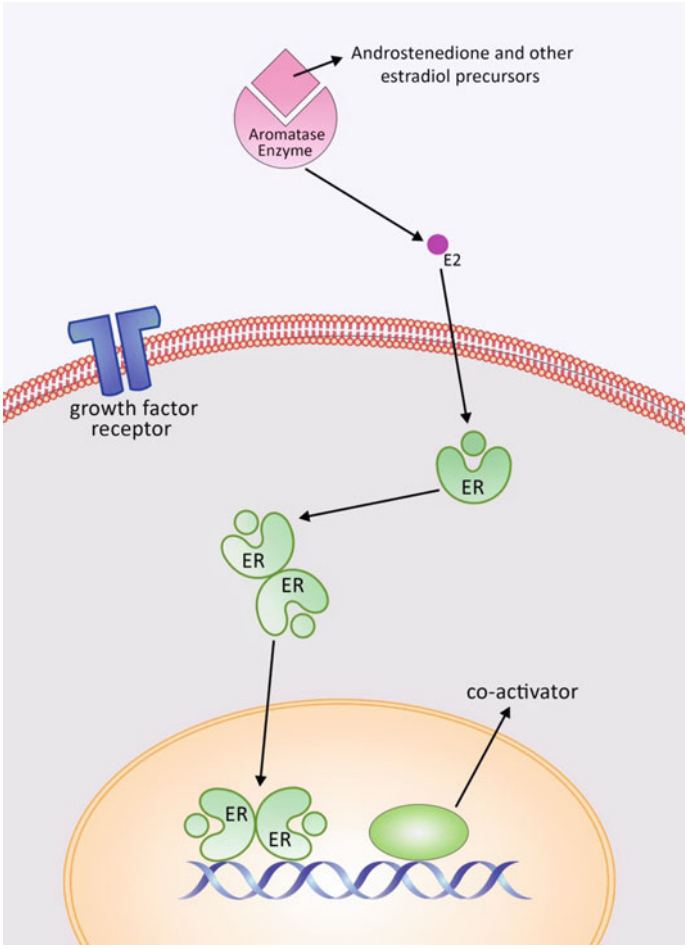

Fig. 17.1 Estrogen receptor genomic pathway. Estradiol precursors activate Aromatase Enzyme and it in turn, produces estrogen (E2) to initiate the genomic pathway inside the normal breast cell. After estrogen binding to its related receptor in the cytoplasm or nucleus, the receptor will be dimerized and regulate the expression of particular genes about cell growth and proliferation. This genomic pathway is highly controlled via inhibitory regulatory pathways

patients do not demonstrate compliance with treatment. It was shown that one-third to half of breast cancer patients left the endocrine therapy during 5 years from therapy initiation (Hershman et al. 2010; McCowan et al. 2008; Owusu et al. 2008; van Herk-Sukel et al. 2010). Living endocrine therapy before completion of the standard treatment accompanies with more mortality rate (Hershman et al. 2011; Ma et al. 2008; Yood et al. 2008). According to NCCN guidelines, when a patient develops Tamoxifen resistance, shifting therapy on Letrozole or Exemestane is the first choice. In patient with visceral crisis, adding chemotherapy regimen to the above treatment strategy is essential.

17.2 Challenges in Endocrine Therapy

17.2.1 Adherence to Hormone Therapy

Although all patients were reported to completely adhere with hormone therapy, only 40–70% of them were adhered to treatment according to prescriptions (Ziller et al. 2009). Many studies have conducted to compare adherence to Tamoxifen with Aromatase inhibitors. Two systematic reviews showed patients less adhesion to Tamoxifen compared with Aromatase inhibitors (AIs) (Huiart et al. 2013; Murphy et al. 2012). Two independent cohort studies examining around 4000 early breast cancer patients showed that non-adherent patients experienced higher mortality rates compared to the ones which continued therapy to the end (McCowan et al. 2008; Yood et al. 2008). Another cohort of more than 3300 patients showed the same with non significant statistics (Makubate et al. 2013). On the other hand, around one forth to one third of eligible patients for initiating endocrine therapy, did not start their treatment (Haque et al. 2012; Livaudais et al. 2012).

As adherence to hormone therapy effects on prognosis, many social and clinical factors have been proposed to impress adherence to treatment. Some of these factors include age, education, having a partner, family history of cancer, alcohol drinking, smoking and stage of disease. Being older, having higher education levels, having a partner, having a family history of cancer, more psychotherapy consultations, undergoing breast cancer surgery and consulting mastologist and/or oncologist associates with more adherences to the therapy. Contrarily, more examinations and tests, higher stage of disease, alcohol consumption, receiving chemotherapy or concurrent Tamoxifen and AI hormone therapy regimen and more hospitalizations resulted in lower adherence to treatment (Brito et al. 2014).

17.2.2 Tamoxifen Resistance and Hormone Therapy Failure

Tamoxifen resistance is classified as either de novo, with disease progression within 2 years of treatment initiation or acquired with an initial clinical benefit of at least 2 years according to ASCO guideline. Host's genes signature, pattern of drug metabolism and environmental factors have been suggested as the most important parameters in Tamoxifen resistant occurrence. Main molecular patterns of Tamoxifen resistance involve deregulation or intensification of growth signaling pathways and underlying mediators, over-expression of oncogenes and amplification of interrelated receptors including ERα36 and GPR30. It seems that molecular interactions between ER dependent and independent pathways confer complex resistance network. ER dependent and independent cascades are in two plateaus of balance; ER outweighed in ER positive tumors (Mansouri et al. 2017b). Here, it will be explained how molecular mechanisms causes tumor cells to become resistant to Tamoxifen.

17.2.2.1 HER2 and HER2 Downstream Mediators Over-Expression in Tamoxifen Resistant Cells

Studies have demonstrated that HER2 over-expression either in HER2 positive tumor, or HER2 negative breast cancer cells can result in Tamoxifen resistant occurrence (Cui et al. 2012; Garcia-Becerra et al. 2012; Shou et al. 2004). Some studies have proposed that physiological elimination of estradiol can results in HER2 over-expression and subsequent development of Tamoxifen resistance (Block et al. 2012; Massarweh et al. 2008; Moi et al. 2012; Zhang and Wang 2013). However, studies with opposite results also exist. For instance, it has been shown that Tamoxifen administration can suppress HER2 expression through activation of PAX2, an important transcription factor in tumor cells (Hurtado et al. 2008). In any case, gene expression signature in resistant tumor cells overlaps with those involve in HER2 growth signaling cascade (Massarweh et al. 2008). Also, it has been shown that phosphorylation of tyrosine position 1221/1222 of HER2 accompanies with poor prognosis in ER+ patients (Frogne et al. 2009).

Many studies have performed with the purpose of identifying the key factors involved in over-expression of HER2. MUC1C has been proposed as an inducer of HER2 over-expression in the resistant tumor cells (Merikhian et al. 2017). This was approved by administration of GO-203, a MUC1-C inhibitor and reduction in HER2 phosphorylation. Furthermore, this MUC1-C inhibitor also demonstrates synergistic effect with Tamoxifen when administered concurrently (Kharbanda et al. 2013; Raina et al. 2014).

The most important HER2 cascade downstream mediators include PI3K, PDK1, AKT, PTEN, mTOR and S6K1. Gain of function mutations in PIK3CAgene, form the most frequent oncogene in breast carcinomas, present in about 25–40% of breast tumors (Bachman et al. 2004; Campbell et al. 2001; Miller et al. 2010). Phosphorylating PDK1, PI3K initiates phosphorylation of downstream protein kinases such as AKT and PKC (Dillon et al. 2007). It has been shown that PI3K mostly results in Tamoxifen resistance occurrence through activating PDK1 but not AKT (Iorns et al. 2009; Maurer et al. 2009; Miller et al. 2011; Stemke-Hale et al. 2008).

PI3K mutations in exon 9 and 20 have shown to accompany with the worse outcomes (Barbareschi et al. 2007; Lai et al. 2008). Mutated PI3K through activating AKT leads to activation of ER in an estrogen independent manner (Campbell et al. 2001). Newly developed PI3K blocking agents, including NVP-BYL719 and NVP-BKM120, has shown to significantly enhance Tamoxifen therapeutic effects in ER positive breast cancer cells (Chu et al. 2005). Consequently, PI3K inhibitory agents can mostly restore Tamoxifen sensitivity in resistant cells (DeGraffenried et al. 2003). Nevertheless, a meta-analysis on prognostic role of PIK3CK mutations and their association with overall survival in 5719 cases of ER+ breast cancer patients didn't demonstrate any significant correlation (Beelen et al. 2014b; Lopez-Knowles et al. 2010; Pang et al. 2014).

Although Tamoxifen suppresses AKT expression in sensitive cells, phosphorylated AKT (pAKT) concentration has shown to become increased in resistant cells (Block et al. 2012; Bostner et al. 2013). AKT is activated by Guanine nucleotide phosphorylation which in turn initiates further phosphorylation of other downstream molecules (Chu et al. 2005; McCubrey et al. 2007). pAKT activation take place by different molecular interactions including those with ERα, IGF-IR, EGFR, and HER2 (Leary et al. 2010).

Mammalian Target of Rapamycin (mTOR), is also activated by pAKT which in turn, activates S6K1 or 70S6K, ending in ribosomal s6 protein phosphorylation and induction of protein synthesis (Zhang et al. 2014). mTOR can also phosphorylate Serine 167 position of estrogen receptor which in turn activates ER in an estrogen independent manner (Holz 2012). Although Rapamycin as an mTOR inhibitor prevents Tamoxifen sensitive tumor cells from proliferation, it is not effective in cells expressing AKT at high levels. However, concurrent administration of CCl-779 or Temsirolimus with Tamoxifen results in induction of apoptosis even in highly expressing AKT cells (deGraffenried et al. 2004). Everolimus, the FDA approved mTOR inhibitor, also demonstrates the same effect as Temsitolimus do. Although, Everolimus increase the amounts of pAKT, pHER3, pERK1/2 but less in the case of pS6K1, results of concurrent administration of Tamoxifen and Everolimus in xenograft models could not prove these findings (Gutteridge et al. 2010; Martin et al. 2012). In another study, addition of LY294002, a PI3K inhibitor, to the cancer cells media and xenograft models, significantly enhanced the efficacy of concurrent Tamoxifen and Everolimus administration (Chen et al. 2013; Ghayad et al. 2010). Decreasing concentrations of pAKT in the presence of LY294002 inHER2-cancer cells takes place time independently, however, in HER2 + cancer cell lines this respond happens in a time depended manner (Chen et al. 2013). In another words, initially pAKT level is decreased, but overtime pAKT is increased again. mTOR also activates 70S6K, a serine/threonine protein kinase (Yamnik et al. 2009) and through a negative feedback loop reduced pAKT concentrations (Gutteridge et al. 2010). Consistent with this theory, Kim et al. (2011) and Beelen et al. (2014b) reported that p70S6K over-expression in ER+/HER2- patients is together with better prognosis although being Tamoxifen resistant (Beelen et al. 2014a; Ghayad et al. 2010; Hong et al. 2013; Maruani et al. 2012; Zhang et al. 2014). S6K1 phosphorylates at serine position 167 of ER and activates Estrogen receptor in the absence of estrogen (Bostner et al. 2013). In addition, ER acts as a transcription factor for RPS6KB1 gene which encodes S6K1.

Phosphatase and Tensin homolog (PTEN) is also another effective PI3Kinhibitor. PTEN suppression results in over-expression of cyclin E1 and reduction of cyclin D1, p21 and p27, acceleratingcell proliferation (Lopez-Knowles et al. 2010; Raina et al. 2004). PTEN under-expression may be resulted from DNA Methyl Transferase 1 (DNMT1) and S-Adenosyl Methionine (SAM) over-expression in tumor cells, which in turn methylate the promoter region of PTEN gene and reduce PTEN expression. Administration of 5-Aza-2′-deoxycitidine a DNMT1 inhibitor, has shown to significantly reverse Tamoxifen sensitivity (Phuong et al. 2011).

Investigating the role of EGFR and HER2 in Tamoxifen resistance, Leary et al. demonstrated that administration of dual EGFR and HER2 inhibitors, such as Erlotinib and Lapatinib, in combination with Tamoxifen effectively re-sensitized Tamoxifen resistance in cells (Leary et al. 2010). Other studies have also reported similar results by combined administration of dual EGFR and HER2 inhibitors with Tamoxifen (Johnston et al. 2009; Konecny et al. 2006; Leary et al. 2010; Shou et al. 2004). In multi-central phase III clinical trial EGF30008, studying 1286 patients in two groups, one receiving Lapatinib and Letrozole concurrently and the other Letrozole alone, the group receiving combination therapy demonstrated a significantly longer disease free survival compared to the ones receiving Letrozole monotherapy (8 months and 3 months respectively Johnston et al. 2009).

17.2.2.2 EGFR and EGFR Cascade Components Over-Expression and Over-Activity in Resistant Cells

Along with over-expression of EGFR and increment in pEGFR levels, downstream genes are also over-expressed in resistant cells (Chong et al. 2011; Ignatov et al. 2010; Leung et al. 2010; Massarweh et al. 2008). Nevertheless, in some studies, it has been demonstrated that EGFR over-expression does not necessarily change during resistance occurrence (Block et al. 2012; Fan et al. 2007). Therefore, it is not surprising that expression of EGFR ligands gene including Amphiregulin (AREG), Betacellulin (BTC), Epithelial mitogen homolog (EPGN), Heparin-binding EGF-like growth factor (HBEGF), Neuregulin2 (NRG2), and Neuregulin3 (NRG3) become elevated in resistant cells compared to sensitive ones (Ghayad et al. 2010). For instance, administration of Gefitinib, a potent EGFR inhibitor, significantly decreases levels of pEGFR, p-ERK1, 2, p-MAPK and Ki67, without influencing expression of ER, PR, pAKT, HER2 and IGF1R (Gutteridge et al. 2010). Furthermore, EGFR over-expression in Tamoxifen treated patients is associated with a poor prognosis and shorter survival (Gutteridge et al. 2010; Kim et al. 2015). pEGFR mostly results in activation of ERK1 and ERK2. Few studies have also claimed that ERK1 phosphorylation demonstrates more significant effects in Tamoxifen resistance occurrence compare to pAKT either in long-term Tamoxifen therapy or in the absence of estrogen (Block et al. 2012; Leung et al. 2010; Ripple et al. 2005). pERK level is mostly adjusted by estrogen concentration, however, estrogen threshold for maximum amount of pERK is less in Tamoxifen resistant cells. Efforts for understanding the underlying mechanisms resulted in identification of Gαs which is activated in the presence of low Tamoxifen concentration (Shou et al. 2004; Wang et al. 2013).

pERK activates MAPK (Linderholm et al. 2011), another important factor in Tamoxifen resistance occurrence (Baselga et al. 2005; Ghayad et al. 2010). Just like ERK1, ERK2 and AKT, MAPK phosphorylates serine 118 position of ERα66 estrogen independently (deGraffenried et al. 2004; Ghayad et al. 2010; Qi et al. 2012) which in turn, through activating E6AP, induces more ERα66 proteasomal degradation. As a result, there exist an inverse relationship between ERα expression

levels and ERK6. On the other hand, some studies have proposed that peptidyl-prolyl isomerase (Pin1) may stabilize serine 118 position phosphorylation of ERα66 which in turn, facilitates ERα66 transcriptional activity in the presence of Tamoxifen (Mantovani et al. 2015; Rajbhandari et al. 2014; Yde et al. 2012). Therefore, it can explain how 118p-ERα increment correlates with Tamoxifen resistance occurrence. Alongside with serine 118 position phosphorylation of ERα66 as a consequence of EGFR-ERα interaction, other molecules were introduced to explain the relation between EGFR and ERα. It has been reported that EGFR over-expression results in phosphorylation of nuclear protein MED1 which in turn affects genes transcription via negotiating with ERα. EGFR induces phosphorylation of MED1 through activation of MAPK (Mansouri et al. 2017c). It has also been reported that treating cells with AG825, a HER2 inhibitor, and PD98059, a MAPK inhibitor can intensely reduce pMED1 (Beelen et al. 2014a). As discussed earlier, higher expression levels of SRC3 results in induction of Tamoxifen agonistic effect on ERα with similar mechanisms (Mc Ilroy et al. 2006; Smith et al. 1997). However, Contrarily, Brandt et al. demonstrated an inverse relation between SRC3 and ERα expression levels (Burandt et al. 2013).

Although few studies have reported that MAPK inhibition cannot restore Tamoxifen sensitivity, pMAPK rising in the resistant cells reduces ERα66 expression level (Moerkens et al. 2014). Studies on different cancer cell lines, have suggested that Gefitinib results in complete inhibition of serine 118 position phosphorylation of ERα66 induced by EGF and Heregulin, but partially in the presence of Tamoxifen and none in the presence of estrogen (Ripple et al. 2005; Shou et al. 2004). Similar result were observed in animal models too (Sachdev et al. 2010; Shou et al. 2004). In addition, Rapamycin also suppress phosphorylation activity of AKT on serine 118 position of ERα (deGraffenried et al. 2004). On the other hand, MAPK inhibits ERα66 related classic gene expression, while non-classical pathways related genes such as cyclinD1 is over expressed through the c-Jun and AP1 over-activity in resistant cells (Qi et al. 2012). MED1 phosphorylation by MAPK takes place in switching genes expression from classical to non-classical genes. Treating cells with AG825, a HER2 inhibitor, and PD98059, a MAPK inhibitor, significantly reduce pMED1 (Ignatov et al. 2010).

Figure 17.2 summarizes the RTKs cascades over-activity in Tamoxifen resistance.

17.2.2.3 SRC3 Over-Expression and Over-Activity in Resistant Cells

Steroid Receptor Co-activators (SRCs), including SRC1, SRC2, and SRC3 belong to p160 nuclear receptors super-family, located in plasma membrane, cytoplasm and nucleus (Karmakar et al. 2011; Phuong et al. 2011). MAPK is responsible inactivating the most famous member of this family, SRC3 or AIB1. AIB1 over-expression has shown to result in ER activation in an estrogen independent manner (Karmakar et al. 2011) in recent studies, role of AIB-1 in expression of HER2 related genes has been proved (Nikolai et al. 2016). Also, it has been

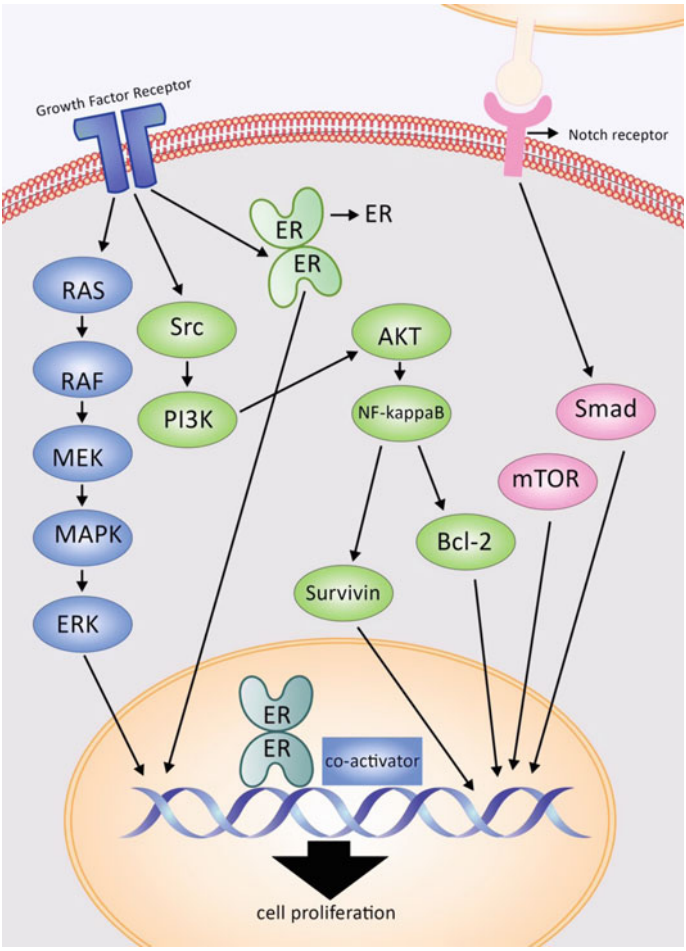

Fig. 17.2 Growth factor over-activity and cell over-proliferation in cells with Resistance. Three main proliferation stimulating pathways have been shown above: (i) Ras cascade (ii) PI3K/AKT pathway (iii) Notch pathway. Altogether, they induce cell proliferation even in the presence of Tamoxifen. Ras cascade is a quick transduction pathway consisting of proteins such as RAF, MEK, MAPK and ERK that become activated successively after Ras. PI3K/AKT pathway is a complex and multi-branching signaling pathway but all of the branches are not focused in the figure above. It activates mTOR complex resulting into cell proliferation. AKT also initiate NF-kappa B (NF-κB) pathway to induce anti-apoptotic agents and survivin. And the last pathway displayed above is Notch pathway which is usually present in highly proliferative tumor cells and it induces the expression of genes related to cell stemness

demonstrated that HER2 and AIB1 simultaneous over-expression, increases ERα66 phosphorylation, subsequent activation of ERα66 in an estrogen independent manner and association with a poor prognosis (Beelen et al. 2014a; Fu et al. 2013; Mc Ilroy et al. 2006; Osborne et al. 2003; Shou et al. 2004; Smith et al. 1997). It

appears that Tamoxifen demonstrate its agonistic activity on ERα through the same way (Smith et al. 1997). For instance, Tamoxifen agonistic effects on endometrial carcinoma have shown to be explained similar to those observed with AIB1 activation (Beelen et al. 2014a). Also, SRC3 Depletion has shown to end in cell sub-population arrest in G0/G1 phase and induces apoptosis (Leung et al. 2010).

17.2.2.4 ERα66 Activation Estrogen Independently in Resistant Cells

It has been shown that SRC and ERα66 interactions may also result in counter-activation. EGFR and HER2 phosphorylate SRC through activating ERα66in the presence of both estrogen and Tamoxifen (Mc Ilroy et al. 2006). In addition, ERα66 is activated in an estrogen independent manner by SRC3 which in turn, brought about Tamoxifen resistance (Shou et al. 2004; Smith et al. 1997).

Anterior gradient-2 (ARG2) is a pro-metastatic protein downstream of Estrogen Response Element (ERE) over-expressed in the presence of Tamoxifen. ERE expression itself, is induced by ERα66 (Hengel et al. 2011; Hrstka et al. 2010). Since there is a correlation between pAKT and higher expression of ARG2, administration of AKT inhibitors may reduce ARG2 expression levels. However, surprisingly, AKT inhibitors have failed to suppress ARG2 expression in the presence of Tamoxifen compared with AKT inhibitors monotherapy (Hrstka et al. 2013). Therefore, it is concluded that Tamoxifen has agonistic effect on ARG2 expression by activation AKT. The Fig. 17.3 shows the interactions between estrogen dependent and independent pathways in Tamoxifen resistance network. It also reveals that ERα36 activates RTKs.

17.2.2.5 ERα36 Activation in Resistant Cells

ERα36 functions in a non-genomic manner and interacts with cytoplasmic growth cascade components and binds to DNA as an ERα66 co-regulator and causes estrogen hypersensitivity (Garcia-Becerra et al. 2012; Yin et al. 2015; Zhang et al. 2014). ERα36 was first discovered in a study in which pAKT increment took place in the presence of Tamoxifen (Clark et al. 2002). Administration of siERα36 has shown to significantly suppress pAKT expression levels, mostly due to the inhibition of ERα36 (Yin et al. 2014). Although it has been revealed that ERα36 and ERα66 are inversely regulated, ERα36 can pass in the nucleus and binds with DNA, exactly to the same position of ERα66 (Wang et al. 2005, 2006). In fact, ERα36 does not have any transcriptional activity, however by competing with ERα66 to attach with its associated binding site on DNA, demonstrates its effects (Wang et al. 2006). Furthermore, ERα36 dimerizes with ERα66 which prevents ERα66 to enter the nucleus and accumulates ERα66 in the cytoplasm (Wang and Yin 2015). ERα66 in turn inhibits the transcription of ERα36 by blocking its promoter (Wang and Yin 2015).

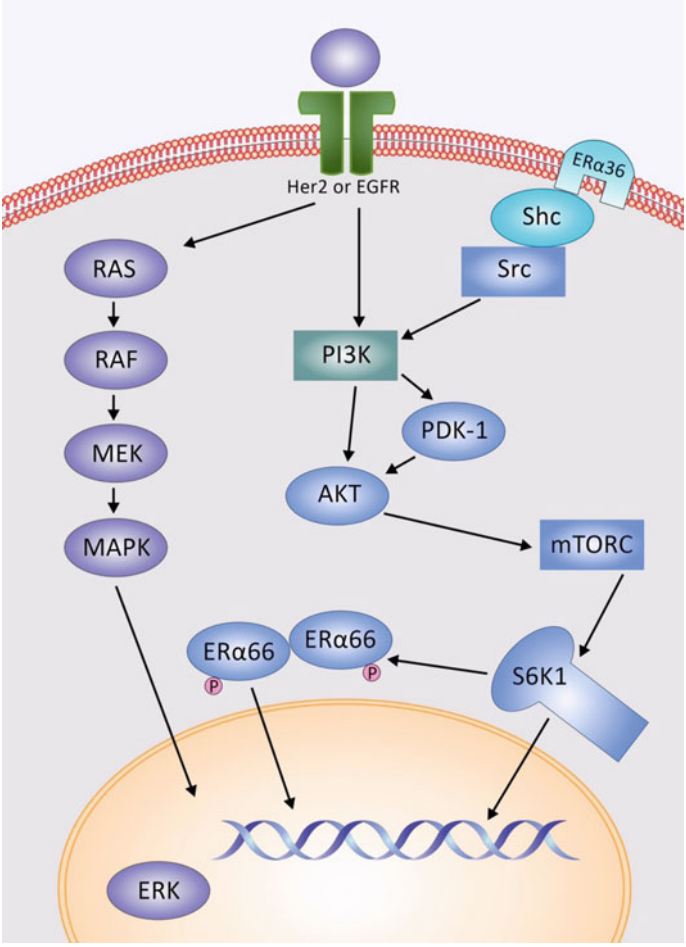

Fig. 17.3 Interactions between ERα36 and Her2/EGFR pathway. PI3K/AKT is activated simultaneously by ERα36 and growth factor receptors. It has different consequences mentioned before. This figure displays that activation of mTOR complex and S6K1 protein lead to ER-α66 phosphorylation on serine167 residue. This phosphorylation activates Estrogen receptor and makes it able to enter to the nucleus and induce proliferation without estrogen. As a result, ER-α36 and Her2/EGFR pathways can turn the cell hormone independent for proliferation which exacerbates the resistance

17.2.3 Resistance to Letrozole

Aromatase has long been focused as target for breast cancer hormone therapy in post-menopausal patients due to its crucial role in estrogen biosynthesis (Lonning 2004). Aminogluthetide were the First generation of Aromatase inhibitors which were developed (Santen et al. 1978). Nevertheless, this molecule lacked the

required selectivity to Aromatase as an ideal Aromatase inhibitor and could inhibit the biosynthesis of cortisol, aldosterone and thyroid hormone and induce hepatic enzymes activity too (Hausler et al. 1989; Murray et al. 1993; Pittman and Brown 1966). Therefore, the second generation of AIs was developed consisting of two different subtypes, the steroidal and non-steroidal Aromatase inhibitors. Fadrozole was the prototype of non-steroidal type second generation AIs. Although It was more potent and selective compared to Aminogluthetide, still it was not enough selective for this enzyme (Santen et al. 1991). However, this was Letrozole, the third generation of AIs which alongside with its high potency could selectively enough inhibit Aromatase and demonstrate specific anti-estrogenic effects. Based on result of many clinical trials evaluating and comparing this drugs potency and selectivity with other Aromatase inhibitors, Letrozole has now entered the marketing and became the most potent and effective anti-hormonal therapy for post-menopausal women with early or advanced ER+ breast cancers (Bhatnagar et al. 1990). As it was mentioned earlier, in post-menopausal women, Aromatase enzyme activity in the adipose tissue is the main source of estrogen production. As, Letrozole specifically inhibit this enzyme, results in suppressing estrogen production. Therefore, estrogen as the initiator of signaling cascade does not attach to its receptor and cell proliferation is notably reduced.

In normal breast tissue of a post-menopausal woman, Aromatase produces estrogen, which in turn enters to the cell and binds with its associated receptor, Estrogen receptor will be duplicated and acts as a transcription factor to induce the genes related to cell proliferation.

Similar to other medications, resistance to Letrozole develops after administration. Development of resistance to Letrozole can be divided into intrinsic and acquired categories. Albeit, in some cases it is not possible to state a border between these two. But this type of classification, makes us cable of focusing on different mechanisms involved in resistance development. Studying different Letrozole resistant cells have demonstrated that somatic cell mutations, ESR1 gene alteration, alternative signaling pathway, resistance to cell death, induction of cells with stem cell characteristics and cell cycle dysregulation are the most important factors involved in development of resistance.

17.2.3.1 ESR1 Acquired Alteration

One of the changes which have been studied in many AI resistant cases was estrogen receptor alterations. These are acquired changes assisting tumor cell to overcome the inhibitory effects of AIs such as Letrozole. This mutation is mainly known as an “acquired” mutation. Because it is totally absent at the initiation but it is present in about 11 to 55% of metastatic advanced tumor cells (Li et al. 2013; Merenbakh-Lamin et al. 2013; Robinson et al. 2013; Toy et al. 2013; Zhang et al. 1997). The most common mutations are on ligand binding domain of this receptor

and lead to ligand-independent activation of receptor (Li et al. 2013; Robinson et al. 2013; Toy et al. 2013; Zhang et al. 1997). So AIs and SERMs which are effective on estrogen will be ineffective. One of the suggested theories to overcome this kind of resistance is to escalate the dose of anti-estrogen medications such as AIs, Tamoxifen or Fulvestrant (Ma et al. 2015).

17.2.3.2 Alternative Pathway Changes

One of the most interesting chapters in AI resistance is Alternative pathways. Alternative pathways are the proliferation pathways which are activated mostly by Growth Factor Receptors (GFRs). These receptors can activate downstream molecules such as PI3K, AKT and mTOR and reduce the expression of ER (Miller et al. 2010; Sanchez et al. 2011).

In a normal breast tissue, the most important pathway for growth and proliferation is managed by Estrogen Receptors. But in these cells, Growth Factor Receptors (GFRs) play the most effective role in cell proliferation. The most known growth factor receptors are: EGFR (Epithelial Growth Factor Receptor) (Fan et al. 2007), FGFR (Fibroblast Growth Factor Receptor) (Turner et al. 2010), IGFR (Insulin-like Growth Factor Receptor) (Stephen et al. 2001) and HER2 (also known as ERBB2) (Creighton et al. 2006; Lopez-Tarruella and Schiff 2007; Oh et al. 2001). In addition to all of these, GFRs can activate kinases on their downstream which are able to phosphorylate ER on Serine 167 and Serine 118 residue. These are important sites for activation of Estrogen Receptor without attachment to Estrogen; this pathway is known as "non-genomic way of ER activation" (Font de Mora and Brown 2000; Osborne et al. 2003). Therefore, dominancy of these receptors in tumor cells is associated with resistance to AIs.

17.3 Adverse Effects of Hormone Therapy

17.3.1 Hormone Therapy Induced Menopause Symptoms

Endocrine therapy has shown to induce menopausal symptoms including vaginal dryness, hot flashes, night sweats, urinary incontinence, weight gain, and psychological distress (Duijts et al. 2012). Furthermore, Hormone replacement therapy is contraindicated in breast cancer (Duijts et al. 2012; Mann et al. 2012) and around half of patients receiving AIs experience musculoskeletal symptoms which can be suppressed by yoga (Bakoyiannis et al. 2016).

17.3.2 Tamoxifen Induced Central Nervous System Impairment

Preclinical studies have proposed that estradiol and Tamoxifen demonstrate neuroprotective effect too. For instance, Tamoxifen demonstrated agonistic effects in ovariectomized animals on CNS function, contrary to the observations in non-ovariectomized ones (Buwalda and Schagen 2013). Aromatase inhibitors have demonstrated similar effects in preclinical studies too. Furthermore, in a dose dependent manner, AIs can improve the spatial learning and memory (Bakoyiannis et al. 2016; Henneghan 2016). Nevertheless, it has been shown that AIs may reduce axonal plasticity in hippocampus (Buwalda and Schagen 2013). Clinical studies focusing on identifying AIs probable CNS impairment inducing effects have not come up with enough evidence to confirm the CNS impairment inducing effects of AIs (Phillips et al. 2011). Cognitive dysfunction has been reported in breast cancer survivors receiving systemic therapies inducing brain malfunction (Ahles et al. 2012; Buwalda and Schagen 2013; Falleti et al. 2005). A systematic review on breast cancer patients, demonstrated that patients receiving endocrine therapy demonstrate more cognitive dysfunction compared to the ones receiving other chemotherapy regimens and control group (Bakoyiannis et al. 2016). Studies have demonstrated that Tamoxifen consumption, negatively affects both risk and ambiguity of decision-making function (Bechara 2004; Brand et al. 2006). Further investigations demonstrated that breast cancer patients receiving Tamoxifen demonstrate some degrees of impaired memory, information processing, verbal memory and executive functions. These were reported to result from amygdale, hippocampus and basal ganglia structure destruction by Tamoxifen (Chen et al. 2014). Also, it has been shown that the negative impact of Tamoxifen on cognition is prominent in patients older than 65 years (Espeland et al. 2004). Ahles et al. demonstrated that these negative impacts on patients without history of receiving chemotherapy prior to Tamoxifen, were worse than controls in processing speed, verbal memory and verbal ability (Ahles et al. 2010). This study distinguished the pure effect of hormone therapy on CNS impairment by omitting the impact of chemotherapy. However, there are little studies on hormone therapy induced CNS malfunction with excluding the effect of chemotherapy on CNS (Phillips et al. 2011). CNS impairments which are induced by Tamoxifen are listed in Table 17.1.

Table 17.1 CNS impairments which are induced by Tamoxifen

Adverse effects	References
Verbal memory	Jenkins et al. (2004), Schilder et al. (2009), Shilling et al. (2003)
Executive function	Chen et al. (2014), Schilder et al. (2010)
Visuospatial ability and processing speed	Palmer et al. (2008), Shilling et al. (2003)
Learning deficit (animal model)	Walker et al. (2011)
Cognitive dysfunction	Buwalda and Schagen (2013), Phillips et al. (2011)

17.3.3 Biological, Psychological and Behavioral Factors Affected Cognition

Currently no study exists on association between modifiable factors and induction of cognition impairment in breast cancer survivors. Nevertheless, a mixed meta-analysis on finding the relation between modifiable biological, behavioral, psychological and cognition impairment demonstrated that there exists some correlations between cortisol, neural metabolites, hemoglobin, loneliness, avoidance/hyper-arousal and extend of cognitive deficit (Henneghan 2016).

17.4 Management the Menopause Symptoms by Psychotherapy

Cognitive behavioral therapies (CBT) mostly consisted of relaxation and physical exercise (PE) for 2.5–3 h per week could significantly reduce both primary and secondary symptoms of menopause. Also, it was shown that combination of CBT and PE improved sexual and physical function (Duijts et al. 2012). Another randomized clinical trial on relieving hot flushes and night sweat in breast cancer survivors, demonstrated that CBT could significantly reduce the menopausal symptoms. During this study, paced breathing, psycho-education, and cognitive and behavioral strategies were employed to control the symptoms (Mann et al. 2012).

17.5 Conclusion

It would be concluded that whereas endocrine therapy is administered commonly in the breast cancer patients, there are several problems which will be confronted. As the main focus of this chapter was on the explanation of molecular pathways which are involved in the resistance to the endocrine therapy, the molecular network will be summarized here. There are many preclinical studies to figure out the underlying molecular mechanisms take place in the development of resistance. Briefly, it seems that over-activation of alternative growth cascades can compensate the estrogen dependent pathways which is inhibited by medications. RTKs related cascades, ERα66 alterations, and ERα36 over-expression are the main mechanisms of initiating resistance (Teymourzadeh et al. 2017). A comprehensive studying on the molecular network of resistance will be warranted.

17.6 Summery

Estrogen induces breast cancer cell proliferation through initiating transcription of ERα target genes. In addition, the majority of breast cancer risk factors may increase the level of plasma estrogen. Endocrine therapy was administered to prevent tumor cell growth, since the role of estrogen was introduced in the development of breast cancer cells. Tamoxifen is administered in both pre and post menopause patients while Letrozole is commonly used in the post menopause ones. However, there are some challenges in endocrine therapy. Although adherence to the treatment is crucial, around half of patients leave the therapy and they will face worse prognosis. Many social and clinical factors have been suggested to influence on the adherence to therapy. It would be conclusive that modifying these factors can improve patients' adherence to treatment. Another challenging issue in the field of endocrine therapy is the drug resistance. Based on the literature, one of the prominent determinants of Tamoxifen resistance is over-activation of cytoplasmic cascades which can rescue the cell proliferation in the presence of ERα ablation. The most studied cytoplasmic growth cascades is RTKs which includes HER2/PI3K/PDK1/AKT/mTOR/S6K1 and EGFR/MAPK/ERK1, 2; they have many molecular interactions with ERα which lead to phosphorylation of ERα at Ser167 and as consequence ERα is activated in an estrogen independent manner. Hence, it would be conclusive that not only RTKs activation outweighs in the resistant cells, but also they can induce ERα to transcript the target genes. Recently, a new variant of ERα which is named ERα36 was introduced to be activated by Tamoxifen and interact with many growth cascades components such as AKT which should be investigate more in the future studies. In addition, resistance to Letrozole was focused in this chapter. Letrozole belongs to Aromatase inhibitors which are used widely in the post menopause patients. ESR1 acquired mutations and EGFR/HER2 dependent growth pathways over-activation are the most studied role players in the Letrozole resistance development. Another challenging issue which is faced in the endocrine therapy is adverse effects which will be managed by psychotherapy. Inducing menopause symptoms and central nervous system impairment are the discussed adverse effects of endocrine therapy; cognitive behavioral therapies were administered to alleviate both primary and secondary symptoms of menopause. However, there are few evidences on the magnitude of psychotherapy in the management of adverse effects of cancer therapies. Furthermore, there is a great need to investigate the influence of psychotherapy to increment the efficiency of cancer therapies.

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Chapter 18
Skin Cancer: Genetics, Immunology, Treatments, and Psychological Care

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Abstract Non-melanoma skin cancer (NMSC), consisting of basal cell carcinoma and squamous cell carcinoma, is considered the most common form of malignancy in humans. Melanoma is another major skin cancer, accounting for a small percentage of skin cancers, but is the deadliest form of skin cancer. Several players contribute in the pathogenesis of skin cancers, including genetics and epigenetics, various types of immune cells, and environmental risk factors. During recent years, some novel genes, numerous new therapeutic options, and also some new approved drugs associated with skin cancer have been introduced in the literature. Moreover, psychological care, which might be underestimated and remains unmet in case of many patients, is being emphasized of late. Considering the fact that skin cancer is a multifactorial condition that could be developed or influenced by genetic alterations, immune system alterations, and also environmental and lifestyle changes, it needs to be discussed from different points of view. In this chapter, all the mentioned factors were discussed both in case of NMSC and melanoma. After giving a clinical insight, risk factors, clinical manifestations, and the pathology of these conditions, recognized mutated genes as well as rare syndromes associated with NMSCs and melanoma have been discussed. Additionally, epigenetic factors (methylation, histone modifications, and microRNAs) have also been introduced. Since immune responses are the determinants of outcome, the role of the most studied immune cells in skin cancer patients such as T cells, natural killer cells, dendritic cells, macrophages, and mast cells have been discussed in detail. There is also some limited evidence of the contribution of autoimmune diseases and some

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viruses in the pathogenesis of skin cancer. Having an overview of the signaling pathways involved in skin cancer development and several clinical trials has led to the approval of some drugs belonging to various treatment strategies, including targeted therapy, immunotherapy, and the oncolytic viruses. These treatments seem to be potent enough to replace traditional non-surgical treatment options for skin cancer. Since patients with cancer not only need physical improvement but also psychological Interventions, the last section of this chapter covers the psychological issues in patients with skin cancer. Psychological outcomes, psychoneuroimmunology, and supportive cares for those who suffer from psychological problems are covered in this chapter.

Keywords Non-melanoma skin cancer · Basal cell carcinoma · Squamous cell carcinoma · Melanoma · Psychology · Target therapy · Immunotherapy

Abbreviations

5-FU	5-fluorouracil
AA	Alopecia areata
ACT	Adoptive cell transfer
AK	Actinic keratosis
ALA	Δ-5-aminolevulinic acid
ALM	Acral lentiginous melanoma
APCs	Antigen-presenting cells
BCC	Basal cell carcinoma
BCNS	Basal cell nevus syndrome
CM	Cutaneous melanoma
CMV	Cytomegalovirus
COX	Cyclooxygenase
CTLA-4	T-lymphocyte-associated protein 4
DCs	Dendritic cells
DDEB	Dominant dystrophic epidermolysis bullosa
DEB	Dystrophic epidermolysis bullosa
EB	Epidermolysis bullosa
EBV	Epstein–Barr virus
EGFR	Epidermal growth factor receptor
EV	Epidermodysplasia verruciformis
FAMMM	Familial atypical multiple mole-melanoma
FAMMM-PC	Familial atypical multiple mole-melanoma-Pancreatic Cancer
FDA	U.S. Food and Drug Administration
FSD	Ferguson-Smith disease
GG-NER	Global genome nucleotide excision repair
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HBV	Hepatitis B virus
Hh	Hedgehog

HHV8	Human Herpesvirus 8
HLA	Human leukocyte antigen
HPA	Hypothalamic–pituitary–adrenal axis
HPV	Human papillomavirus
HSV	Herpes simplex virus
HWs	Hispanic whites
IDO	Indoleamine 2,3-dioxygenase
IFN-γ	Interferon-gamma
IL	Interleukin
iNKT cells	Natural killer T cells
JEB	Junctional epidermolysis bullosa
KIR	Killer cell immunoglobulin-like receptor
KS	Kaposi Sarcoma
LAG-3	Lymphocyte-activation gene 3
LMM	Lentigo maligna melanoma
MAL	Methyl ester
MAS	Melanoma-Astrocytoma syndrome
MBAITs	BAP1-mutated intradermal tumors
MCC	Merkel Cell Carcinoma
MCs	Mast cells
MIS	Melanoma in situ
miRNA	MicroRNA
MM	Malignant melanoma
MSSE	Multiple self-healing squamous epithelioma
NBCCs	Nevoid basal cell carcinoma syndrome
NF-κB	Nuclear factor-kappa-B
NGF	Nerve growth factor
NHWs	Non-Hispanic whites
NK cell	Natural killer cell
NKT cell	Natural killer T cell
NMSCs	Non-melanoma skin cancers
non-HS RDEB	Autosomal recessive dystrophic epidermolysis bullosa, Non-Hallopeau-Siemens type
NSAIDs	Non-steroidal anti-inflammatory drugs
OCA	Oculocutaneous albinism
OTRs	Organ transplant recipients
PD-1	Programmed death 1
PDT	Photodynamic therapy
PFS	Progression-free survival
PGE2	Prostaglandin E2
PUVA	Psoralen and ultraviolet A radiation
RA	Rheumatoid arthritis
RDEB-HS	Autosomal recessive dystrophic epidermolysis bullosa, Hallopeau-Siemens type

RT	Radiation therapy
SA	Sympathetic axis
SCC	Squamous cell carcinoma
SLE	Systemic lupus erythematosus
SLNB	Sentinel lymph node biopsy
SNPs	Single-nucleotide polymorphisms
SP	Sub-stance P
SSM	Superficial spreading melanoma
T4N5	T4 endonuclease V
TAMs	Tumor-associated macrophages
Tc	Cytotoxic T-cell
TGF-β	Transforming growth factor-beta
Th	T helper
TILs	Tumor-infiltrating lymphocytes
TIM-3	T-cell immunoglobulin and mucin-domain containing-3
TNF-α	Tumor necrosis factor-alpha
T-VEC	Talimogene laherparepvec
UV	Ultraviolet
UVR	Ultraviolet radiation
VDR	Vitamin D receptor
VEGF	Vascular endothelial growth factor
XP	Xeroderma pigmentosum

18.1 Introduction

The most common types of skin cancers are basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and cutaneous melanoma (CM). BCC and SCC are referred to as non-melanoma skin cancers (NMSCs), the most common form of malignancy in humans. It is estimated that, in the U.S., the total number of those with NMSCs and those treated for NMSCs are more than three and two million, respectively (Rogers et al. 2010). BCC is the most common skin cancer and accounts for more than 70% of the NMSCs. Considering the uncommon metastasis rate of BCC, mortality is low. However, this malignancy causes considerable morbidity. Moreover, those diagnosed with BCC are at a high risk of developing further BCC and other malignancies. After BCC, SCC is the most frequently diagnosed form of NMSC, representing around 20% of all NMSCs, while its overall mortality rate is higher compared to the BCC. CM, also known as melanoma is less common than NMSCs. It is the most dangerous form of skin cancer and frequently metastasizes, which could potentially be fatal. It develops from the melanocytes, the pigment-containing cells, and more than 95% of melanomas arise from the skin. Although it is the least common among the three main types of skin cancers, it

causes the most number of deaths. If melanoma is diagnosed and treated at its early stages of development, it is almost always curable. However, if it has enough time to advance and develop and, subsequently, spread to other areas of the body, it becomes hard to treat and can be fatal. In addition to exposure to ultraviolet (UV) light, the most identified risk factor for skin cancer, genetics and epigenetics also play their roles in causing susceptibility to melanoma development. Approximately 5–10% of all melanomas have a genetic background and may be inherited in an autosomal dominant fashion. In addition to these three major types of skin cancers, there are some other, less common, such as merkel cell carcinoma, sebaceous gland carcinoma, etc., which will not be discussed in this chapter.

There are several environmental risk factors for both NMSCs and melanoma, including UV light, family history, immunosuppressive therapies, ionizing radiation, certain chemical carcinogens, and multiple other factors. However, there remains a minority of cases where skin cancer occurs in the setting of hereditary cancer syndromes, highlighting the critical role of genes. The prevention of skin cancer among those with a familial background or exposed to environmental risks, and the sealing of the chances of a new tumor in individuals with previous skin cancer history, is not possible without a clear insight into the risk factors associated with skin cancer development. Different conventional treatments for skin cancer are available, including surgery, radiation therapy, and chemotherapy, but they are not the best approaches to treat all skin cancer patients. As the role of genes is becoming more clear, especially in genetic syndromes, these genes can be used for screening the high-risk populations. On the other hand, the genes can be used to develop novel treatment modalities. Additionally, the immune system plays a critical role in the suppression of skin cancer. Although immune responses are not directly related to the development of skin cancer, an immune system failure could impair immune surveillance and, finally, cause skin cancer. Hence, the recognition of the role of immune cells in fighting skin cancer has led to the development of new types of treatments called immunotherapy. Moreover, immune signatures could be used to predict the prognosis and even response to treatment in patients with different types of cancer, such as melanoma (Daud et al. 2016; Hsu et al. 2010). Psychological care is also important to improve the quality of life of patients diagnosed with any type of skin cancer. In addition to having a positive impact on the quality of life, a reduction of psychological stress seems to be beneficial to reversing impaired immunity during cancer (Repasky et al. 2015). Indeed, this approach does not directly lead to tumor shrinkage but tends to promote a better outcome.

In this chapter, an attempt has been made to cover different aspects of involved factors in three major types of skin cancer, such as risk factors, genes, syndromes, genetics, epigenetics, and immunology. Moreover, the prevention of skin cancers and the majority of available treatments, including the traditional ones and the novel, emerging treatments have been discussed. Owing to the importance of psychological care for patients with skin cancers, a separate section has been assigned for a discussion on the psychological outcome and care in patients with skin cancers.

18.2 Skin Structure and Functions

18.2.1 Skin Structure

Skin is composed of several layers and components, including the epidermis, dermis, subcutis layer, and skin adnexa (Fig. 18.1). Epidermis is the outer layer of the skin. The main cellular population of this layer is keratinocytes. The lower portion of keratinocytes forms the basal layer of epidermis, having the ability to divide. As basal keratinocytes migrate toward the skin surface, they make spinous cell layer, the granular cell layer, and the keratinized outer layer, or stratum corneum (Vandergriff and Bergstresser 2012). Additionally, melanocytes are distributed singly along the basement membrane in the epidermis. Melanocytes are derived from neural crest cells and migrate to the epidermal compartment towards the eighth week of the gestational age. Langerhans cells, or dendritic cells (DCs), are other cell types in the epidermis, whose primary function is antigen presentation. These cells reside in the skin for an extended period and respond to different stimuli such as UV radiation (UVR) or topical steroids, which cause them to migrate out of the skin (Koster et al. 2012).

The dermis is largely composed of an extracellular matrix. Prominent cell types in this compartment are fibroblasts, endothelial cells, and transient immune system cells. When transformed, fibroblasts form fibrous soft tissue tumors, and endothelial cells form vascular tumors. There are a number of immune cell types that move in

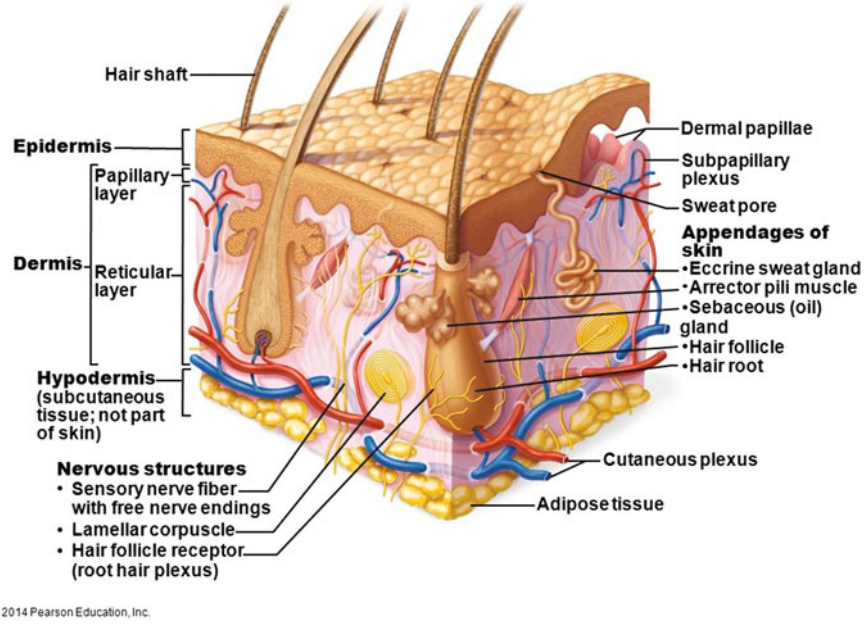

Fig. 18.1 Basic structure of normal skin

and out of the skin to blood vessels and lymphatics; these include mast cells (MCs), lymphocytes, mononuclear cells, histiocytes, and granulocytes. These cells can increase in number during inflammatory diseases and can also form tumors within the skin. Mastocytosis and cutaneous T-cell lymphoma are some examples. Another important component of the skin is skin adnexa such as hair follicles, sweat glands, and the sebaceous glands associated with the hair follicles. These can form a large variety of benign or malignant tumors with diverse biological behaviors called "adnexal tumors". Several of these tumors are associated with familial syndromes (Kaddu and Kohle 2012). Finally, the subcutis fat is a layer that extends below the dermis with varying depths, depending on the anatomic location. This deeper layer includes muscle, fascia, bone, and cartilage. The subcutis can be affected by inflammatory conditions such as panniculitis and malignancies such as liposarcoma (Kaddu and Kohle 2012). Internal malignancies also commonly metastasize to the skin. The dermis and subcutis are the most common locations, but the epidermis can also be involved in conditions such as pagetoid breast cancer. A schematic representation of the normal skin has been shown in Fig. 18.1.

18.2.2 Function of the Skin

The skin has a wide variety of functions. Perhaps the most important function is its role as an active barrier. On the one hand, skin prevents extensive water and temperature loss and, on the other, prevents body penetration by microorganisms, toxins, and UVR. Furthermore, skin participates in body temperature regulation via sweating, fluid balance, and peripheral circulation. Another unique role of the skin is its ability to repair itself after different physical injuries. The skin is an important communication organ with internal and external environments. This communication is mediated with the help of nerve fibers, cytokines, and hormones. Hence, skin is a basic organ of the body, which can detect the different sensations of heat, cold, pressure, contact and pain. Sensation is felt through the nerve endings in the dermis, which are easily affected by wounds. The skin's sensation can protect us from first and second degree burns, but in cases of deeper, third degree burns, it is less effective, as we do not feel any pain, as nerve endings are destroyed by heat.

The skin has also multiple endocrine functions. The skin, probably, is one of our main sources of vitamin D, and its role in different autoimmune diseases and tumors is being increasingly specified. The skin is a big source of immunological cells of both the adaptive and innate immune system, having a dynamic relation with lymphoid tissues. The most prominent role of the skin immune system is fighting infection and cancer. Thus, immunosuppression, which occurs during organ transplant, HIV infection, and similar afflictions, are significant risk factors for skin cancer. Finally, skin is one of the most notable components in beauty, attraction, and interpersonal communication. This role is so important that, nowadays, cosmetic dermatology has become one of the most important branches of the dermatological sciences.

18.3 Skin Cancer

18.3.1 Basal Cell Carcinoma

18.3.1.1 Introduction

Basal cell carcinoma (BCC) is the most common malignancy in people of European descent, with an associated lifetime risk of 30%. BCC represents approximately 74% of NMSC, while SCC is less common at 23%. The incidence of BCC has also been noted to increase in European countries. The incidence rate of BCC is the highest in Australia (>1000/100 000 person-years) and lowest in some parts of Africa (<1/100,000 person-years). It is more common among the elderly males: more than 80% of the cases occur in people older than 60 years (Lomas et al. 2012). In contrast to CM, metastatic spread of BCC is very rare. With early detection, the prognosis for BCC is excellent. The true cytologic origin of BCC is not exactly known. Considering many histologic similarities between BCC and basal cell keratinocytes and the outer root sheath cells of the hair follicle, these cells have been proposed as the cell of origin for BCC (Schirren et al. 1997).

18.3.1.2 Risk Factors

Up to now, numerous risk factors associated with an increased risk of BCC have been identified; some of the most important factors and their brief descriptions are given below.

Skin Type

The most important factor related to the development of these neoplasms appears to be skin phenotype. One study showed that individuals with Fitzpatrick Type I or II skin were shown to have a 2–3 fold greater risk of BCC (Gon and Minelli 2011). Blond or red hair color was associated with increased BCC risk in two large cohorts (Wu et al. 2013).

Family History

Apart from defined genetic disorders with an increased risk of BCC, a positive family history of any skin cancer (BCC, SCC, and CM) is a strong predictor of the development of BCC (Berlin et al. 2015). A study of 376 early-onset BCC cases and 383 controls found that a family history of any type of skin cancer increased the risk of early-onset BCC (odds ratio [OR], 2.49; 95% CI, 1.80–3.45) (Berlin et al. 2015).

Sun Exposure

Sun exposure is the most important known environmental factor associated with the development of skin cancer of all types. Genetic mutation induced by Ultraviolet A and B (UVA and UVB) radiation is the main mechanism of photocarcinogenesis. For BCCs, intermittent and intense episodes of UV exposure and sunburns at any age appear to increase the risk, whereas a cumulative long-term UV exposure and cumulative effect of UV exposure increase the risk for developing SCCs and actinic keratosis (AK) (Almahroos and Kurban 2004; Zanetti et al. 2006).

Immunosuppression

Organ transplant recipients (OTRs) have a markedly increased incidence of NMSCs, primarily SCC. The incidence of BCC in OTRs is up to five to ten times greater than in the general population, while the incidence of SCC is 40–250 times greater (Berg and Otley 2002; Comeau et al. 2008). The use of immunosuppressant drugs other than for organ transplantation also increases the risk for the development of NMSCs (SCC more than BCC). In one study, the risk of SCC was found to significantly increase among recipients of oral glucocorticoids (odds ratio = 2.31) and the risk of BCC was also elevated (odds ratio = 1.49) (Karagas et al. 2001).

Arsenic

Chronic exposure to arsenic is a well-known risk factor contributing to the development of multiple SCCs and BCCs (Wong et al. 1998; Simeonova and Luster 2000). It is characterized by mottled hyper and hypopigmentation, palmoplantar pitting, and multiple NMSCs, particularly Bowen's disease. The NMSCs may be mostly on sun-protected areas of the body with a latent period of 30–50 years (Schwartz 1997).

Ionizing Radiation

Exposure to ionizing fractionated doses (>12–15 Gy) leads to a three-fold increased risk of NMSC. Most tumors appear more than 20 years after the initial exposure (Stante et al. 2002). A historical source of scalp radiation was the use of X-ray for the treatment of tinea capitis many years ago, which eventually led to the development of multiple BCC and SCC several years after the exposure (with a relative risk of 3.6% in a study for BCC) (Shore et al. 2002).

Previous Personal History of Non-Melanoma Skin Cancer

A personal history of BCC or SCC is strongly associated with a subsequent BCC or SCC. There is an approximate 20% increased risk of a subsequent lesion within the first year after a skin cancer has been diagnosed. The mean age of occurrence for these NMSCs is the mid-60s (Karagas et al. 1992; Robinson 1987; Bergstresser and Halprin 1975; Moller et al. 1975; Epstein 1973). Individuals who have had a BCC or SCC are at an increased risk of developing additional BCCs and SCCs (Marcil and Stern 2000) and CM (Marghoob et al. 1995). Additionally, several studies have found that individuals with a history of skin cancer have an increased risk of a subsequent diagnosis of a non-cutaneous cancer (Frisch et al. 1996; Wheless et al. 2010; Efird et al. 2002). Thus, a regular follow-up is recommended for the general population for sites other than the skin.

18.3.1.3 Clinical Manifestation

Clinically, more than 20 subtypes of BCC have been described, but the most important subtypes are nodular, superficial, morpheaform, and fibroepithelial. A shiny pearly papule or nodule with an elevated rolled border and arborizing telangiectasias is the presenting lesion in many subtypes of BCC. With time, the lesion enlarges very slowly and may ulcerate. Each subtype may have specific additional features, which are beyond the purpose of this chapter (Soyer et al. 2012a) (Fig. 18.2).

Nodular BCC is the most common BCC subtype. It appears as a translucent waxy papule or nodule with telangiectasia. Superficial BCC is the least invasive subtype of BCC, which appears as a well-circumscribed erythematous and scaly patch or plaque with pinpointed hemorrhage mostly on the trunk and back. It is considered a differential diagnosis of dermatitis. Morpheaform BCC is an uncommon subtype of BCC, which presents itself as a sclerotic flat or depressed waxy lesion. Since there is no ulceration and crusting, it can be very similar to a scar and its diagnosis may be challenging. Fibroepithelial BCC is another rare subtype of BCC, which usually presents as a skin-colored or pink, sessile plaque or pedunculated papulonodule. Pigmented BCC is an uncommon variant of nodular BCC, which has an irregular and incomplete pigmentation. Clinical characteristics of BCC, like pearl and arborizing telangiectasia, can be seen in the non-pigmented parts of the tumor, thus helping to differentiate pigmented BCC from its most important differential diagnosis, CM (Betti et al. 2009; Griffin et al. 2007).

18.3.1.4 Pathology

The main determining features in the pathology of different subtypes of BBC are nests of basaloid cells within a variably fibromyxoid stroma (Soyer et al. 2012d). The basaloid tumor cells have large, hyperchromatic, oval nuclei and little

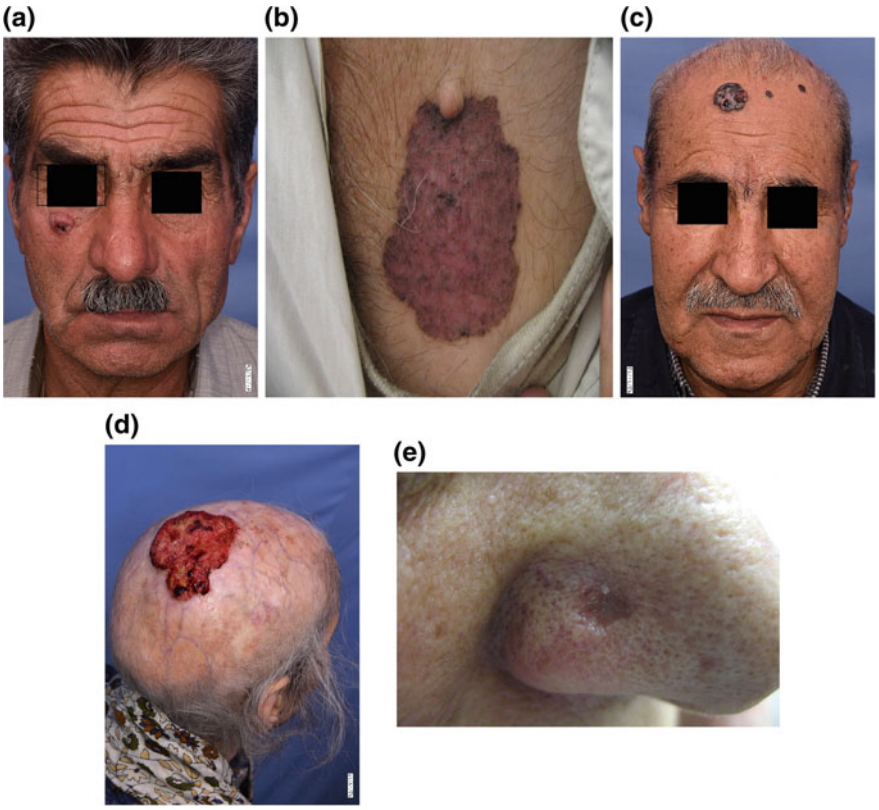

Fig. 18.2 Clinical spectrum of basal cell carcinoma (BCC): Nodular BCC (**a**), Superficial BCC (**b**), Pigmented BCC (**c**), Ulcerative BCC in context of radiodermatitis (**d**) Morpheaform BCC (**e**)

cytoplasm similar to basal layer keratinocytes. Mitotic figures are usually few. A microscopically visible cleft between BCC nests and fibromyxoid stroma, caused due to the retraction of the stroma around the tumor islands is another characteristic feature of BCC pathology (Soyer et al. 2012d).

18.3.2 Squamous Cell Carcinoma

18.3.2.1 Introduction

SCC accounts for almost 20% of skin tumors and is the second most common cutaneous malignancy. The estimated annual incidence of SCC is between 1.0 and 5.4 million in the U.S. (Rogers et al. 2010). Despite increased knowledge and public education, the incidence of SCC continues to rise worldwide. SCC has a

good prognosis until metastasis to the lymph nodes, but it can cause significant morbidity and disfigurement.

18.3.2.2 Risk Factors

Skin Phenotype

Skin types Fitzpatrick 1 and 2 are at an increased risk of developing SCC (Armstrong and Kricker 2001). Individuals with heavy freckling on the forearm were found to have a 14-fold increase in SCC risk if freckling was present in adulthood, and an almost three-fold risk if freckling was present in childhood (Kricker et al. 1991; English et al. 1998). SCC is less prevalent in darker skin. However considering the low incidence of BCC in black individuals, SCC is the most prevalent type of skin malignancy in these populations (Asuquo and Ebughe 2012; Halder and Bang 1988).

Sun Exposure and Radiations

Sun exposure is the major known environmental factor associated with the development of skin cancer of all types. Unlike BCC, SCC is associated with a chronic exposure, rather than intermittent intense exposure to UVR. Occupational exposure is a characteristic pattern of sun exposure linked to SCC (Armstrong and Kricker 2001). In addition to environmental radiation, exposure to therapeutic radiation like psoralen and ultraviolet A radiation (PUVA) (6-fold increase in risk of SCC) (Lindelof et al. 1999), UVB (relative risk 1.37) (Lim and Stern 2005), and tanning beds (Karagas et al. 2002) are the other risk factors for SCC. There are mixed results considering the relationship between SCC and ionizing radiation. It has been shown that, after a long latent period of more than 20 years, the prevalence of SCC is increased in patients with a history of therapeutic radiotherapy (especially patients with tinea capitis who had undergone radiotherapy) and some special occupations such as airline pilots, textile workers, sailors, locomotive engineers, and agricultural workers (Soyer et al. 2012e). However, this has not been seen in medical staffs such as radiology technicians and atomic survivors, who face an increased exposure to radiation (Ron et al. 1998; Yoshinaga et al. 2005).

Immunosuppression

Immunosuppression is a well-established cause of NMSCs. After solid organ transplantation, the incidence of BCC goes up five to ten times and for SCC 40–250 times compared to the general population with more aggressive behavior and more recurrence (Lindelof et al. 2000; Krynitz et al. 2013). This increased risk has been

linked to an interaction between the level of immunosuppression and UVR exposure (Alam et al. 2011; Frezza et al. 1997). The drug agents mostly implicated in this process are prednisolone, azathioprine, cyclosporine, voriconazole, muromonab (anti-CD3) (Jensen et al. 1999; Lampros et al. 1998).

Personal History of Nonmelanoma and Melanoma Skin Cancer

As mentioned in the BCC risk factors above, personal history of any NMSC and even CM is strongly associated with subsequent SCC and BCC (Helgadottir et al. 2015; Bergstresser and Halprin 1975; Moller et al. 1975; Epstein 1973; Cantwell et al. 2009), which is more prominent in the first year after a skin cancer has been diagnosed. On the other hand, people with family history of SCC (Hussain et al. 2009), BCC, and CM (Hemminki et al. 2003) in their first degree relatives have an increased risk of invasive and in situ SCC compared to the general population. Data shows that both genetic and environmental factors account for this familial clustering, so the database estimates genetic risk effects of 8% and familial shared-environmental effects of 18% (Totten 1980).

Chronic Wound and Inflammation

SCC is an uncommon complication of burn scar and long-standing chronic ulceration. The behavior of SCC arising in the context of chronic inflammation is more aggressive. Some of the chronic inflammatory lesions, which are prone to SCC development, are Marjolin ulcers, burn scars, or thermal injuries, venous ulcers, lymphedema, discoid lupus erythematosus, erosive oral lichen planus, lichen sclerosus et atrophicus, mutilating keratoderma, necrobiosis lipoidica, acne conglobate, hidradenitis suppurativa, dissecting cellulitis of the scalp, lupus vulgaris, and chronic deep fungal infection.

Human Papillomavirus Infection

Human papillomavirus (HPV) is a known implicated factor in anogenital SCC (HPV 16, 18) and in epidermodysplasia verruciformis (EV) induced SCCs (HPV 5, 8, etc.) that is explained below.

HIV Infection

HIV infection is a risk factor behind many cancers including cutaneous SCC. Perianal SCC is especially important in HIV/AIDS patients. Hence, serial anal cytological examination is recommended for them (Clifford et al. 2005).

Other Environmental Factors

As mentioned in BCC risk factors, there is an increased risk of SCC in patients with high concentrations of arsenic in toenails (Gailani et al. 1992). Here, again, there is a long latency period from the manifestation of clinical symptoms of SCC (20–40 years) (Wong et al. 1998). Considering different available data, in summary, it seems that smoking is associated with increased risk of both mucosal and skin SCC, in contrast to BCC (Odenbro et al. 2005; De Hertog et al. 2001). Treatment with hydroxyurea has also been associated with multiple SCCs (De Simone et al. 1998). There is scattered evidence of a relation between SCC and pesticides, asphalt, tar, and polycyclic aromatic hydrocarbons (Lei et al. 2001).

18.3.2.3 Clinical Manifestation

The initial presentation of SCC typically includes a history of a non-healing ulcer or tumoral lesion in sun-exposed area. The precursor of many of SCC cases is AK (Padilla et al. 2010), which appears as scaly plaques or papules, often with an erythematous to brown base. Patients with multiple AKs have a 6–10% lifetime risk of developing SCC (Fig. 18.3).

SCC in Situ (Bowen's Disease)

Bowen's disease usually presents as a solitary erythematous scaly patch or plaque in a sun-exposed area, except for arsenic-related Bowen's disease, which is usually multiple and located in non-sun exposed areas of the body (trunk) (Soyer et al.

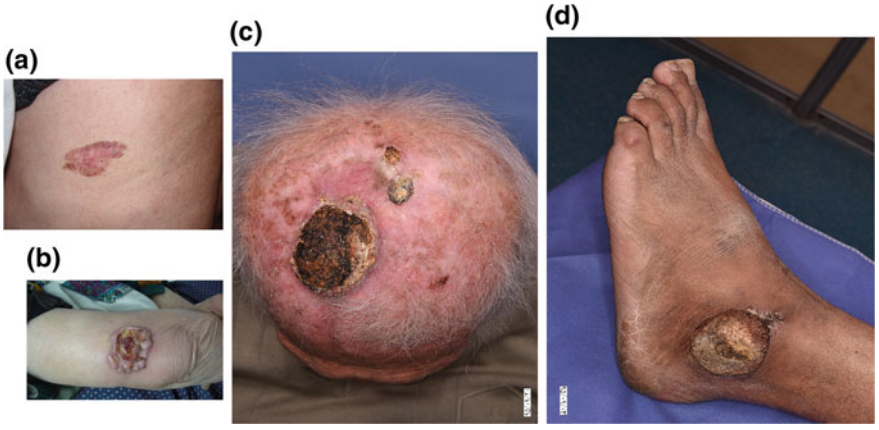

Fig. 18.3 Clinical spectrum of Squamous cell carcinoma (SCC). SCC in situ (**a**), Invasive SCC (**b**), SCC in the context of radio-dermatitis (**c**), SCC in the context of scar tissue (**d**)

2012c). At times, clinically differentiating between Bowen's disease, superficial BCC, and dermatitis might be very difficult.

Invasive SCC

Invasive SCC usually arises within severely photo-damaged skin as a hyperkeratotic exophytic papule, plaque, nodule and erosion; crust and ulcer may override the primary lesion (Soyer et al. 2012b). The first clinical evidence of malignancy is induration that is seen by examining the border of a lesion between a finger and the thumb.

18.3.2.4 Pathology

SCC in Situ

The main changes in the microscopic view of the Bowen's disease are acanthosis with full-thickness atypia, pleomorphism, and apoptosis of the epidermis over a broad zone.

Invasive SCC

Here, atypical keratinocytes breach the epidermal basement membrane and invade the dermis. The tumor spreads by a downward proliferation of lobules of eosinophilic keratinocytes. Pleomorphism, apoptosis and atypia are seen.

18.3.3 Cutaneous Melanoma

18.3.3.1 Introduction

Cutaneous melanoma (CM) results from a malignant transformation of skin melanocytes. It is the deadliest skin malignancy with a population-adjusted melanoma death rate of 3.1 per 100,000 population in the U.S. (Glazer et al. 2016). Its incidence and mortality rate has been rising over the past decades worldwide. The increased incidence may be partly attributed to better screening and lower threshold for biopsy of suspicious lesions. According to this speculation, higher incidence of melanoma in situ (MIS) is expected. In fact, the possibility of this trend has been mentioned in various studies. Meanwhile, melanomas of greater thicknesses have also shown an increased incidence. The observed increased incidence of melanomas of higher thicknesses (in addition to MIS) and the melanoma associated mortality shows that increased scrutiny is not the sole cause of the increased overall incidence of CM (Wei et al. 2016). The disease is more common and with a worse prognosis

in men (Cavanaugh-Hussey et al. 2015; Khosrotehrani et al. 2015). CM increases with age and the mean age at diagnosis is 63 years, but it is also seen in young adults and responsible for the majority of cancer-related deaths (Key Statistics for Melanoma Skin Cancer 2017).

CM is most common among the Caucasians. It is less common in non-Caucasian Whites and Asians, and rare in Blacks. This is true of all subtypes of CM except acral lentiginous melanoma (ALM). In a study on racial differences among six major subtypes of CM in the U.S., non-Hispanic whites (NHWs) had the highest incidence rates of all subtypes except ALM, followed by Hispanic whites (HWs). Blacks had the lowest rates. As for ALM, HWs had the highest rate, followed by NHWs (Wang et al. 2016). ALM is also found to be the most common subtype among Iranians (Kamyab et al. 2017).

18.3.3.2 Risk Factors

Sun and Ultraviolet Light

Sunlight and UV light exposure is the most important risk factor for CM. Intermittent sun exposure leading to sunburn at a young age is a major culprit. This happens especially in people with less pigmented phenotypes, who are less able to tan and burn easily (Gandini et al. 2005b). The role of artificial UV tanning through sunbed and sunlamps have also been investigated in recent years and it is known that use of tanning beds at an young age is a risk factor for melanoma (Gallagher et al. 2005; Colantonio et al. 2014; Le Clair and Cockburn 2016; Ghiasvand et al. 2017). Accordingly, the use of tanning beds is banned for teenagers in many countries as well as many states in the U.S. Living in equatorial latitudes, which exposes the skin to higher level of UV light, is also important and is the cause of a greater incidence of CM among whites residing in Australia (Queensland) and New Zealand. Notably, the role of chronic cumulative sun exposure in melanoma is less important than in SCC, with the exception of the LMM subtype. Prolonged PUVA therapy is also a possible risk factor (Stern 2001).

Phenotype

People with fair skin, who burn easily and are less able to tan (Fitzparick skin types I and II), are most susceptible to developing CM. Those carrying the MCR1 gene variants showing red hair, fair skin, poor tanning, and freckling have the highest risk of CM (Williams et al. 2011). However, even those MCR1 variants with normally pigmented skin and hair have a modest increased risk of CM (Williams et al. 2011). The presence of large number of acquired melanocytic nevi is the most important phenotypic risk factor for CM (Gandini et al. 2005a; Chang et al. 2009; Newton-Bishop et al. 2010). More than a hundred nevi are associated with an 8–10-fold increased relative risk of CM (Gandini et al. 2005a). A quick count of

more than 11 nevi in one arm is shown to correlate with total body nevi of more than 100 (Ribero et al. 2016). The risk related to atypical melanocytic nevi is also significant and a count of more than five is associated with a 4–6-fold increase in relative risk of CM (Gandini et al. 2005a). Nevogenesis is influenced by both genetic and environmental (especially sun exposure) factors. Familial forms of atypical nevi are discussed below. The presence of a high density of ephelides and multiple solar lentigines are the other known risk factors of CM (Gandini et al. 2005c).

Large congenital melanocytic nevus (by definition larger than 20 cm in largest width of projected adult size) is another risk factor of CM (Kopf et al. 1979). According to a systematic review, melanoma developed in 2% of patients with large congenital melanocytic nevus, the majority of which were cutaneous. Most associated nevi were located on the trunk, were larger than 40 cm, and had multiple satellite nevi (Vourc'h-Jourdain et al. 2013). In a retrospective study, two age periods were noted for the detection of CM—less than 10 years and older than 20 years (Lacoste et al. 2015).

Family History

Only about 10% of CM cases have a family history (Gandini et al. 2005c). Most familial cases are not single gene diseases; in fact, shared sun exposure in susceptible phototypes may underlie these familial clusterings (Goldstein and Tucker 2001). About 45% of true hereditary cases are due to CDKN2A or CDK4 germline mutations (Soura et al. 2016b).

Family history of a melanoma in at least one relative poses a two-fold risk of CM. Multiple CM in one first-degree relative or two or more relatives with one CM both increase this risk; for one first-degree relative with one primary melanoma the risk was 2.2, but increased to 16.3 for 5 or more melanomas. For two affected first-degree relatives with a single melanoma, the familial risk was 5.5-fold, and increased to 23.9 for two or more melanomas (Chen et al. 2014). The familial risk was significantly higher for SSM than LMM and for multiple parts and trunk than head/neck (Chen et al. 2014). In a study on twins, a heritability of 58% was observed for CM (Mucci et al. 2016).

Personal History of Cutaneous Melanoma and Non-melanoma Skin Cancer

Personal history of melanoma is considered a risk factor for subsequent melanoma development. A study on 4484 patients diagnosed with a first primary melanoma had been enrolled, clearly showed a high rate of second and third melanoma development (Ferrone et al. 2005). In addition to the history of melanoma, those with a history of NMSCs, including BCC and SCC are at substantial increased risk of developing CM (Marghoob et al. 1995).

Childhood Cancer History

Childhood cancer survivors are at an increased risk (2.5-fold) of developing melanoma (Pappo et al. 2013).

Immunosuppression

OTRs are at an increased risk of developing CM. The relative risk is 5-fold in case of heart transplants and 2.5-fold in kidney transplants (Green and Olsen 2015). The risk of CM also increases in lymphoma and chronic lymphocytic leukemia. HIV/AIDS patients are also more prone to developing CM (RR = 1.8) (Kubica and Brewer 2012; Silverberg et al. 2011, 2015).

Unproven Risk Factors

Sildenafil was found to be associated with CM in a cohort study (Li et al. 2014); however, a causal association between phosphodiesterase inhibitors and CM was not supported more recently (Pottegard et al. 2016). Some studies have shown that the consumption of coffee may reduce the risk of CM (Caini et al. 2017; Lukic et al. 2016). The beneficial role of vitamin D in melanoma is not proven (Park et al. 2016). An association between Parkinson's disease and CM has been observed (Liu et al. 2011).

18.3.3.3 Cutaneous Manifestation

As a rule of thumb, any lesion with the well-known ABCDE red flags (A: Asymmetry, B: Border irregularity, C: different Colors, D: Diameter >5 mm and E: Evolving, which means any new change in the lesion) should be excised and CM should be ruled out (Tsao et al. 2015). Some authors suggest adding "F" for family or personal history of melanoma (Levit et al. 2000). Many do not agree with a 6-mm limit since many patients diagnosed with CM have smaller lesions and many benign lesions such as seborrheic keratosis are actually larger than 6 mm (Goldsmith and Solomon 2007). The substitution of "Dark" standing for "D" is suggested (Stanwick and Hallum 1974). Another helpful clue is the "ugly duckling" sign; this means the recognition of the pigmented lesion that looks different from the patient's other nevi (Gaudy-Marqueste et al. 2017). This odd lesion is suspicious and should be biopsied.

CM has four major subtypes that are discussed briefly.

Fig. 18.4 Clinical spectrum of cutaneous melanoma (CM): Nodular melanoma (**a**), Superficial spreading melanoma (**b**), Lentigo malignant melanoma (**c**), Acral lentiginous melanoma (**d**)

Superficially Spreading Melanoma

A superficially spreading melanoma (SSM) is the most common subtype (60–70% of all CMs) in fair-skinned populations. It begins as a pigmented lesion with ABCDE red flags most commonly on the trunk, grows, and may become ulcerated. SSM is the most common CM arising in a pre-existing nevus (about 50% of SSM cases) (Skender-Kalnenas et al. 1995) (Fig. 18.4).

Nodular Melanoma

Nodular melanoma (NM) is the second most common subtype of CM in fair-skinned individuals (15–30% of MM cases). NM presents as a blue to black, but sometimes pink to red, nodule, which may be ulcerated (Demierre et al. 2005).

Lentigo Maligna Melanoma

Lentigo maligna melanoma (LMM) is a rare subtype of CM. The most specific clinical point about LMM is its association with chronic sun damage. It is generally seen in chronically sun-damaged skin, most commonly on the face, with a preference for the nose and cheek (Tannous et al. 2000).

Acral Lentiginous Melanoma

ALM is the most common subtype of CM among African and Asian individuals, but is uncommon among whites. It presents as an asymmetric, brown to black macule with color variation and irregular borders in the acral areas and mucosa.

Melanoma of the nail matrix can present as a pigmented band (longitudinal melanonychia) (Metzger et al. 1998). ALM is the most common subtype of CM in Iranian patients (Kamyab et al. 2017).

18.3.3.4 Pathology

CM progresses in two phases—the radial growth phase (also called melanoma in situ) and the vertical growth phase. Initially, the growth shows a radial phase of pagetoid spread in the epidermis and, later, malignant cells invade the dermis in the vertical growth phase. Histologically, melanomas are asymmetrical and poorly circumscribed lesions with architectural disturbance, marked cytological atypia, pleomorphism, and mitosis. There are some specific features in the epidermis: pagetoid spread of melanocytes, nests of melanocytes with variable size and shape, and ulceration. The dermis maturation within the architecture of the lesion is not seen. In other words, nests of melanocytes do not become smaller with progressive descent (Skender-Kalnenas et al. 1995; Clark et al. 1969). Breslow tumor thickness is measured from the granular layer of the epidermis to the deepest part of the tumor and is the most important prognostic factor in CM (Balch et al. 2009).

18.4 Genetics and Skin Cancer

In addition to the environmental exposure factors, including UVR, radiotherapy, viral infections, immunosuppression, previous cigarette smoking, and phenotypic traits, such as nevus count, red hair, freckling, genetic risk factors also have a substantial role in skin cancer development. Mutation in several genes has been accepted as the culprit of skin cancers and different associated syndromes. In this section, the most commonly reported genes and syndromes will be discussed.

18.4.1 Mutated Genes and Associated Syndromes

18.4.1.1 Genes

Several genes and hereditary syndromes associated with the development of skin cancer have been identified. Genetic alterations could increase cancer risk via three major mechanisms, including the activation of oncogenes, loss of tumor suppressor genes, or genomic instability. Oncogenes activation could occur through three genetic mechanisms, including mutation, gene amplification, and chromosome rearrangements. However, more alterations in genome (such as mutations in another gene), or environmental factors (such as a viral infection) are required for the

formation of many kinds of human cancer cells. When oncogenes are active, abnormal cell proliferation is driven. However, tumor suppressor genes (also known as antioncogene) normally act to inhibit cell proliferation to prevent tumor development. It seems that loss of these genes may be even more important than activation of oncogenes for cancer development. The most frequently mutated identified tumor suppressor genes are p53, INK4, and PTEN that contribute to the development of different types of cancer, such as lung cancer, prostate cancer as well as melanoma (Cooper 2000). A mutated allele of tumor suppressor genes (for example, p53) could be inherited. Second mutation in the remaining wild type allele or even prevention of normal protein function from the un-mutated allele by the mutated p53 protein (dominant negative) may drive a tumor. CDKN2A and CDK4 are two well-recognized genes in melanoma pathogenesis, which are tumor suppressors and oncogenes, respectively. These genes are associated with the hereditary melanoma syndrome, but do not cover a high percentage of familial melanoma. Genomic instability refers to a high frequency of mutations within the genome of a cellular lineage and is a characteristic of most cancer cells. Genomic integrity is closely monitored by several surveillance mechanisms, including a DNA damage checkpoint, DNA repair machinery, and a mitotic checkpoint. Thus, it is not surprising that a defect in the regulation of each of these mechanisms leads to genomic instability. The DNA repair process is critical to the survival of the cell, as it prevents increased persistent mutations in daughter cell generations, genomic instability, and, ultimately, in driving cancer. In Xeroderma pigmentosum (XP), the ability to repair damage caused by UV light is inadequate, which lead to a greatly increased risk of developing skin cancer.

Major Genes for Basal Cell Carcinoma

PTCH1

The most effective data in accessing the genetic aspects of BCC was the demonstration of allelic loss of PTCH1 gene in chromosome 9q22 (Shanley et al. 1995; Gailani et al. 1992). PTCH1 is the most important gene involved in BCC pathogenesis. All patients with the nevoid basal cell carcinoma syndrome (NBCCs) and up to 30% of sporadic BCCs demonstrate PTCH1 pathogenic variants (Gailani et al. 1996). PTCH1 codes an inhibitory effect on the seven-transmembrane protein Smoothened (SMO), which is a component of the hedgehog (Hh) pathway (Tabata and Kornberg 1994). The binding of the Hh ligand to PTCH1 releases inhibition of SMO, with the resultant activation of transcription factors (GLI1, GLI2), cell proliferation genes (cyclin D, cyclin E, c-Myc), and regulators of angiogenesis (Tojo et al. 2003; Lum and Beachy 2004). Thus, the balance of PTCH1 (inhibition) and SMO (activation) manages the essential regulatory downstream Hh signal transduction pathway. The loss-of-function of pathogenic variants of PTCH1 or

gain-of-function variants of SMO tips this balance toward activation, a key event in potential neoplastic transformation.

PTCH2

PTCH2 (mapping to chromosome 1p32.1–32.3) is another candidate gene, which displays 57% homology with PTCH1 (Shakhova et al. 2006; Smyth et al. 1999). While the exact role of PTCH2 remains unclear, its mutation has been demonstrated in both BCC and medulloblastoma (Rahnama et al. 2004).

SUFU

SUFU is a major negative regulator of the Hh pathway and its mutation has been seen in small numbers of patients with NBCC features (Pastorino et al. 2009).

BAP1

Although the BAP1 gene is well known to be associated with skin and uveal melanoma, there are several reports of BAP1 protein expression loss in families that report diagnoses of BCC (Mochel et al. 2015; de la Fouchardiere et al. 2015; Carbone et al. 2015; Wadt et al. 2015).

Major Genes for Squamous Cell Carcinoma

p53

It is generally accepted that the p53 tumor suppressor gene is the most commonly mutated gene in human cancer. It normally functions as a negative regulator of the cell cycle, arresting cells in the G1 phase. Hence, mutations in this critical gene may result in an unrestrained proliferation of keratinocytes. There is some evidence that p53 mutations arise early during the development of NMSC. It has been shown that UVR is responsible for the induction of p53 mutations and seems to be involved in the development of both aggressive and nonaggressive BCCs and SCCs (Bolshakov et al. 2003). Following the inactivation of p53, apoptotic keratinocytes could be generated by an overexposure to UV (Ziegler et al. 1994). Mutations in p53 induced by UVR found in more than 90% of human SCCs were present in AKs (Ziegler et al. 1994). During the early stages, this may manifest as AKs, which can then possibly progress to cutaneous SCCs. Several studies have shown that the p53 protein is over-expressed in sun-exposed skin of patients with cutaneous SCC (Coulter et al. 1995).

NOTCH1/NOTCH2

In addition to p53 mutations, NOTCH1 genes were recently identified as one of the most frequently mutated genes in cutaneous SCCs. NOTCH1 is a human gene,

encoding a single-pass transmembrane receptor. It was proposed that the NOTCH1 gene is a p53 target with a role in human tumor suppression (Lefort et al. 2007). Interestingly, NOTCH1 is proposed to be an oncogene or tumor suppressor gene in human cancer development, depending on the cellular context (Lobry et al. 2011). Recently, considerable NOTCH gene mutations were identified in cutaneous SCC. Wang et al. (2011) had identified NOTCH1 or NOTCH2 mutations in approximately 75% of patients with cutaneous SCC. Moreover, a significant NOTCH1 mutation was identified as an early event in squamous cell carcinogenesis (South et al. 2014). It was suggested that most of the NOTCH1 gene alterations were loss-of-function mutations (Zhang et al. 2016). Hence, the activation of the NOTCH signaling pathway seems to be growth-repressive and differentiation-inducing in cutaneous SCC. Besides mutations, substantially down-modulated NOTCH1 gene expression and activity in keratinocyte cancer cell lines and tumors were observed (Lefort et al. 2007).

CDKN2A

The silencing of the CDKN2A gene in SCCs and its significant role in the development of this cancer were noted in previous studies (Pacifico and Leone 2007). Usually, inactivation of both p16INK4a and p14ARF tumor suppressor genes, which are encoded within the CDKN2A, was reported in cutaneous SCC (Brown et al. 2004). That inactivation seems to be mainly to be the consequence of promoter methylation, an epigenetic event (more descriptions have been provided in the next section).

Major Genes for Melanoma

CDKN2A

CDKN2A has high penetrance and the most frequent high-risk melanoma susceptibility gene. Mutation in this gene is associated with an increased risk of melanoma. CDKN2A is a gene, which, in humans, is located in chromosome 9p21.3. It has been reported that mutations in CDKN2A account for 35–40% of familial melanomas (Goldstein et al. 2006). Interestingly, mutation in CDKN2A has also been found to be related to the increased risk of pancreatic cancer. Owing to the importance of this gene, its testing for mutations is commercially available. CDKN2A encodes two distinct proteins, including INK4A (also known as p16INK4a) and ARF (also known as p14ARF in humans), which act as tumor suppressors by regulating the cell cycle. p16INK4a and p14ARF inhibit CDK4 and CDK6 (followed by the activation of the retinoblastoma family of proteins and then inhibiting the G1 phase of the cell cycle) and activate p53 genes, respectively.

CDK4 and CDK6

Like CDKN2A, CDK4 is a high-risk susceptibility gene for cutaneous malignant melanoma. Generally, the cycle-independent kinases (CDK) family plays a critical role in the progression of cells from G1 to S phase. CDK4 and CDK6 accelerate the function of the cell cycle and are also in the same signaling pathway as CDKN2A. In a study that included 17 families with CDK4 germline mutations, similar risk of melanoma cancer with CDK4 mutations, and those with CDKN2A variants were observed (Puntervoll et al. 2013). As the families with CDK4 germline mutations could not be distinguished phenotypically from the CDKN2A melanoma families, it was suggested that the CDK4 gene should always be examined when a melanoma family tests negative for CDKN2A mutation. Surprisingly, in spite of relatively similar functions of the CDK6 to CDK4, germline mutations in CDK6 were not found to contribute to melanoma predisposition (Shennan et al. 2000).

TERT

There is some evidence that the TERT promoter mutation contributes in familial and sporadic melanoma development (Horn et al. 2013; Huang et al. 2013). These results imply the presence of mutations in the TERT gene in some melanoma patients.

POT1

POT1 seems to be a major susceptibility gene for familial melanoma in several populations. Indeed, POT1 mutations were found in some melanoma families in the absence of CDKN2A or CDK4 mutations, suggesting it may be another gene in hereditary melanoma (Shi et al. 2014; Robles-Espinoza et al. 2014). Carriers of POT1 mutations had increased telomere lengths and also numbers of fragile telomeres compared to melanoma cases without the POT1 variants. Hence, it can be speculated that there is a link between the disruption in the normal telomere length and melanoma. In contrast to CDKN2A, the clinical utility of testing this gene has not yet been established.

BAP1

BAP1 is a tumor suppressor gene that was found to be inactivated in 84% of patients with metastatic uveal melanoma (Harbour et al. 2010). There is some evidence suggesting that the BAP1 gene implicated both in sporadic and hereditary melanomas. Although the majority of the found BAP1 mutations were somatic, both germline and somatic BAP1 mutations were found in melanoma patients.

BRAF

Studies have identified mutations in the BRAF gene that appears to be the most common event in the process that leads to melanoma. The most prevalent BRAF mutations detected in melanoma are missense mutations (V600). Approximately 50% of melanoma patients harbor activating BRAF mutations. Identification of the mutations in the BRAF gene has led to the emergence of target therapy via selective

inhibitor of mutant BRAF, including vemurafenib and dabrafenib, the two U.S. Food and Drug Administration (FDA) approved BRAF inhibitors for the treatment of melanoma.

PTEN

PTEN is a tumor suppressor gene that could be mutated in human melanomas (Palmieri et al. 2009). However, mutation in PTEN is not limited to melanoma but was observed in different other cancers, such as endometrial, breast, thyroid, and prostate cancers. An effect on lipid and protein phosphatase activity in several identified PTEN alterations could lead to abnormal cell growth and also abnormal cell spread and migration.

18.4.1.2 Syndromes

Generally, genetic syndromes associated with skin cancer can be categorized into different groups. Some of them may be associated with immunodeficiency or could affect pigmentation. Defects in key signaling pathways involved in the pathogenesis of NMSCs or melanomas are another category. List of syndromes associated with BCC, SCC, MM, and other rare syndrome, are shown in Tables 18.1, 18.2, 18.3 and 18.4, respectively.

Syndromes Associated with a Predisposition to Basal Cell Carcinoma

Nevoid Basal Cell Carcinoma Syndrome

Nevoid basal cell carcinoma syndrome, also called Gorlin syndrome or Basal cell nevus syndrome, is an autosomal dominant cancer genetic syndrome. It is caused by pathogenic variants in PTCH1 and PTCH2. Recently, SUFU was recognized as the other involved gene in NBCCs. This syndrome is characterized by the development of multiple neoplasms and is associated with an increased risk of BCC (Fig. 18.5). The estimated prevalence of NBCCs is 1 in 57,000 individuals. This syndrome has complete penetrance and high levels of variable expressivity (Bale 1997; Shimkets et al. 1996; Farndon et al. 1992). More details about the clinical features of NBCCs are given in Table 18.1.

Brooke-Spiegler syndrome

The Brooke-Spiegler syndrome was first described in 1842 by Ancell (Ancell 1842). This rare autosomal dominant syndrome is characterized by multiple skin tumors that develop from sweat glands and hair follicles. Mutations in the CYLD gene, which are involved in the regulation of nuclear factor-kappa-B (NF-κB), were found to be the culprit in this syndrome (Hu et al. 2003).

Table 18.1 Basal cell carcinoma syndromes

Syndrome	Gene(s)	Clinical feature
– Basal cell nevus syndrome, also known as Gorlin syndrome	– PTCH1, PTCH2, SUFU	– BCC (before age 20 year), Medulloblastoma, odontogenic keratocyst, skeletal abnormalities, palmo-plantar pits, calcification of the falx cerebri, macrocephaly
– Brooke-Spiegler syndrome	– CYLD	– Cylindroma (forehead, scalp, trunk, and pubic area), trichoepithelioma (around nose), spiradenoma, and BCC
– Bazex-Dupré-Christol syndrome		– Hypotrichosis (variable), hypohidrosis, milia, follicular atrophoderma (dorsal hands), and multiple BCCs (aged teens to early 20 s)
– Rombo syndrome		– Milia, atrophoderma vermiculatum, acrocyanosis, trichoepitheliomas, and BCC (age 30–40 year)
– Multiple hereditary infundibulocystic BCC		– Multiple BCC (infundibulocystic type)
– Schopf-Schulz-Passargesyndrome		– Ectodermal dysplasia (hypotrichosis, hypodontia, and nail dystrophy [anonychia and trachyonychia], hidrocystomas of eyelids, palmo-plantar keratosis and hyperhidrosis, and BCC
– Xeroderma pigmentosum (XP)	– Global genome nucleotide excision repair (GG-NER) – XP genes	– Cutaneous abnormalities: photosensitivity, xerosis, multiple lentigines, early onset multiple NMSCs, and melanoma – Ocular abnormalities: keratitis, corneal opacification – Neurological abnormalities: hyporeflexia, deafness, seizures, and (most common in groups and D; do not usually occur in XP variant) – Internal malignancies: brain, lung, oral cavity, gastrointestinal tract, kidney and hematopoietic system
– Muir-Torre syndrome (MTS)	– MLH1, MSH2, MSH6	– Sebaceous adenomas, carcinomas, and keratoacanthomas or BCCs with sebaceous differentiation – Gastrointestinal malignancies (colorectal, stomach, small bowel, liver, and bile duct) and/or genitourinary malignancies (endometrial and bladder)

Table 18.2 Squamous cell carcinoma associated syndromes

Syndrome	Gene(s)	Clinical features
– Xeroderma pigmentosum (XP)	– Global genome nucleotide excision repair (GG-NER) – XP genes	– Cutaneous abnormalities: photosensitivity, xerosis, multiple lentigines, early onset multiple NMSCs, and melanoma – Ocular abnormalities: keratitis, corneal opacification – Neurological abnormalities: hyporeflexia, deafness, seizures, and (most common in groups and D; do not usually occur in XP variant) – Internal malignancies: brain, lung, oral cavity, gastrointestinal tract, kidney and hematopoietic system
– Werner syndrome	– WRN/RECQL2	– Premature aging, diabetes mellitus, atherosclerosis and vascular calcification, canities during the 2nd decade of life – Sclerodermoid changes, ulcers, short stature, increased risk of malignancies including SCC and soft tissue sarcomas
– Kindler	– FERMT1 (KIND1)	– Cutaneous involvement: congenital onset, trauma induced acral bullae, progressive poikiloderma, skin atrophy, photosensitivity, palmo-plantar keratoderma, webbing of the fingers and toes, SCC – Mucosal involvement: gingivitis, colitis, ectropion – Bladder: transitional carcinoma of the bladder
– Muir-Torre syndrome (MTS)	– MLH1, MSH2, MSH6	– Sebaceous adenomas, carcinomas, and keratoacanthomas or BCCs with sebaceous differentiation

(continued)

Table 18.2 (continued)

Syndrome	Gene(s)	Clinical features
		– Gastrointestinal malignancies (colorectal, stomach, small bowel, liver, and bile duct) and/or genitourinary malignancies (endometrial and bladder)
– Dyskeratosis congenita	– DKC1,TERC, TINF2, NHP2/NOLA2 NOP10/NOLA3, TERT, WRAP53, C16orf57, RTEL1	– Cutaneous involvement: Dysplastic nails, reticular pigmentation of the chest and neck, and oral leukoplakia – Hematologic involvement: anemia, increased risk of myelodysplastic syndrome, acute leukemia, and bone marrow failure – Increased risk of cancers: Head and neck cancers and cutaneous SCC – Others: Ocular, dental, neurologic, gastrointestinal, pulmonary, and skeletal abnormalities
– Fanconi anemia	– FANCA, FANCB, FANCC, FANCD1/BRCA2, FANCD2, FANCE, FANCF, FANCG/XRCC9, FANCI, FANCJ/BRIP1/BACH1, FANCL, FANCM, FANCN/PALB2, FANCO/RAD51C, FANCP/SLX4/BTBD12, FANCQ/ERCC4/XPF, FANCS/BRCA1	– Susceptibility to DNA cross-linking agents (e.g., mitomycin-C or diepoxybutane) and ionizing and UVR – Increased incidence of cancers: Hematologic and gastrointestinal, head and neck, and gynecologicsl, skin SCC
– Epidermodysplasia verruciformis	– EVER1/TMC6, EVER2/TMC8	– Photo-distributed warts and SCCs in situ and invasive SCCs
– Oculocutaneous albinism (OCA)	– TYR, OCA2, TYRP1, SLC45A2/MATP/OCA4, Locus 4q24, SLC24A5, C10Orf11	– Skin: White hair, milky white skin, and blue-gray eyes, amelanotic melanocytic nevi, extreme sensitivity to UV light, and a strong predisposition to skin cancer – Reduced visual acuity

(continued)

Table 18.2 (continued)

Syndrome	Gene(s)	Clinical features
– Bloom syndrome	– BLM/RECQL3	– Cutaneous abnormalities: Malar erythema and telangiectasias, skin SCC, cafe-au-Lait macules, areas of hypopigmentation – Skeletal abnormalities: Elongated face with malar hypoplasia and prominent nose, short stature – Other features: Diabetes mellitus, recurrent respiratory and GI infections. Increased frequency of leukemia, lymphoma, GI adenocarcinoma, reduced fertility but normal intelligence
– Huriez syndrome	– Unknown; Locus 4q23	– Palmo-plantar keratoderma with scleroatrophy, increased risk of SCC and MM
– Epidermolysis bullosa dystrophic	– COL7A1	– Cutaneous abnormalities: Skin blister and scar pseudosyndactyly and limb amputation, SCC – Other abnormalities: Short stature, anemia, strictures of the gastrointestinal and genitourinary tracts, corneal scarring and blindness
– Epidermolysis bullosa junctional	– LAMA3, LAMB3, LAMC2, COL17A1	– Skin and mucosal blistering
– Ferguson-Smith syndrome (multiple self-healing keratoacanthoma)	– TGFBR1	– Multiple spontaneously resolving keratoacanthomas which ends to scar, increased risk of SCC
– Olmsted syndrome		– Palmo-plantar keratoderma with periorificial plaques, increased risk of SCC and MM
– Mal de meleda	– SLURP-1	– Palmo-plantar keratoderma, Atopic dermatitis, increased risk of SCC and MM

(continued)

Table 18.2 (continued)

Syndrome	Gene(s)	Clinical features
– Rothmund-Thomson syndrome	– RECQL4, C16orf57	– Chromosomal instability – Skin abnormalities: Erythema, edema and vesicles on the cheeks and face during the first few months of life, poikiloderma, which typically affects the dorsal aspect of the hands/forearms and the buttocks – Sparse hair (scalp, eyebrows, eyelashes), hypoplastic nails, acral keratoses (in adolescents and adults) – Osteosarcoma (10–30%) – Normal immune function, intelligence and lifespan (in the absence of malignancy) – Other abnormalities: Short stature, skeletal (e.g., radial ray defects, osteoporosis) and dental abnormalities, juvenile cataracts, chronic diarrhea/vomiting during infancy, pituitary hypogonadism (may be associated with midface hypoplasia/"saddle nose")
– Hermansky-Pudlak syndrome (HSP)	– HSP1, AP3B1, HPS3, HPS4. HPS5, HPS6, DTNBP1, BLOC1s3, PLDN	– Cutaneous abnormalities: pigmentary dilution of the skin, hair, and eye – Ocular manifestations of albinism, such as nystagmus and reduced visual acuity – Bleeding tendency – Interstitial pulmonary fibrosis – Granulomatous colitis – Renal failure, Cardiomyopathy develop
– Gracilis syndrome	– MY05A, RAB27A, MLPH	– Decreased cutaneous pigmentation with hypomelanosis and

(continued)

Table 18.2 (continued)

Syndrome	Gene(s)	Clinical features
		neurologic deficits, Increased risk of SCC
– Pachyonychia Congenita	– Keratin 6a, 6b,16, 17	– Painful focal palmo-plantar keratoderma hypertrophic nails, oral leukokeratosis, steatocystoma multiplex and vellus hair cysts, hair shaft abnormalities

Table 18.3 Malignant melanoma associated syndromes

Syndrome	Gene(s)	Clinical features
– Familial atypical multiple mole-melanoma syndrome (FAMMM)	– CDKN2A, CDK4	– High number of body nevi (>50), multiple atypical nevi, personal and family history of cutaneous melanoma in one or more 1st degree relatives, onset of melanoma at young age
Familial atypical multiple mole-melanoma—pancreatic cancer syndrome	– CDKN2A	– Similar to FAMMM syndrome – Pancreatic cancer
– Melanoma-Astrocytoma syndrome	– CDKN2A, CDKN2B, CDKN2BAS	A variant of FAMMM; associated with multiple types of nervous system tumors.
– Hereditary malignant melanoma	– CDK4, CDKN2A, POT1,TERT	
– Xeroderma pigmentosum	– XPC, XPD, XPA	– Photosensitivity from infancy, freckles and lentigines, multiple SCC, BCC, keratoacanthoma, melanoma
– PTEN hamartoma tumor syndromes.	– PTEN	– Multiple hamartoma including trichilemmoma, breast carcinoma, thyroid carcinoma; cutaneous melanoma rare.
– BAP1 tumor syndrome	– BAP1	– Multiple melanocytic proliferations, multiple cutaneous melanoma, uveal melanoma, internal malignancies including renal cell carcinoma and malignant mesothelioma
– Hereditary breast and ovarian cancer syndrome	– BRCA1, BRCA2	
– Li-Fraumeni syndrome	– p53	– Premenopausal breast cancer, soft tissue sarcoma, osteosarcoma, central nervous system (CNS) tumor, and adrenocortical carcinoma – Skin cancer is not a typical feature: melanoma rare

Table 18.4 Rare skin cancer syndromes

Syndrome	Gene(s)	Clinical features
– Hereditary leiomyomatosis and renal cell cancer (HLRCC), Reed's syndrome	– Fumarate Hydratase (FH)	– Cutaneous leiomyomas – Uterine Leiomyoma, renal cell carcinoma
– Li-Fraumeni syndrome	– p53	– Breast cancer, brain tumors, osteosarcoma and leukemia. NMSCs are not typical features
– Birt-Hogg-Dube syndrome	– FLCN	– Multiple adnexal tumors (fibrofolliculomas, trichodiscomas and skin-tag-like lesions) – Renal cell carcinoma, colonic adenomas, pulmonary cysts, spontaneous pneumothorax, – Medullary thyroid carcinoma – Connective tissue nevi
– Cowden syndrome	– PTEN	– Thyroid, breast, gastrointestinal and genitourinary benign and malignant tumor – Mucocutaneous: Pigmented macules of genitalia (glans and shaft of penis, vulva) lipomas, vascular anomalies, neuromas (facial, acral, mucosal), epidermal nevi Cafe-au-Lait macules – Skeletal: Macrocephaly down-slanting palpebral fissures, frontal bossing, high-arched palate, long philtrum, pectus excavatum, joint hyperextensibility
– Tuberous sclerosis	– TSC1, TCS2	– Hypopigmented macules, facial angiofibromas, collagenomas, and periungual fibromas – Gingival fibromas and dental enamel pits – Hamartomas can be found in the eye as well as several internal organs, including the brain, kidneys, heart and lungs
– Neurofibromatosis	– NF1	– Cafe-au-Lait macules, neurofibromas, freckling in the axillae and groin, Lisch nodules, and bony defects
– Pilomatrix carcinoma	– CTNNB1	– Pilomatricoma
– Fabry disease	– GLA	-Mucocutaneous finding: Multiple angiokeratomas – Others: Pain, paresthesia, renal failure, hypohidrosis, coronary insufficiency
– Multiple endocrine neoplasia (MEN1)	– MEN1	– Pituitary, parathyroid, pancreas tumors – Mucocutaneous abnormalities: Multiple angiofibromas, collagenomas, lipomas, hypopigmented macules, cafe-au-Iait

(continued)

Table 18.4 (continued)

Syndrome	Gene(s)	Clinical features
		macules, multiple gingival papules, confetti-like – Less common: foregut tumors, adrenal (cortical) tumors, follicular adenomas (thyroid)
– Multiple endocrine neoplasia, type 2A (MEN 2A)	– RET	– Parathyroid, thyroid, adrenal tumors – Lichen amyloidosis, macular amyloidosis
– Multiple endocrine neoplasia, type 2B (MEN 2B)	– RET	– Endocrine tumors: Thyroid, adrenal tumor cushing syndrome, – Skin and skeletal abnormalities: Hyperpigmentation around mouth and overlying small joints of hands and feet, cafe-au-Lait macules, lentigines, Proximal myopathy, reduced subcutaneous fat, Marfanoid habitus, pectus excavatum, bilateral pes cavus, high-arched palate, high patella, scoliosis, kyphosis, joint laxity, everted lips and eyelids, hypertrichosis, synophrys, diffuse ganglioneuromatosis, multiple mucosal neuroma
– Buschke–Ollendorff syndrome	– LEMD3	– Skin tumors: collagenoma, elastoma, osteopoikilosis
– Gardner syndrome	– APC	– Skin abnormalities: Epidermoid cysts, osteomas, desmoid tumors and fibrous tumors – Congenital hyperpigmentation of the retinal pigment epithelium (CHRPE) – Adenomatous polyposis coli
– Proteus syndrome	– AKT1	– A mosaic overgrowth of multiple tissues and multiple hamartomatous and lipomatosis
– Familial glomuvenous malformation	– GLMN	– Multiple glomuvenous malformations

Bazex-Dupré-Christol syndrome

Bazex-Dupré-Christol, also known as the Bazex syndrome, is a rare X-linked dominant inherited disorder, which is related to BCC. It is characterized by follicular atrophoderma, multiple BCCs, hypotrichosis, milia, and localized hypohidrosis (Kidd et al. 1996). It was found that trichoepitheliomas were an early sign of this syndrome and could guide the diagnosis before the development of BCCs (Castori et al. 2009).

Fig. 18.5 Clinical spectrum of Nevoid basal cell carcinoma syndrome (NBCCs). Multiple nevus-like BCC s (**a**), Right ear BCC (**b**), Palmar pits (**c**), Skeletal deformities (**d**)

Rombo syndrome

The Rombo syndrome was first described in 1981 by Michaëlsson et al. (1981), followed by some other limited reports until now. It is a very rare syndrome, characterized mainly by hair anomalies and skin problems and increases the risk of BCC development. The lesions become visible in late childhood and are most pronounced on the face.

In addition to the mentioned BCC associated syndromes, multiple hereditary infundibulocystic BCC, Schopf-Schulz-Passarge syndrome, and XP (described in the next section) are other syndromes that are associated with BCC.

Syndromes Associated with a Predisposition to Squamous Cell Carcinoma

Xeroderma pigmentosum

XP is an autosomal recessive syndrome, which is caused in the context of global genome nucleotide excision repair (GG-NER) mutation. Since the function of GG-NER is to recognize and repair photoproducts from UVR, it results in severe sensitivity to UV-induced carcinogenesis. This syndrome is characterized with

multiple NMSCs, and melanomas. A total of eight XP genes have been identified, which led to the subdivision of this disease into seven subgroups (Nikolaou et al. 2012). In XP, the ability to repair damage caused by UV light diminishes. Thus, patients are extremely sensitive to UV rays from sunlight. Approximately half of children with XP develop their first skin cancer by the age of 10 and the risk of subsequent skin cancers during their lifetime is high. Those who are young (less than 20 years-old) are more susceptible to the development of NMSCs and melanoma. The clinical manifestation of the syndrome begins with xerosis and early lentigines in the first year of life and more than 100-fold increased risk of NMSCs and MM (Lim and Hawk 2012). Approximately one-half of individuals with this disorder will develop NMSCs, and approximately one-quarter will develop melanoma (Kraemer et al. 1994). Seven subtypes of XP have been introduced so far, and XPC accounts for 40% of the XP cases. XPC has the highest incidence of ocular involvement. Skin cancers may be more common in XPC, XPE, and XPV groups (Fassihi et al. 2016). The diagnosis of XP is made on the basis of clinical findings and family history. Management consists of extremely rigorous photoprotection. Destructive treatment such as cryotherapy, curettage, and excision is the treatment of choice for NMSCs. Oral retinoids can be used as chemopreventive agents (Lim and Hawk 2012). Recently topical application of the liposomal T4 endonuclease V (T4N5), a bacterial DNA repair enzyme, has effectively decreased the formation of BCC and AK in XP patients (Yarosh et al. 2001). However, no effect was seen on the formation of SCC (Fig. 18.6).

Multiple self-healing squamous epithelioma

Multiple self-healing squamous epithelioma (MSSE) (also known as the Ferguson-Smith disease [FSD]) is a rare inherited autosomal-dominant skin cancer condition, characterized by the development of multiple locally invasive skin tumors resembling keratoacanthomas in sun-exposed areas. They usually heal spontaneously and rapidly leave pitted scars. In this disease, germline inactivating mutations in TGFBR1 was introduced as a culprit (Goudie et al. 1993, 2011).

Oculocutaneous albinism

Oculocutaneous albinism (OCA) associated syndromes are genetic syndromes with diffuse pigmentary dilution of the skin, hair follicles, and eyes due to some disturbances in melanin synthesis. The melanocytes in this syndrome are normal but lack melanin. OCA, the most common inherited pigmentary disorder predisposes a person to SCC, particularly on the sun-exposed head and neck. Mutated genes in six from seven types of OCA (OCA1-7) were identified, including TYR, OCA2, TYRP1, SLC45A2, SLC24A5, and C10Orf11 (gene for OCA5 remained unidentified) (Kamaraj and Purohit 2014). OCA1 and OCA2 are extremely prone to early age skin cancers, mostly SCC, followed by BCC and MM (Mabula et al. 2012) (Fig. 18.7).

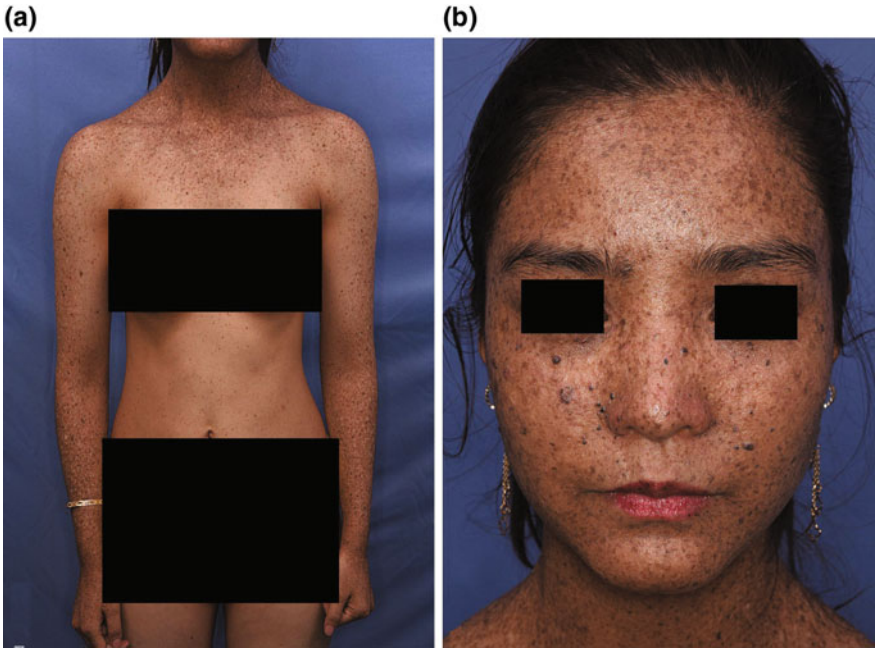

Fig. 18.6 Clinical features of Xeroderma pigmentosum syndrome (XP): Multiple lentigines and xerosis in a photo-distributed pattern (**a**), multiple lentigines and BCC (**b**)

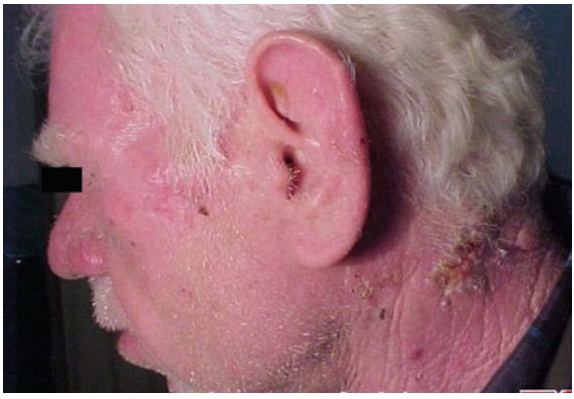

Fig. 18.7 Oculocutaneous albinism with multiple actinic keratosis and squamous cell carcinoma

Epidermolysis bullosa

Epidermolysis bullosa (EB) is a group of rare genetic diseases, characterized by severe blistering after minor trauma and frictions. A few subtypes of EB syndromes, discussed below, are associated with an increased risk of skin cancer, particularly SCC (Fine et al. 2014).

- *Dystrophic epidermolysis bullosa*

Dystrophic epidermolysis bullosa (DEB) is the most severe form of EB causing many complications and life-threatening events. There are three main subtypes of DEB, including recessive dystrophic EB (Hallopeau-Siemens type) (RDEB-HS), the recessive dystrophic EB (non-Hallopeau-Siemens type) (non-HS RDEB), and a predominantly inherited form (DDEB).

The RDEB Hallopeau-Siemens type is the most severe type of EB, resulting from the COL7A1 (collagen VII) mutation located in 3p21.3. It is characterized by severe skin and mucosal blister, scar, pseudosyndactyly, limb loss, and many mucosal scars (Fine et al. 2014). RDEB carries 85% SCC risk in non-healing ulcers by the age of 45 (Fine et al. 2004, 1999). Unfortunately, SCC overriding in RDEB has a very aggressive behavior with metastasis and is the leading cause of mortality in the middle age cases of RDEB. These cancers arise in non-healing wounds and usually metastasize to cause death within five years of the diagnosis of SCC (Fine et al. 2008b). The exact cause of this aggressive behavior of an SCC tumor in this setting may be related to specific mutations in the type VII collagen gene, or to elevated levels of the basic fibroblast growth factor (Soyer et al. 2012c). Diagnosis of EB may be accomplished by immunofluorescence or electron microscopy (Fine et al. 2008a) (Fig. 18.8).

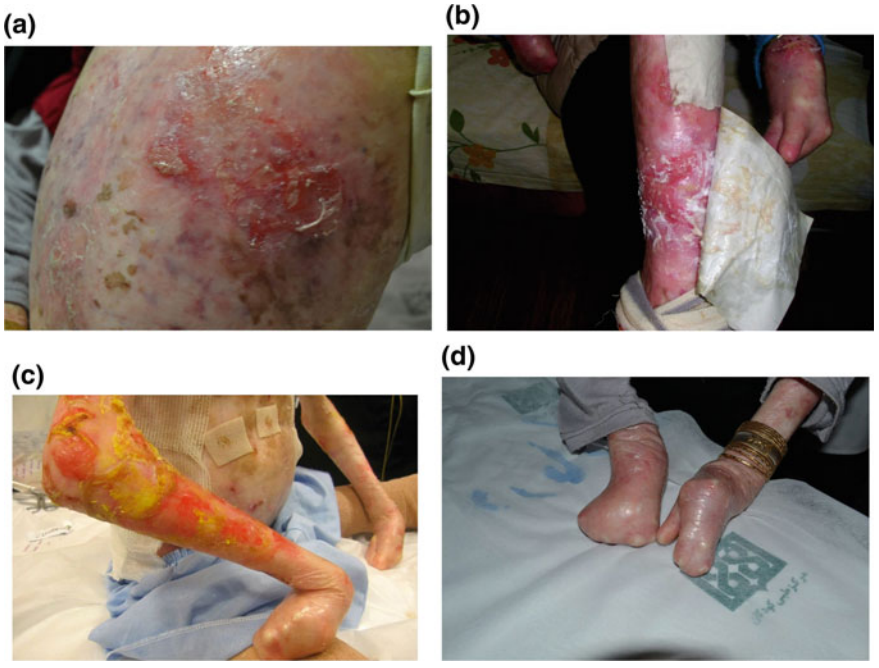

Fig. 18.8 Clinical pictures of patient with recessive dystrophic EB, Hallopeau-Siemens type. Multiple erosions and bullae in the limb (**a**) and (**b**), severe mutilation and spontaneous limb amputation, (**c**) and (**d**)

- *Junctional epidermolysis bullosa*

Junctional epidermolysis bullosa (JEB) is an autosomal recessive type of EB, arising due to mutations in laminin 332, or variants in COL17A1. The most severe subtype of JEB is Herlitz JEB, which is associated with an increased risk of SCC, with a cumulative risk of 18% by the age of 25 years (Kiritsi et al. 2011; Fine and Mellerio 2009) (Fig. 18.9).

Epidermodysplasia verruciformis

Epidermodysplasia verruciformis (EV) is a rare autosomal recessive syndrome, characterized by multiple warts and multiple SCCs in the sun-exposed area. One-third to one-half of the patients develops SCCs as adults, usually in the sun-exposed regions, and several decades earlier than is typical for SCC development in the general population (Soyer et al. 2012c). Infection with specific HPV subtypes (mostly HPV 5, 8, 14, 17, 20, 47) leads to multiple warts in the sun-exposed areas. Subsequently, in situ and invasive SCC can develop in 30–60% of patients (Karagas et al. 2006; Majewski and Jablonska 1995). The genes associated with this disorder, EVER1 and EVER2, encode transmembrane proteins participating in the regulation of the zinc balance (Lazarczyk et al. 2008; Ramoz et al. 2002) (Fig. 18.10).

Syndromes Associated with a Predisposition to Malignant Melanoma

Although most cases of melanoma are sporadic, some individuals have an inherited risk of melanoma. Approximately 8% of those newly diagnosed with melanoma have an affected first-degree relative, which highlights the critical role of genetic background and the chance of passing melanoma risk from generation to generation. Malignant melanoma associated syndromes are listed in Table 18.3.

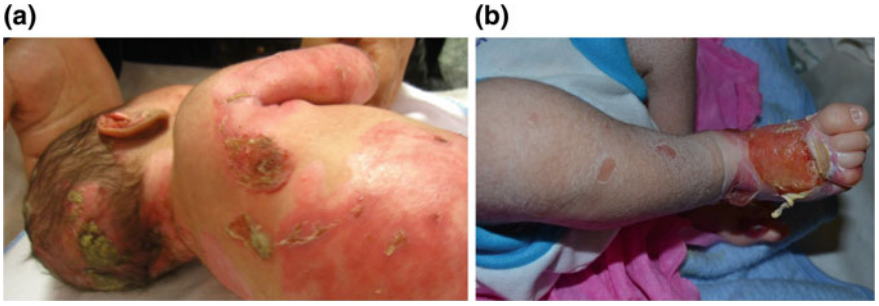

Fig. 18.9 Clinical features of patient with junctional epidermolysis bullosa- Herlitz type. Multiple erosions and bullae (**a**) and (**b**)

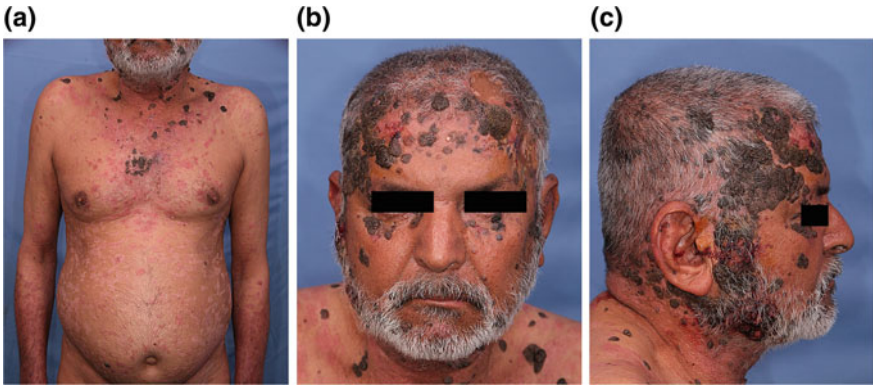

Fig. 18.10 Clinical features of a patient with Epidermodysplasia verruciformis (EV) syndrome, Multiple warts in the body (**a**). Multiple Warts and SCCs in the head and neck (**b**), and (**c**)

Familial atypical multiple mole-melanoma (*FAMMM*) *and familial atypical multiple mole-melanoma—pancreatic cancer* (*FAMMM-PC*) *syndromes*

FAMMM syndrome is a predominantly inherited genodermatosis, characterized by multiple nevi (moles)—some of them atypical—and a family history of CM. Germline mutations of CDKN2A have been detected in 40% of cases and of CDK4 only rarely (Goldstein et al. 2006). The criteria for the diagnosis of FAMMM are as follows (Soura et al. 2016b):

- CM in one or more first- or second-degree relatives.
- High total body nevi count (>50), multiple atypical nevi.
- Specific histological features present on nevi (these include: asymmetry, subepidermal fibroplasia, lentiginous melanocytic hyperplasia with spindle or epithelioid melanocytes, variable dermal lymphocyte infiltration, and presence of the shouldering phenomenon).

Cutaneous melanomas develop at a young age in these patients and most commonly on normal skin, although atypical nevi are also more prone to become malignant than normal looking nevi (Soura et al. 2016b). Second primary melanoma is also more common in these patients than in the general population (van der Rhee et al. 2011; Soura et al. 2016b). In addition, families with CDKN2A mutations have a significant risk of pancreatic cancer (Goldstein et al. 2006). It has been found that 60–90% of the carriers of CDKN2A develop CM by the age of 80 and 17% show pancreatic cancer by the age 75 (Eckerle Mize et al. 2009).

Melanoma-Astrocytoma Syndrome

The Melanoma-Astrocytoma Syndrome (MAS) is a variant of FAMMM, mostly due to the mutation of p14ARF (Randerson-Moor et al. 2001). CM appears at an early age before or after the neural tumor (Soura et al. 2016b).

Xeroderma pigmentosum

Patients with XP are at an increased risk of CM, along with NMSCs (discussed above) (Blankenburg et al. 2005).

PTEN hamartoma tumor syndromes (*including Cowden syndrome*)

Patients with PTEN hamartoma tumor syndromes have an elevated incidence ratio of CM (28 in women and 39 in men) (Bubien et al. 2013). This figure was 8.5 in another study (Tan et al. 2012) (Table 18.2).

BAP1 tumor syndrome

BAP1 tumor syndrome is an autosomal-dominant syndrome linked to the mutation of the BAP1 gene (3p21) (Wiesner et al. 2011). It is characterized by multiple atypical melanocytic proliferations, multiple CM, uveal melanoma and internal malignancies. Melanocytic proliferations seen in these patients clinically resemble dermal nevi but lack BAP1 staining and are called melanocytic BAP1-mutated intradermal tumors (MBAITs) (Soura et al. 2016a).

Other Rare Skin Cancers Associated Syndromes

In addition to the mentioned syndromes, there are some other ones, which usually do not lead to BCC, SCC, and melanoma. The names, possible involved genes, and their clinical features are listed in Table 18.4.

18.4.2 Polymorphisms

Genetic variations or polymorphisms in the human genome can lead to genetic susceptibility to cancer. Polymorphisms in genes could drive an increased risk of cancers through different pathways, such as cell cycle control, carcinogen metabolism, DNA repair, apoptosis, inflammation, and epigenetic regulation. The ability to identify polymorphisms in genes that are associated with cancer or segregate with disease in families allows high-risk loci to be identified. However, a positive correlation between gene polymorphism and a designated trait does not necessarily means that the polymorphism is responsible for that trait. In fact, it could imply that the causal variant is linked and may lie within the locus of interest (Binstock et al. 2014). Binstock et al. (2014) have shown that single-nucleotide polymorphisms (SNPs) in genes of the pigmentation pathway, which are associated with clinical skin phenotype, are associated with NMSC. Additionally, other SNPs, independent of the clinical skin phenotype (rs1805007 and rs1805008 in the MC1R gene), are associated with NMSC. Thereby, it could be speculated that alterations of biochemical pathways outside of or beyond pigment production may predispose people to NMSC. Considering a significant increase in the risk of defect in the DNA

repair process, it is expected that SNPs in different DNA repair genes would affect human cancer susceptibility. Mocellin et al. (2009) systematically reviewed available evidence of the role of DNA repair gene polymorphisms in the risk of melanoma development. As a result of that study, it was demonstrated that there was sufficient evidence to suggest that a DNA repair gene, such as the XPD and ERCC2 genes might be a low-penetrance CM risk gene. Vitamin D receptor (VDR) polymorphisms were also found to be related to several types of cancers, including both NMSC and melanoma. Furthermore, Gandini et al. (2014) have done a systematic review of the literature to evaluate the relevance of greater VDR polymorphisms for different cancers. They found significant associations with VDR polymorphisms (Fok1, Bsm1, Taq1) and skin cancer. These polymorphisms were selected to highlight the role of gene polymorphism during the development of skin cancers, while there is a wide range of gene polymorphism associated with different types of skin cancers that are not discussed here.

18.4.3 Epigenetics

In addition to genetic events (e.g., mutation), epigenetic modification can abolish normal gene functions. Epigenetic is defined as the reversible heritable changes in gene expression that occur independent of changes in the primary DNA sequence. For example, DNA methylation and histone modification could alter gene expression, with no change in the underlying DNA sequence. In other words, epigenetics can modulate the human genetic program and can emphasize or silence genes. Hence, considering the deep involvement of genetic expression in cancer, manipulation with epigenetic modifiers could hold a great promise for cancer prevention, detection, and therapy. In contrast to a mutation, which is almost always irreversible, epigenetic alterations are potentially reversible. Overall, epigenetic events could be categorized into three broad groups: DNA methylation, histone modifications, and non-coding RNAs, particularly microRNAs (miRNAs).

One of the well-studied epigenetic changes is DNA methylation. In this process, the covalent addition of a methyl group generally occurs in the CpG islands, which causes the regulation of gene expression and stable gene silencing. Hypermethylation within the promoter regions of tumor suppressor genes is now firmly established as an important mechanism for gene inactivation. Additionally, loss of methylation (global hypomethylation) and the increasing of the mutation rate as a result of predisposing methylated cytosine to spontaneous, UVR, or chemical carcinogen-induced deamination could also contribute to epigenetic alterations (van Doorn et al. 2005). This could affect several critical processes, such as cell cycle, proliferation, DNA repair, carcinogen-metabolism, cell-adherence, and apoptosis. Stamatelli et al. (2014) analyzed the promoter methylation status of six genes in 52 sporadic BCCs and compared it with 26 matching normal tissues. They detected methylation at a variable frequency of 44, 33, 32.5, 32 and 14% of DCR2, APC, DCR1, RASSF1 and DAPK promoters, respectively. Methylation in

other genes, which could contribute to cell adhesion (CDH13, CDH1, CDH3, LAMA3, and LAMC2) (Sathyanarayana et al. 2007; et al. 2002b), negative regulator of the cell cycle (14-3-3 sigma) (Lodygin et al. 2003) and tumor suppressor genes (FHIT, SHH, APC, and SFRP5) (Brinkhuizen et al. 2012; Goldberg et al. 2006) were reported in those with BCC (Sathyanarayana et al. 2007; Goldberg et al. 2006; Brinkhuizen et al. 2012; Takeuchi et al. 2002b; Lodygin et al. 2003). Li et al. (2015) observed that DAPK1 methylation, a tumor suppressor candidate likely to be involved in tumor progression of cutaneous SCC, was associated with sun-exposure. Moreover, CDH13, which codes for a member of the cell-cell adhesion proteins, was also detected to be methylated mainly in lesional tissues in SCC patients. Interestingly, it was suggested that DAPK1 and CDH13 methylations were early and late events, respectively. In other studies with a fewer number of SCC, aberrant promoter methylation of tumor suppressor genes [p16INK4a, p14ARF (Brown et al. 2004), EXT1 (Ropero et al. 2004)], those involved in cell-cell adhesion [CDH1 (Chiles et al. 2003), CDH13 (Takeuchi et al. 2002a)], or cell growth and differentiation [FOXE1 (Venza et al. 2010)] were reported. In addition to NMSCs, different studies support the role of DNA methylation in melanomas. Hypermethylation of the different tumor suppressor genes [WIF1, SOCS1, RASSF1A, TFPI2, RAR-β2, MGMT, RASSF1A, and DAPK (Tanemura et al. 2009; Hoon et al. 2004)] were reported in two distinct studies. Additionally, there are other studies that covered a great numbers of cancer-linked genes in patients with melanomas [reviewed in (de Unamuno et al. 2015)]. Among some of the discussed studies, an association between sun-exposure and DNA methylation in critical genes in skin cancer were found. For example, it was found that methylation in some selected genes associated with cell adhesion was significantly related to sun exposure, while little or no methylation was reported in sun-protected specimens (Sathyanarayana et al. 2007). This could shed light on the involvement of UV exposure to the development of skin tumors. DNA methylation patterns could be employed as predictive biomarkers for treatment responses in patients with skin cancers. Changes in epigenetic modifications could be linked to histone modification, which is involved in both dynamic cellular processes (e.g., transcription and DNA repair), and stable maintenance of repressive chromatin.

Analogous to DNA methylation, histone modifications influence various involved processes in the development or suppression of tumor cells. However, data available on aberrant histone modification in skin cancer are limited. In contrast, despite the newly discovered roles of miRNAs, several studies have focused on their role in different types of cancer. miRNA is a small non-coding RNA molecule that functions in RNA silencing and post-transcriptional regulation of gene expression. These molecules influence critical processes in tumorigenesis, including inflammation, cell cycle regulation, stress response, differentiation, apoptosis, and invasion through prevention of miRNAs translation. miRNA expression is dysregulated in human cancer through various mechanisms, which make them the potential biomarkers for human cancer diagnosis, prognosis as well as therapeutic targets. In recent years, there have been suggestions to down-regulate or up-regulate a broad list of miRNAs in patients with NMSCs and melanomas. For example,

miR-1, miR-34a, miR-124/214, miR-125b, miR-155, miR-193b/365a, miR-199a, miR-361-5p, and miR-483-3p or miR-21, miR-31, miR-205, and miR-365 were reported to be down-regulated or up-regulated in cutaneous SCC, respectively [reviewed in (Yu and Li 2016)]). Moreover, de Unamuno et al. (2015) have prepared a list of different miRNAs, involved in melanoma development, which influence cell cycle proliferation, apoptosis, invasion, and immune response. There are some evidence of roles of miRNAs in BCC, while this field has not been studied equally well in case of SCC and melanoma (Sand et al. 2012, 2016, 2017).

18.5 Immunology of Skin Cancer

18.5.1 Immune Responses During Skin Cancer

The immune system is one of the main players in different diseases such as cancer, autoimmune and allergic diseases, and infections. Disruption of the immune response balance could lead to autoimmunity or failure in tumor elimination. While autoimmune diseases are the result of an aberrant immune system, the impairment of immune responses during cancers contributes indirectly to the promotion of tumor cells. Although genetic mutation is the well-known culprit of cancer development, impaired immune responses allow tumors to grow and spread. Regulatory T cells (Tregs) were found to be related to poorer overall survival in different cancers (Terme et al. 2013; Huang et al. 2012; Curiel et al. 2004). The host immune system shapes tumor fate through three phases—elimination, equilibrium, and escape (Mittal et al. 2014). The escape phase is the final one, indicating the inability of the immune system to eliminate cancer cells. Different immune responses are involved in each of these phases. One of the most critical cells involved in cancer are the natural killer (NK) cells, and different types of T cells. These cells can produce several cytokines, at the same time be activated or inhibited through several other signaling pathways. T-cells, which could be effective (anti-tumor) or Tregs, DCs, MCs, and macrophages, are the other involved immune cells that are discussed below. Immune cells can exert both positive and negative impacts on the outcome of a wide range of diseases, such as cancer. For example, NK cells, DCs, and effector T cells inhibit tumor cells, while Tregs promote tumor progression via exhaustion of the immune system. Ladanyi (2015), who discussed the roles of different cells in malignant melanoma (MM), concluded that patients benefit from prominent infiltration of mature DCs, B cells, and activated T lymphocytes. However, plasmacytoid DCs, or neutrophil granulocytes are considered the unfavorable prognostic factors in this disease.

These cells establish very complex immune networks, which could significantly influence cancer outcomes. Hence, the recognition of these networks is critical to shed light on novel therapeutic approaches as well as the prevention of cancer development. Considering various facts about melanoma, including different identified melanoma-specific antigens, involvement of several immune responses

during this disease, responding of patients with metastatic melanoma to immune-stimulating agents, and its reverse correlation with simultaneous onset of vitiligo, it was referred as one of the most immunogenic types of cancer (Jacobs et al. 2012; Daneshpazhooh et al. 2006). Moreover, there is some evidence of immune system involvement in NMSCs (SCC and BCC), such as the changing of the profile of immune cells and a close relation with autoimmunity/infections (which have been discussed in the rest of this chapter).

Natural Killer Cells

NK cells, which control several types of tumors by limiting their growth and dissemination, are critical players in the innate immune system. There is some evidence that NK cells are involved in the elimination of tumor cells. Moreover, these cells can promote an adaptive immune system through interactions with DCs (Degli-Esposti and Smyth 2005). NK cells lyse the tumor cells via perforin and granzymes as well as proinflammatory cytokines, including the tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ). Thus, the decreased number or dysfunction of these cells could make individuals susceptible to the development of cancer. Skin cancers are not exceptions. It was found that functional interactions between killer cell immunoglobulin-like receptor (KIR), NK cell MHC class I specific receptors and human leukocyte antigen (HLA) modify the risks of BCC and SCC (Vineretsky et al. 2016). That study clearly implies the critical involvement of NK cells during the NMSCs. To support the role of NK cells during the NMSC, Carroll et al. (2010) have shown that a low number of NK cells in kidney transplant recipients increases more than six-fold the risk of developing a new SCC within 200 days.

Although NK cells often efficiently recognize melanoma cells in vitro and tumor surveillance in vivo, tumor cells employ various strategies to evade immunosurveillance by these cells. However, an infiltration of NK cells in regressing melanoma lesions could contribute to anti-tumor immunity. These functions of NK cells have led to the idea of NK cell application for melanoma therapy (Holsken et al. 2015). It was shown that melanoma cells alter the expression of triggering receptors, such as NKp30, NKp44, and NKG2D, toward inhibition through indo leamine 2, 3-dioxygenase (IDO) and prostaglandin E2 (PGE2). Consequently, NK cell-mediated cytolytic activities against melanoma cells are impaired (Pietra et al. 2012).

Regulatory T cells

Regulatory responses are critical to prevent the development of autoimmunity in individuals, who are susceptible to autoimmunity against self-antigens. In contrast, they are deemed culprit for the persistence of chronic infections, such as the hepatitis B virus (HBV) as well as cancer progression (Tavakolpour 2016; Tavakolpour et al. 2016; Ha 2009). Tregs infiltrate into the tumor microenvironment and dampen the immune responses to tumor cells via contact-dependent and contact-independent mechanisms. These cells or their associated genes enter in the escape phase. For example, forkhead box P3 (FoxP3), the master regulator of Tregs, is critical to immune evasion/escape.

Generally, it is believed that the dominant regulatory response is associated with different types of cancer. It seems that the recruitment of the tumor could be regulated by tumor-derived chemokines (e.g., CCL17, CCL18, CCL22) as well as the secretion of regulatory cytokines (e.g., transforming growth factor-beta [TGF-β], interleukin [IL]-10, IL-35) to induce differentiation of Tregs and enhance their functions. There is some evidence implying that immunosuppression in OTRs increases the risk of the development of SCC and BCC (Athar et al. 2011). This may be explained by drug-induced regulatory responses, inducing T helper (Th)2 dominance in the microenvironment of SCC in OTRs, shown by a higher expression of Th2-related genes.

It was recently found that intratumoral recruitment of Tregs, which may be due to a high expression of two well-known regulatory responses,TGF-β1 and IL-10, as well as a decrease in the $CD8^+$/$FoxP3^+$ $CD25^+$ T-cell ratio may contribute to the aggressiveness of cutaneous SCC (Azzimonti et al. 2015). Interestingly, an association of the increase in Treg/$CD8^+$ T cells ratio (could also be seen as a decrease in $CD8^+$ T cells/Treg) is associated with an aggressive growth of transplant-induced SCC (Zhang et al. 2013). A higher prevalence of Tregs was also reported in SCC and Bowen's disease, when compared to the AK (Jang 2008). Moreover, the altered immune responses significantly increase the risk of metastasis in SCC patients. It was demonstrated that high Treg ($FoxP3^+CD4^+CD127^{low}$) and low $CD8^+$ T cells are predictors of subsequent SCC (Carroll et al. 2010). The cytokines also have important roles in inducing regulatory responses. These cells can usually be interesting targets to reverse anti-tumor immunity. For example, increased IL-22 and IL-22 receptor expression could provide a proliferative stimulus and accelerate tumor growth in transplant patients (Zhang et al. 2013). Accordingly, targeting IL-22 was suggested as a new therapeutic option for patients with highly aggressive and sometimes fatal forms of SCCs (Zhang et al. 2013). Moreover, in another study, it was demonstrated that high levels of IL-22 and IL-17 in the BCC and SCC microenvironment promote tumor progression (Nardinocchi et al. 2015). Regulatory cytokines, such as IL-10 and TGF-β, contribute to the suppression of effector T-cells, which could be reduced via imiquimod. Another well-known cytokine that have a key function in the progression of skin cancer is IL-6. This cytokine promotes tumor growth by inhibition of apoptosis and induces tumor angiogenesis. In fact, although IL-6 does not cause the promotion of regulatory responses, it regulates a complex cytokine and protease network and stimulates malignant progression by autocrine and paracrine mechanisms in skin SCCs (Lederle et al. 2011). The important role of this cytokine was also discussed in the development and progression of melanoma (Hoejberg et al. 2012). It is suggested that IL-23 has also a strong tumor-promoting role in the skin (Langowski et al. 2006).

UVR, a well-recognized risk factor for the development of skin cancer may mediate immune responses. In fact, UVR not only cause DNA damage, but also suppress protective cellular anti-tumoral immune responses (Urbach 1997). Chen et al. (2011) speculated that UVR may have a direct impact on FoxP3 expression and/or transcription, resulting in anti-tumoral immune restraint. The impact of

FoxP3 via UVR is not limited to the mentioned signaling pathways. Other possible pathways include manipulation and interaction of the epidermal receptor activator of NF-κB ligand, VDR as well as an estrogen receptor. Similar to SCC, the contributions of Tregs and Th2 associated cytokines was suggested in the tumor microenvironment during the BCC (Kaporis et al. 2007).

In melanoma, an increased number of Tregs was reported by several authors. Correll et al. (2010) observed a higher number of Tregs in the peripheral blood of patients at Stage IV melanoma when compared to healthy donors, and was correlated with a reduction in T-cell responsiveness. Interestingly, they have found that those patients benefited from DC-based immunotherapy, which causes the restoration of an antigen-specific immunity and a decrease in Tregs frequencies. Similar to those studies, several other articles reported increased in Treg frequency in peripheral blood of patients with metastatic melanoma compared with healthy controls [reviewed in (Jacobs et al. 2012)].

There is a consensus on the significant contribution of Tregs in melanoma, nevertheless it was suggested that Treg functions might be replaced by other immunosuppressive cells and factors during melanoma progression. Indeed, Kimpfler et al. (2009) used the transgenic mouse spontaneous melanoma model and Treg from lymphoid organs were depleted by anti-CD25 monoclonal antibody. Despite the confirmation of successful Treg depletion, melanoma was not significantly delayed in comparison to the non-treated mice. This was an unexpected occurrence, which led to the hypothesis that involvement of other immunosuppressive cells and factors could be replaced by the Treg during melanoma progression. This speculation could have a positive impact on more effective targeted therapies for melanoma. It is worthy to note that an increase in regulatory responses is not limited to the above mentioned skin cancer types. In fact, T-cell exhaustion via the activity of Tregs as well as an increase in the programmed death 1 (PD-1) expression on non-activated T cells was reported in merkel cell carcinomas, which is a rare but highly malignant skin cancer (Dowlatshahi et al. 2013). As discussed, Tregs contribute to the impairment of T-cell-mediated immune responses through different cytokines and chemokines during skin cancers. However, this is not limited to these factors but also the exhaustion of T cells via co-inhibitory molecules, such as PD-1 and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) (Mishra et al. 2016b; Yuan et al. 2011). This has led to the emergence of novel immunotherapy approaches for skin cancer, including anti-CTLA-4 and anti-PD-1 therapies. Ipilimumab (anti-CTLA-4), pembrolizumab (anti-PD-1), and nivolumab (anti-PD-1) have been approved for melanoma patients. This aspect will be discussed in the treatment section.

Anti-tumor T-cell Responses

In contrast to Tregs, effector T cells can stimulate immune responses. There are different subsets of these cells, including Th1, Th2, Th9, Th17, Th22, and cytotoxic T-cell (Tc). The majority of these cells contributes to the development of autoimmunity. However, their roles are completely different during cancer. In the published studies, Th1, Th17, and Tc were found to be critical players in skin cancers.

For example, Yokogawa et al. (2013) have shown that de novo growth of UVB-induced SCC could be attenuated through the activation of Th17/Th1 cells and Tc via imiquimod treatment. In another study, imiquimod was found to be an effective treatment for SCC, which causes a greater production of IFN-γ, granzyme, and perforin, but less IL-10 and TGF-β (Huang et al. 2009). In contrast to protective role of the mentioned Th cells, Th2 cells are associated with the impairment of immunity to human BCC. This negative impact may be explained by the skewing Th1:Th2 balance toward greater differentiation of Th1 cells (Kaporis et al. 2007). Surprisingly, it was suggested that these undesirable cells could promote tumor elimination through recruitment of eosinophils and contribute to the rejection of melanoma (Eftimie et al. 2010).

There are several critical cytokines that play a role during anti-tumor immunity in skin cancers. Although TNF was first described as a cytotoxic molecule for cancer cells, nowadays, it is considered a cytokine with a controversial role in melanoma (Donia et al. 2016). Nevertheless, systemic IFN-α-2b and high-dose IL-2 are both being used as adjuvants for late-stage melanoma (Mocellin et al. 2010; Tsai 2007). It was found that they have an anti-tumor role in skin cancer, which may be explained by the contribution of these cytokine to the differentiation of Th2 towards Th1 cells, or promotion of infiltrated cytotoxic T cells, which are followed by a greater production of IFN-γ and anti-tumor responses (Langowski et al. 2006).

IL-21, a proinflammatory cytokine, is able to induce the immune activation of NK cells as well as $CD8^+$ T cells in patients with metastatic melanoma. Accordingly, a systemic IL-21 is a potential therapy for metastatic melanoma (Frederiksen et al. 2008). IL-27 could promote anti-tumor responses in melanoma via Tc or NK cells and its antiangiogenic effect (Nagai et al. 2010).

$CD8^+$ T cells are another arm of anti-tumoral immunity required for promoting NK cell infiltration within SCCs. However, these cells may be exhausted during SCC through the expression of inhibitory receptors, PD-1 and lymphocyte-activation gene 3 (LAG-3). To restore the tumor suppression ability of the immune system, these molecules could be blocked via the employment of different available biological agents. Interestingly, in a mouse model of SCC reversing this process via dual blockade of both PD-1 and LAG-3 led to a significant suppression of tumor growth (Mishra et al. 2016a).

Dendritic cells

DCs play a pivotal role in the tumor microenvironment, influencing disease progression in many human malignancies. However, similar to various other immune responses, tumor cells can escape in such a way that immunosuppressive DCs are recruited. A correlation of DCs and the prevalence of Tregs as well as the locating of these cells in direct proximity to Tregs is another finding made while analyzing skin samples obtained from patients with SCC and Bowen's disease. Moreover, a greater number of DCs was found in these two diseases compared with AK (Jang 2008). In line with the critical roles of DCs in skin cancer, it was shown that those with the late antigen-specific immunity were restored via DC-based immunotherapy (Correll et al. 2010).

Macrophages

Macrophages may either inhibit or stimulate tumor growth. In fact, these immune cells are considered the double-edged sword during cancer. There is evidence of participation of tumor-associated macrophages (TAMs) in the early eradication of tumor cells in vitro (Romieu-Mourez et al. 2006), as well as contribution to carcinogenesis (Bingle et al. 2002). Analogous to other cancers, TAMs have apparent paradoxical effects in MM. In a way, activated TAMs can eradicate the neoplastic cells through different signaling pathways, which is followed by the induction of tumor inhibition. In contrast, TAMs can also produce tumor growth-promoting factors, which along with the contribution of immune response alteration could lead to tumor growth promotion (Hussein 2006). Moreover, recent studies have suggested the contribution of macrophages in the development of resistance to BRAF inhibitors in melanoma patients (Smith et al. 2014). In contrast to studies supporting the negative impact of macrophages on skin cancer, it was found that cutaneous macrophages in the tumor microenvironment exerted an anti-tumor effect on BCC.

Mast cells

MC is another immune cell involved in cancer, which seems capable of modulating tumor biology via infiltrating tumors. The role of MC infiltration into tumors remained unclear. Similar to other cancers, MCs are major players in cutaneous malignancies. These cells could exert their effects on the development and spread of cutaneous malignancies through various pathways, including immunosuppression, enhancement of angiogenesis, disruption of the extracellular matrix, and the promotion of tumor cell mitosis (Ch'ng et al. 2005). It was reported that MC tissue density in some cases of invasive melanoma increased compared to benign nevi and in situ melanoma (Duncan et al. 1998). Moreover, a greater number of MCs were detected in melanoma lesions of poor prognosis, compared to those with good prognosis (Duncan et al. 1998). Additionally, the critical role of MCs in the angiogenesis of BCC through the production of the vascular endothelial growth factor (VEGF) and IL-8 was suggested (Aoki et al. 2003). The prevalence of high dermal MCs is a predisposing factor for the development of BCC in humans. According these facts, although MCs can either be beneficial or detrimental to tumor development, targeting MCs is suggested as a potent therapeutic option in the treatment of cancer (Groot Kormelink et al. 2009).

18.5.2 Skin Cancers and Autoimmunity or Infections

18.5.2.1 Autoimmunity

Although no direct correlation between the autoimmunity and skin cancer has been found, there is paucity of evidence implying the relation of some types of skin cancers and some autoimmune diseases or their treatments, (including vitiligo,

rheumatoid arthritis [RA], systemic lupus erythematosus [SLE], and alopecia areata [AA]). These relations could be explained by the alteration in immunological responses under any of these conditions that could affect the other one. Moreover, the involvement of drug interactions, possible genes mutations, or epigenetic alterations cannot be neglected. Interestingly, it was suggested that autoimmunity could improve the survival in melanoma (Senior 2006). Moreover, the development of vitiligo clearly portends enhanced survival in those with melanoma and reduces the risk of both melanoma and NMSC (Byrne and Turk 2011; Teulings et al. 2013). It was also demonstrated that AA was associated with a decreased risk of skin cancers (Mostaghimi et al. 2016). Recently, a meta-analysis has found a reduced risk of skin melanoma, but increased risk in NMSCs among patients with SLE (Cao et al. 2015). However, there are some other studies that revealed the increased risk of skin cancers in those with some autoimmune diseases. For example, SCC was found to be more prevalent in RA patients (Hemminki et al. 2008). Moreover, there is some evidence of a significantly increased risk of NMSC in patients with AIH on immunosuppression (Leung et al. 2010). Therefore, a careful recording of skin cancer development is essential during the treatment of some autoimmune diseases, such as RA and psoriasis (Raaschou et al. 2016; van Lumig et al. 2015). In a few studies, a high risk of skin cancer development was shown in patients who had received methotrexate, azathioprine, natalizumab, and TNF-inhibitors (Buchbinder et al. 2008; Pedersen et al. 2014; Bergamaschi and Montomoli 2009).

18.5.2.2 Infections

Several mechanisms are used by bacterial and viral pathogens to subvert and exploit immune systems. The majority of persistent infections seem to be able to manipulate the immune system to prevent clearance, usually through the exhaustion of immune responses. Thus, infections could promote Tregs, which are considered a barrier to anti-tumor immunity. Among chronic infection, HIV infection has a special role. During this infection, a gradual loss of peripheral $CD4^+$ T cells and the depletion of the cellular immune arm lead to the development of tumors. Different AIDS-related cancers, including Kaposi sarcoma, non-Hodgkin lymphoma, and cervical cancer have been identified. There is paucity of evidence to support the potential role of HIV infection in the development of NMSCs as well as melanomas. For example, Silverberg et al. (2013) have shown twice the incidence rate of BCCs and SCCs among HIV-positive subjects. Additionally, a meta-analysis led to the conclusion that both NMSCs and melanoma occurred at increased rates in patients with HIV/AIDS (Grulich et al. 2007). HPV, the most ubiquitous of the human viruses, is related to skin cancers and several studies have found HPV as a trigger for the development of skin cancers (McLaughlin-Drubin 2015).

Another well-known relation between skin tumors and viruses is the Merkel Cell Carcinoma (MCC). MCC is a primary neuroendocrine carcinoma of the skin with

aggressive behavior. It presents as a solitary, rapidly growing nodule, usually in the head and neck, and can become ulcerated. More than 80% of MCC cases are associated with a kind of polyomavirus, called "Merkel cell Polyomavirus" (Kassem et al. 2008). Another important proven relation between skin cancers and virus is the association between Kaposi Sarcoma (KS) and the Human Herpesvirus 8 (HHV8). Interestingly, nearly all cases of KS are nearly all cases of KS are associated with previous infection of HHV8 (Carroll et al. 2004; Wang et al. 2004). There is limited evidence to support the contribution of other viruses in the development of skin cancers. For example, Zafiropoulos et al. (2003) analyzed cytomegalovirus (CMV), herpes simplex virus (HSV) and Epstein–Barr virus (EBV) genomes in patients with NMSCs. They suggested that CMV, but not the two other viruses, could possibly be linked to NMSCs.

18.6 Prevention and Treatment

18.6.1 Skin Cancer Prevention and Screening

18.6.1.1 Prevention

Strict sun avoidance

Considering UV as the main factor in cutaneous carcinogenesis, extremely rigorous photoprotection is the main intervention in the lifestyle of patients with NMCSs. The main recommendations are the avoidance of sun exposure at peak hours, and the use of protective clothing, sunglasses and sunscreen.

Chemoprevention

Vismodegib, an inhibitor of the Hh pathway, is the outstanding new development in the treatment and prevention of BCCs especially in NBCCs. Two separate trials of patients with NBCCs showed that 150 mg/day of vismodegib could reduce rate of new BCCs requiring surgery and could induce thcombination of these actionse regression of existing BCCs. Although tumors progressed after the discontinuation of the therapy, patients who resumed treatment again experienced tumor response (Tang et al. 2012, 2016). The adverse events included taste disturbance, muscle cramps, hair loss, and weight loss. It led to the discontinuation of medication in 54% of the subjects (Tang et al. 2012). On the other hand, there are some new reports that vismodegib can reduce the size of keratocystic odontogenic tumors in NBCCs (Ally et al. 2014; Goldberg et al. 2011).

Oral retinoids

There are some evidence that oral retinoids (acitretin and isotretinoin) are effective in the chemoprevention and treatment of multiple BCCs, particularly in NBCC

patients. Retinoids are prescribed to OTRs to prevent the development of SCC due to immunosuppression. The activation of the retinoid pathway is responsible for cell growth arrest and cell differentiation, which can lead to apoptosis. Indeed, retinoids act through different signaling pathways, such as the impedance of proliferation, inducing apoptosis, the inducing of differentiation, or a combination of these actions (Asgari et al. 2012; Tang and Gudas 2011; Bushue and Wan 2010; Niles 2000). Goldberg et al. (1989) reported a significant decrease (23%) in the number of new tumors per year in 12 patients with multiple BCCs, treated by isotretinoin without any adverse effect. On the other hand, oral isotretinoin has been successfully used as a chemoprevention agent in XP patients. Oral isotretinoin, with a dose of 2 mg/kg/day, resulted in an average reduction of 63% of NMSCs (DiGiovanna 1998; Kraemer et al. 1988).

Nicotinamide

A prominent new data is the preventive effects of oral nicotinamide (vitamin B3) for NMSCs. In a trial with 386 individuals having a history of at least two NMSCs within five years before study enrollment, Chen et al. (2015) reported that nicotinamide 500 mg administered twice daily for nine months led to a 20% reduction in the incidence of new BCCs and 23% in new NMSCs with no adverse effects.

Non-steroidal anti-inflammatory drugs

There are many controversies regarding the preventative effects of non-steroidal anti-inflammatory drugs (NSAIDs) for NMSCs and MM. Considering the fact that inflammation is one of the main cutaneous responses to the UVR, gives rise to the idea that NSAIDs could be used for preventing the UV-induced inflammatory skin conditions, such as sunburn and cancers. NSAIDs apply their anti-inflammatory effects via the inhibition of NF-κB and cyclooxygenase (COX) enzymes (COX-1, COX-2), both of which are over-expressed in MM (Albano et al. 2013; McNulty et al. 2004; Thun et al. 2002). A few trials have shown that there is a potential protective effect for SCC and BCC, but only when NSAIDs were used for a short term (Clouser et al. 2009). In addition, some other trials have shown that NSAIDs also have preventative effects against MM (Cook et al. 2005; Johannesdottir et al. 2012; Harris et al. 2001), although some others did not show this positive effect (Jeter et al. 2012).

Statins

Statins are inhibitors of the HMG-CoA reductase. In addition to its cholesterol lowering effects, statins have well-known anti-proliferative, anti-invasive, and immunomodulatory properties that inhibit Ras pathway proteins as well as tumorigenic activities (Bonovas et al. 2010; Boudreau et al. 2010). There is some evidence that statins may prevent MM occurrence, while a recent meta-analysis found that there was no evidence of reduced melanoma incidence in statin-using patients (Livingstone et al. 2014).

18.6.1.2 Screening

While the 10-year survival proportion of patients with CM less than 0.75-mm-thick was more than 98%, this figure dropped to less than 46% in those with CM of more than 4-mm-thick (Marghoob et al. 2000). This emphasizes how early detection of CM can be life-saving for the patient as well as financially cost beneficial to the health care systems. A thorough skin examination looking for suspicious lesions using the "ABCDE" rule and "Ugly duckling" sign is the basis of every screening. According to the American Cancer Society, primary-care physicians should check men and women older than 20 years for skin cancer during their periodic health examinations (Mayer et al. 2014). Dermoscopes are valuable aiding tools that increase the sensitivity of cutaneous melanoma diagnosis (Vestergaard et al. 2008). Total body photography and confocal laser microscopy are the other useful, albeit expensive, modalities.

A screening of the general population is not generally recommended; however, there are many guidelines regarding high-risk individuals (Vestergaard et al. 2008). There is no general consensus on the various aspects of screening, among them the definition of risk factors and the intervals of examination (Vestergaard et al. 2008). The strongest risk factors are germline CDKN2A mutations, multiple nevi (greater than 100), >5 atypical nevi, a strong family history of melanoma (i.e., two to three first-degree relatives) and a personal history of melanoma (Vestergaard et al. 2008). High-risk individuals should strictly adhere to norms of protection from sun and other sources of UV and be educated about skin self-examination. They should be screened for new or changing lesions at regular intervals and those at risk of pancreatic cancer should be referred to gastroenterologists. Genetic testing for CDKN2A should be done only after a comprehensive genetic counseling of those at risk.

Dermoscopy is a new noninvasive diagnostic technique that is being recently used for the diagnosis of various dermatological diseases, especially pigmented lesions. Dermatoscope consists of a lens with 10-fold magnification and polarized light. This system can eliminate scattered surface reflections, so that it can provide real views from the basal layers of the epidermis and even superficial dermis. Based on the various patterns seen in the dermatoscope (pigmentation, vascular and various colors), the clinician can conclude whether the lesion is basically melanocytic and, if so, whether it is a benign lesion or suspected to be malignant. Dermatoscope is a new tool in the diagnosis skin lesions, screening of lesions in terms of malignancy, especially helpful for the screening of lesions for BCC, SCC and CM (Zalaudek et al. 2006; Rudnicka et al. 2008).

18.6.2 Conventional Treatments for Skin Cancer

18.6.2.1 Non-melanoma Skin Cancers

As a rule of thumb, the treatment of NMSCs is the destruction of the tumoral tissue with safe margins and the most trustful modality is the total excision with safe margin and Moh's surgery. The standard surgical excision with a 4–6 mm margin is effective for most primary NMSCs; with a 98% response in treatment (Telfer et al. 2008). Moh's surgery is of a specific nature in which all the margins of the excised tumor are checked pathologically for any residue of tumoral involvement, while allowing the maximum conservation of the normal tissue. As a result, Moh's surgery clearly has the highest cure rate and lowest recurrence rate (long-term recurrence rate of 5.6% superior to other modalities, including excision [17.4%], radiation therapy [9.8%] and curettage with electrodesiccation [40%]) (Rowe et al. 1989). Other destructive modalities include: cryosurgery, curettage and electrodessication (Soyer et al. 2012f).

Cryosurgery

Cryosurgery is the destruction of tumoral tissues with liquid nitrogen. It is fast and easy and effective especially in superficial lesions. However, subsequent recurrences may become extensive because of concealment by the fibrous scar tissue (Soyer et al. 2012b).

Curettage with electrodesiccation

Curettage with electrodesiccation is the destruction of the tumor with three cycles of combined curettage and electrodesiccation. In a proper setting of patient and technique, the cure rates can be as high as 97–98% (Honeycutt and Jansen 1973).

Radiation therapy

Radiation therapy (RT) is the destruction of the tumor by X-ray. It is another modality of treatment when excision is impossible. RT can also be used as an adjuvant therapy of NMSCs, when there is a high risk of recurrence, perineural invasion and in cases of recurrent NMSCs (Soyer et al. 2012b).

Chemotherapy

Chemotherapy is the modality of choice in the treatment of metastatic SCC. There is currently no standard treatment of metastatic disease, but cisplatin with either 5-fluorouracil (5-FU), doxorubicin or bleomycin have demonstrated some degree of efficacy, achieving complete responses in some cases. In addition, nowadays, the EGFR inhibitors are new and more effective agents with more successful response (DeConti 2012).

18.6.2.2 Cutaneous Melanoma

Surgical Management

Since melanoma cells may extend subclinically several millimeters to several centimeters beyond the clinically visible lesion, the main treatment strategy for early-stage MM is total excision with a proper free margin. The proper surgical margin depends on the tumor thickness of the lesion (Garbe and Bauer 2012a). Specific surgical margin recommendations for primary invasive melanoma are shown in Table 18.5.

Sentinel Lymph Node Biopsy

Sentinel lymph node biopsy (SLNB) is the detection of micrometastatic melanoma in regional lymph nodes by the aid of radioisotopes or blue dye. As the sentinel node is the initial site of regional metastasis, its tumor status accurately predicts the tumor status of other nodes in the lymphatic basin. In this procedure, technetium sulfur colloid or blue dye is injected into the skin surrounding the melanoma biopsy site. Afterwards, with the help of a hand-held gamma counter and visual inspection the "hot/blue" sentinel node(s) is (are) detected, biopsied, and histologically examined by H & E stains combined with immunohistochemistry (S 100, HMB45). If melanoma micrometastasis is identified, then a complete regional lymph node dissection is normally recommended (Garbe and Bauer 2012b).

Radiotherapy

RT has a limited success in the treatment of CM and its role is confined to just palliative therapy in cases of skin, bone and brain metastases (Perera et al. 2013).

Chemotherapy

Chemotherapy was previously the main strategy for treating metastatic CM. The main agents were cytotoxic agents like dacarbazine, temozolomide, fotemustine, although most of them had limited or even no success (Avril et al. 2004; Middleton et al. 2000).

Table 18.5 Surgical margin recommendations for primary cutaneous melanoma

Tumor thickness	Clinically measured surgical margin (cm)
CM in situ	0.5–1.0
Up to 1.0 mm	1
1.01–2.0 mm	1–2
>2.0 mm	2

From Bichakjian et al. (2011)

[a]Wider margins may be necessary for lentigo maligna subtype

18.6.2.3 Novel and Emerging Treatments

The standard treatments (surgery, radiation and chemotherapy) were found effective for NMSCs. However, melanomas—especially advanced melanomas—are known for their poor response to conventional treatment options. Indeed, these treatments have had minimal impact on the overall survival of patients with metastatic melanoma. Additionally, chemotherapy and radiation predominantly kill proliferating cells without discriminating between cancer and normal host cells. Hence, there arises a need for targeted therapy to specifically target tumor cells in order to reduce normal tissue toxicity and side effects. Those with promising results could replace those treatments with lower efficacy or with a high rate of side effects.

Immunotherapy

Cytokines

Employment of IL-2 and IFN-α is the first steps of immunotherapy in patients with cancers such as advanced melanoma (Dutcher et al. 1989; Kirkwood and Ernstoff 1984). IL-2 is a cytokine that promotes anti-tumor immunity through NK cell proliferation and cytotoxic activity; and causes greater production of critical cytokines, such as IFN-γ and TNF-α. Prolonged disease control seen with IL-2 led the FDA to approve a high-dose IL-2 therapy for metastatic melanoma. However, many subsequent studies did not find it a promising treatment option for melanoma. Hence, due to numerous unwanted side effects and a low response rate, IL-2 has not been widely accepted. IFN-α established a significant improvement in both relapse-free and overall survival in melanoma patients, though several side effects were reported. The side effects (e.g., flu-like symptoms) and a prolonged course of treatment course have a significantly negative impact on patients' quality of life, which limited the use of this treatment. In addition to these approved treatments, some studies have suggested targeting IL-22 as a novel potential treatment, but it needs further studies (Zhang et al. 2013).

Checkpoint inhibitors

Treatment with cytokines, such as IL-2 or IFN therapies, tries to directly stimulate anti-tumor immunity. However, recent efforts aimed at triggering robust immune responses via the inhibition of immune checkpoints, such as CTLA-4, PD-1, T-cell immunoglobulin and mucin-domain containing-3 (TIM-3) and LAG-3. These co-inhibitory molecules are negative regulators of T cell activation and are essential to the prevention of autoimmunity. In contrast, these receptors are responsible of immune system exhaustion during persistent infections (e.g., HBV) and different types of cancer. CTLA-4 (also known as CD152) engagement results in a down-regulation of T cell activation by ablation of co-stimulatory ligands from

antigen-presenting cells (APCs). PD-1 is another co-inhibitory receptor expressed on T cells that is important for tumor immune evasion. Thus, their inhibition could be a logical way to restore impaired anti-tumor immunity. The FDA has approved the checkpoint inhibitor drugs to target CTLA-4 (ipilimumab) and PD-1 (pembrolizumab and nivolumab) to treat melanoma. Moreover, a combination of ipilimumab and nivolumab has also been approved. Monotherapy or combination of other identified checkpoints also could be tested for the treatment of those with advanced melanoma. There is some evidence to support the priority of combination therapy over monotherapy. For example, in phase II trial, 2-year overall survival was reported as 63·8% (95% CI 53·3–72·6) and 53·6% (95% CI 38·1–66·8) for those assigned to nivolumab plus ipilimumab and ipilimumab alone, respectively (Hodi et al. 2016). Although these treatments seem to be promising, not all patients respond to checkpoint inhibitors. Additionally, due to the protective roles of co-inhibitory molecules in autoimmunity, the expected side effect is the development of autoimmune or inflammatory diseases. As predicted, these side effects seem to be observed more in patients who are genetically susceptible to the development of autoimmunity. There are also other immune related adverse effects, including diarrhea/colitis, dermatitis, hepatitis, and endocrinopathy, which mainly affect those treated with ipilimumab (Robert et al. 2015b). The adverse effects of anti-PD-1 agents (pembrolizumab or nivolumab) are less than anti-CTLA-4 (ipilimumab) (Robert et al. 2015b).

Targeted Therapy

Targeted therapy can be classified as a special type of chemotherapy that takes advantage of differences between normal cells and cancer cells. Hence, it would be reasonable to expect that these treatments would be associated with higher efficacy as well as fewer side effects compared to conventional non-surgical interventions. Different targeted therapies are available for NMSCs and melanoma. The Hh pathway inhibitors and epidermal growth factor receptor (EGFR) pathway inhibitors have been suggested as promising treatments for advanced BCC and SCC, respectively. However, BRAF, MEK, and KIT inhibitors have shown promising results in melanoma treatment.

Aberrant activation of the Hh signaling pathway was found to be associated with the development of several types of cancer, particularly BCC. Hh is a key regulator of cell growth and differentiation during embryonic development, which controls the cell cycle in a variety of circumstances. Moreover, it plays crucial roles in adult tissue maintenance, renewal, and regeneration. The majority of basal-cell tumors have mutations in the Hh signaling pathway, which may lead to the inactivation of PTCH1 (more frequently) or constitutively active SMO (less frequently). Thus, it is not surprising that mutations in components of the Hh-induced pathway be the culprit of almost all sporadic BCC. These mutations, which usually occur in PTCH1 and SMO cause aberrant Hh signaling pathways and is followed by unrestrained proliferation of basal cells of the skin. In addition to sporadic BCC,

some of associated syndromes are related to Hh signaling pathways. For example, NBCC is mainly caused by a mutation of PTCH1, which is a member of Hh signaling. Accordingly, this signaling pathway may be a desirable target in BCC patients. Vismodegib is the first oral Hh signal pathway inhibitor that binds selectively to the transmembrane smoothened protein (encoded by the SMO gene), inhibiting the Hh signaling pathway followed by tumor regression. It was suggested as a new treatment option for patients with advanced BCC, which led to the approval of vismodegib by the FDA (Sekulic et al. 2012). Sonidegib, another therapeutic option for the inhibition of Hh signaling pathways, has been recently approved for treating BCC patients.

EGFR, a transmembrane cell surface receptor induces receptor dimerization and tyrosine autophosphorylation by binding to a ligand. Subsequently, different events in line with the promotion of tumor, such as inhibition of apoptosis, promotion of cell growth and proliferation, and angiogenesis could happen (Spallone et al. 2011). Cetuximab is an EGFR inhibitor that was approved for metastatic colorectal cancer and head and neck SCC. Although this drug has not yet received FDA approval for cutaneous SCC, there is some evidence of successful cetuximab monotherapy, which led to its introduction as an alternative treatment for patients with advanced cutaneous SCC (Maubec et al. 2011; Conen et al. 2014; Seber et al. 2016).

Three choices—BRAF inhibitors, MEK inhibitors, and KIT inhibitors—are available for melanoma targeted therapies based on FDA approval. Approximately 40–60% of advanced melanomas harbor activating BRAF mutations (over 90% V600E). Vemurafenib and dabrafenib are two approved BRAF inhibitors for the treatment of melanoma in adult patients with BRAF V600 mutation. Dabrafenib had been approved two years after vemurafenib for advanced melanoma, only for those with V600E mutation. Compared to dacarbazine, an approved chemotherapy medication, used in the treatment of melanoma, an improved response rate and higher median overall survival were reported with vemurafenib and dabrafenib. Despite the promising results of these treatments in metastatic BRAF mutant melanoma patients, around 20% of patients are intrinsically resistant to these therapies and most of the initial responders eventually develop mechanisms of acquired resistance (Manzano et al. 2016). However, several strategies to overcome the resistance have been suggested (e.g., combination with other targeted therapies) (Manzano et al. 2016). The BRAF inhibition is associated with several toxicities including cutaneous toxicity, secondary tumors, arthralgia, fatigue, and alopecia. These adverse effects are serious barriers to the employment of these drugs (Larkin et al. 2014).

Since MEK1 and MEK2 have crucial roles in the progression of tumor cells in melanoma, MEK1/2 inhibition is employed as a therapeutic strategy. Trametinib is a potent, highly specific inhibitor of MEK1/2, which was approved for metastatic BRAF-mutated melanoma. Cobimetinib is another MEK inhibitor, which has been recently approved by the FDA for metastatic melanomas with a BRAF V600E or V600K mutation, in combination with vemurafenib. Binimetinib and selumetinib are two other potent MEK inhibitors, which have not yet received FDA approval. Similar to BRAF inhibitors, MEK inhibitors have some advantages compared to

chemotherapy, but the problems of drug resistance and serious adverse events persist. A combination of BRAF and MEK inhibitors is the gold standard for BRAF-mutated metastatic melanoma. Interestingly, it was found that a combination of BRAF inhibitor (dabrafenib) with MEK inhibitor (trametinib) could improve progression-free survival (PFS), compared to monotherapy with BRAF inhibitors. Moreover, it delays the appearance of alterations involved in resistance (Robert et al. 2015a).

A minority of patients with melanoma have mutated C-KIT gene. The identification of these oncogenic KIT mutations results in the development of a new class of target therapies, which could be effective in melanomas driven by such alterations. Imatinib, nilotinib, and dasatinib are three potent KIT inhibitors. Imatinib treatment results in significant clinical responses among those with advanced melanoma harboring KIT alterations (Carvajal et al. 2011). Additionally, in a phase-II study, it was shown that nilotinib controlled the disease in patients with melanomas harboring KIT alterations as well as in those with disease progression despite imatinib therapy (Carvajal et al. 2015).

Adoptive Cell Transfer

Adoptive cell transfer (ACT) can yield durable responses in patients with metastatic melanoma. During this strategy, autologous or allogenic tumor-infiltrating lymphocytes (TILs) after expansion and modification are administrated to patients to shrink the tumors. Generally, in patients with melanoma, ACT aims to first control the growth and spread of melanoma cells and, secondly, ensure the shrinkage of recurring and metastatic melanoma tumors.

Although ACT has not been approved by the FDA for melanoma, published clinical trials have shown encouraging results to support the efficacy and safety of this approach of treatment. Phan and Rosenberg (2013) reviewed the literature on the use of ACT for metastatic melanoma. Responses were reported in a reasonable percentage of patients, approximately 40–72%. Interestingly, around 40% of patients completely responded to ACT, the effect lasting up to seven years. Recently, in a phase-I study, an adoptive transfer of invariant natural killer T (NKT) cells was used as the immunotherapy for those with advanced melanoma (Exley et al. 2017). Although no clear correlation between the disease outcome and immune parameters could be found, it was reported that adoptive transfer of in vitro expanded autologous invariant NKT (iNKT) cells was a feasible and safe therapy. These cells promote anti-tumor immunity via producing Th1-like responses. Since we are in the early stages of this strategy, some potential logistical and technical hurdles in patient selection, tumor resection, and expansion of TILs exist. Taken together, ACT seems to be a potentially curative therapy for patients with metastatic melanoma and needs further studies and a higher phase of clinical trials of transferring T cells or NK cells in melanoma patients for going under review at the FDA for approval.

18.6.2.4 Other Treatment Options

Studies on skin cancer have revealed that 5-FU, imiquimod, and retinoids could be effective in the treatment and prevention of NMSC (Amini et al. 2010).

5-fluorouracil

Topical 5-FU is a topical chemotherapeutic agent and well-known treatment modality for NMSC that decreases cell proliferation and induces cellular death through interference with DNA synthesis. Although topical 5-FU has shown promising results in the treatment of superficial BCC or SCC, its application in invasive NMSCs has not been recommended (Neville et al. 2007).

Imiquimod

Imiquimod promotes innate and acquired immune responses via the stimulation of releasing a number of cytokines, such as IFN-α, TNF-α, IL-1, IL-2, IL-6, IL-8, and IL-12. It also activates Th1 cell-mediated immunity, stimulates NK cells as well as proliferation of B-lymphocytes. The FDA has approved imiquimod 5% cream for the treatment of superficial BCC, when surgery is a less-appropriate treatment option. Local skin reactions, such as rash and swelling are the most commonly reported adverse events associated with topical imiquimod.

PhotodynamicTherapy

Photodynamic therapy (PDT) refers to a treatment strategy, which needs photosensitizing agents, oxygen, and light, to create a photochemical reaction. During PDT, irradiation of visible light to skin prepared with photosensitizing agent initiates a tissue-toxic photochemical reaction that can selectively destroy cancer cells. The most commonly used photosensitization substances in the PDT approach are δ-5-aminolevulinic acid (ALA) or its methyl ester (MAL) (Ericson et al. 2008). PDT is a non-invasive, generally well tolerated, and effective option for treating AK and superficial BCC, which leads to reasonable cosmetic outcomes. Although not currently approved by the FDA, the efficacy and safety of PDT in superficial BCC were emphasized in numerous studies. Fortunately, PDT side-effects are mild and not persistent, usually are limited to pain during treatment (particularly in facial or scalp lesions) and swelling. Owing to a limited light penetration through tissues, its use is limited to treating superficial BCC and AK.

Oncolytic Virus Therapy

Talimogene aherparepvec (T-VEC), trade-named "Imlygic", is an oncolytic virus that selectively replicates inside and kills cancer cells. Indeed, it is a genetically modified

granulocyte-macrophage colony-stimulating factor (GM-CSF) secreting oncolytic HSV-1, which recruits anti-tumor immunity against tumor cells. As the early steps toward evaluation of the beneficial effects and safety of T-VEC in melanoma, a phase-I study on various metastatic tumors including malignant melanoma has been conducted (Hu et al. 2006). The treatment was well-tolerated and was safely administrated. Three years later, the efficacy and safety of T-VEC were evaluated in a phase II trial study (Senzer et al. 2009). The overall response rate was reported to be 26% and patients also benefited from the durability of the overall objective responses, overall survival rates, and low toxicity. Adverse effects were limited primarily to transient flu-like symptoms. Recently, the therapeutic benefit of T-VEC in advanced MM was demonstrated in a phase-III trial (Andtbacka et al. 2015). Local intralesional injections with T-VEC suppressed the growth of the tumors, ensuring prolonged overall survival. The most common adverse events with T-VEC were flu-like symptoms, such as fatigue, chill, and pyrexia. This treatment was well tolerated and no treatment-related fatality was reported. Following the promising reported results, T-VEC was approved by the FDA, in late 2015, for the treatment of melanoma.

18.7 Psychological Care for Skin Cancer Patients

18.7.1 Psychological Outcomes

If an individual is diagnosed with any form of cancer, his/her quality of life will be influenced significantly. In addition to the financial burden associated with cancer treatment and physical problems (e.g., disfigurement, pain, and swelling of the involved regions in skin cancer patients) of those with cancer, psychological stress is another critical issue, which should be considered for better management of cancer. In fact, cancer diagnosis has been associated with several serious problems, including anxiety, depression, and psychosexual problems. Following the diagnosis of any types of cancer, both patients and their families may experience several unexpected problems.

In spite of higher cure rates for skin cancers, patients may experience serious psychological problems, which are related to the appearance of the skin during these diseases. Skin cancers can change many aspects of a person's life, from personal life to family roles and relationships, which may not necessarily end with treatment. Although skin cancers are the most frequent types of cancer, their psychological impact is underestimated. Considering the aggressive and potential metastatic nature of melanoma, survivors of melanoma are prone to experience distress at some point after diagnosis and need supportive care, more than other skin cancers. Patients treated for melanoma should receive life-long clinical and psychosocial follow-up cares. Nevertheless, most of the patients are only followed-up for clinical screening and no clear recommendations and intervention for psychosocial screening or interventions are available.

Patients with any type of skin cancer experience different concerns, such as worries about future skin cancers, anxiety about the present skin cancer, risk of

cancer spread, appearance-related concerns, social concerns, concern of inheritance of their children, fear of recurrence, pessimism, and self-blame.

One of the most important aspects of life that could be negatively affected in patients with skin cancer is social issue. After diagnosis, patients with any type of skin cancer may experience a greater interference of stressors (physical and emotional) in social activities. Discrimination of cancer patients is a common problem affecting a wide range of cancer patients. Considering the aging of the world's populations as well as improved survival among cancer patients as a result of novel treatment strategies, it is critical to pay to attention to the economic and workplace consequences of cancer more than ever before. Unfortunately, patients diagnosed with cancer have a greater chance of experiencing job loss, which is followed by a loss of income and social status. A meta-analysis of 36 studies and more than twenty thousand cancer patients found an association between the cancer survivor rate and unemployment (de Boer et al. 2009). However, no significant difference in the employment was reported between the cancer survivors (both NMSC and melanoma) and referents (Taskila-Brandt et al. 2004). Although those results were promising, several serious work environmental issues, such as workplace discrimination and social stigma remained unsolved. Furthermore, a study in Korea revealed that cancer patients might experience various negative changes in work status, including workplace discrimination and forced resignation (Park et al. 2010). According to previous researches, a fifth of those diagnosed with cancer face discrimination on their return to work. Unjustified demotion, denial of promotion despite eligibility, and lack of flexibility for getting time off for medical appointments are only some of the possible workplace discriminatory instances.

The majority of MM survivors suffer from an enduring fear of developing new melanoma, which may interfere with regular self and clinical skin examination (Andtbacka et al. 2015). All of these have a significant negative impact on the quality of life. These concerns may not only cause immediate suffering but could also influence a wide range of patient behavior in a negative way. Medical issues are the other important outcomes of psychological distress, if ignored, in patients with skin cancer. It may lead to neglect of regulator examination. Indeed, this delay may cause additional inconveniences, heightening the severity of psychological distress/outcomes in a positive feedback loop. Considering the fact that there is a strong relationship between depression and high-risk behaviors, such as smoking, overeating, substance abuse, and alcohol addictions, patients with psychological problems are significantly threatened by these behaviors. These high-risk behaviors could lead to serious health problems and increase the risk of other types of cancers.

For most patients the worry diminishes after treatment. However, worrying about the development of new skin cancers might cause distress even after the treatment. Considering the facts that early skin cancers in the facial area are primarily treated with surgery as the first line of treatment, leaving conspicuous scars, especially those in the visible areas of the skin on the head and neck, is another disturbing problem even after the removal of the tumor. Noticeable scars are a serious issue, which could lead to psychological problems including feeling overly self-conscious about appearance and social anxiety in the early postoperative period (Lee et al. 2016).

18.7.2 Psychoneuroimmunology and Skin Cancer

As it mentioned earlier, the immune system is required for the elimination of tumor cells. Since the number and function of Th1 cells are critical during tumor elimination, any alteration in the immune system, which leads to an impairment of this arm, could cause the promotion of tumor cells. Psychological stressors can disrupt critical networks between the central nervous system and the immune system through the alteration of chemical messengers associated with neurons, endocrine organs and immune cell functions and differentiation. Research has proved that chronic stress could lead to the promotion of Th2 immune responses through the modification of the hypothalamic-pituitary-adrenal axis (HPA) reactivity, which is followed by a skewing of the Th1:Th2 balance toward fewer Th1 cells. In addition to the HPA alteration, the sympathetic axis (SA) is directly involved in induction of tumor growth (Yang et al. 2009). Modification of other stress mediators, including neuropeptides or neurotrophins, sub-stance P (SP), and nerve growth factor (NGF) may also be involved through different signaling pathways. Jointly, these changes, which were reported in individuals under chronic stress, could lead to the promotion of tumor cells, as the consequence of the inability of tumor-specific T cells to suppress or eliminate the tumor. Impairment of cytotoxic activities and NK cell activity are also the outcomes of chronic stress (Tausk et al. 2008; Dhabhar 2009; Li et al. 1997). Fawzy et al. (1990) have shown that a six-month psychiatric intervention in patients with melanoma led to an increase in the percent of NK cells and their functions. Surprisingly, the levels of $CD4^+$ T cells were reduced, probably due to a decrease in the $CD4^+$ Tregs. Considering the alteration of the immune system toward a reduced capacity of anti-tumor immunity during psychological distress, it is not surprising that the mentioned alteration in immune responses is harmful for those with skin cancer. In a mice model study on the effects of stress and UV-induced SCC, evidence implying the critical effect of stress and the emergence and progression of SCC was observed. This increased susceptibility may be mediated through the suppression of the critical anti- tumor cytokines and T cells as well as an increase in the number of Tregs (Saul et al. 2005).

18.7.3 Psychological Care

As discussed above, after the diagnosis of skin cancer, especially in case of those with advanced melanoma, several life aspects not only of the patients, but also their families could be negatively impacted. All of these cause serious psychological outcomes. Distress can occur at any time following the development of skin cancer, including before diagnosis, after diagnosis, during surgery, and after surgery. Thus, it is essential to include effective intervention to the treatment plan, which could improve both the quality of life as well as the disease outcome (Kissane 2009). However, the adoption of measures to minimize a patient's distress is often neglected.

Accurate detection of distress in patients with skin cancer is as critical as initiation of psychological support. In fact, psychological ill-being first requires detection and then the employment of proper approaches to deliver supportive care. The Identification of patients who are at a high risk of developing maladaptive psychological responses is essential. Previous studies have suggested that those with melanoma need more care compared to SCC and BCC. Other suggested vulnerable groups include those with visible affected parts of the skin (especially head or neck area) females, those young in age, those who live alone, patients with previous mental health problems, those with lower education, and those who lack partnership and social support (Peters 2012; Hamama-Raz 2012; Kasparian et al. 2009; Loquai et al. 2013; McLoone et al. 2012). Following the diagnosis of any form of cancer, such as skin cancer, not only patients and their families may experience several unexpected problems but their relatives, too. Owing to the increased risk of melanoma among those who have relatives with the same cancer, distress evoked by such changes may affect relative of cancer patients. Thus, it is important for patients with cancer as well as their families to cope with such distress. Considering the family members also have psychological needs during cancer (Lederberg 1998), a high risk of melanoma development among the relatives of patients makes such need more essential. Following the lack of or improper supportive care, physical and psychological impairments could lead to substantial social problems, such as the inability to work and/or fulfill other normative social roles.

Unfortunately, care needs may be underestimated and remain unmet in many of the patients with skin cancers. There is some evidence to support the fact that a considerable numbers of patients do not receive the necessary supportive care due to different reasons, such as lack of awareness, not seeking help for patients despite their need, or incompetent staff members. Recently, Fischbeck et al. (2015) suggested that psychosocial care needs for survivors of melanoma could be predicted by age, level of school education, fatigue, illness-related social support, and the time since the initial melanoma diagnosis. According to their study, greater attention should be paid to those who are young, show higher general fatigue, higher symptom of burden, lower general health, and negative social interactions.

Generally, the provision of supportive care for patients with cancer can be categorized as educational techniques, behavioral or skills training, social support as well as psychotherapy (Kasparian 2013b). Psychological care for skin-cancer patients aim to induce emotional well-being as well as improve the quality of life of patients and their families. Additionally, supportive care has a direct (e.g., promotion of anti-tumor immunity) and indirect effects (e.g., the avoiding of high-risk behaviors) on skin cancer toward achieving a desirable outcome. During psychological care, different aspects of psychological outcomes should be considered not only for cancer patients, but also for their families. One of the most challenging aspects is discrimination. In order to improving the quality of life of the majority of cancer patients experiencing workplace discrimination, some recommendations have been made. It has been recommended that special attention be paid to vulnerable patients with cancers, changes be brought about in workplace culture and

anti-discrimination laws passed, and the employment status of cancer patients be closely monitored to eliminate discrimination (Park et al. 2010). Patients with skin cancer could also benefit from these recommendations. Coping with cancer is another vital issue in psychological care. Different factors have been found to be associated with better coping abilities of those with skin cancer. For example, Holland et al. (1999) reported that greater reliance on spiritual and religious beliefs could significantly positively affect using an active-cognitive coping style.

Successive supportive care may lead to the promotion of immune responses related to tumor shrinkage and clinical improvement. Additionally, those with melanoma, who suffer from psychological problems may refuse regular clinical and self-examination of skin. They are threatened by possibilities of recurrence and the development of new primary melanomas. Several studies have demonstrated the beneficial effects of easing emotional stress in skin cancer patients. Although there are contradictory data concerning the improvement of long-term survival rates among patients with skin cancer receiving supportive care, it is generally accepted that such care significantly improves their quality of life. Studies about the association of psychosocial factors and melanoma outcomes have shown different results ranging from none, weak to moderate [reviewed in (Kasparian 2013b)].

Different types of supportive care, such as psychodynamic, cognitive-behavioral, supportive-expressive, and dialectical behavioral therapies could be helpful in the management of patients with skin cancer. These cares could be delivered through different formats (Kasparian 2013b). Owing to misconceptions about cancer risks and recurrence among melanoma survivors, it has been recommended those patients should have clear information about their disease, and, at the same time, receive emotional support (Kasparian 2013a). Even after stabilizing the psychological distress, patients should be closely monitored to prevent the recurrence of psychological problems because even simple regular skin examinations may prove to be stressful for a patient.

18.8 Concluding Remarks

Various risk factors associated with a heightened risk of skin cancer have been identified. The most commonly reported ones that trigger the development of both NMSCs and melanoma is sunlight induces mutations. Family history is another well-recognized risk factor for skin cancers. However, darker skin seems to be a protective factor. Immunosuppression, usually a result of organ transplantation, is also a common risk factor for different types of skin cancers, and is expected to be amplified in those with a family background. Mutations in several genes have been reported in patients with skin cancer. Those genes may belong to oncogenes or tumor suppressor gene families. Today, a large number of syndromes associated to skin cancer have been identified. These syndromes may increase the risk of skin cancer by harboring mutation in critical genes involved in the impairment of tumor suppressor genes functions or the DNA repairing process. CDKN2A, CDK4,

PTCH1, PTCH2, BAP1, and PTEN are the most discussed involved genes in the development of skin cancer. In the pathogenesis of skin cancer, besides genetics, epigenetics is also involved. DNA methylation and miRNAs are the most investigated players in epigenetic modification. The immune system controls tumor progression through different immune cells. Each cell could affect a tumor's fate. Evidence points to the positive impact of mature DCs, NK cells, activated effector T cells, B cells, and macrophages on the tumor elimination. However, plasmacytoid DCs, Tregs, and probably MCs are considered the unfavorable prognostic factors in this disease. Possibly as a result of the recruitment of numerous immune cells and components, autoimmune diseases (e.g., RA), some of viral infections (e.g., HPV and HIV), and immunosuppressive agents (e.g., methotrexate, azathioprine, natalizumab) have been found to be associated with an increased risk of skin cancer. However, vitiligo is associated with a decreased risk of both melanoma and NMSC. Regarding the treatment of skin cancer, non-surgical approaches seems to be replaced by emerging treatments. Target therapies, directed at signaling pathways during NMSCs and melanoma, have shown promising results. Vismodegib, sonidegib, vemurafenib, dabrafenib, trametinib, and cobimetinib are some of the drugs belonging to the target-therapy group that have received FDA approval. Ipilimumab, pembrolizumab, and nivolumab are also checkpoint inhibitors that fall into the immunotherapy approach. Recently, T-VEC was approved by the FDA for melanoma treatment. As the result of being diagnosed with cancer, patients may experience different concerns that could affect their life in a negative way. Developed psychological distress could lead to cancer promotion through different mechanisms, including the impairment of anti-tumor immunity and the patient's refusal to undergo regular clinical and self-examination. These could decrease the quality of life and survival prospects. Psychological problems could also interfere with social activities and lead to workplace discrimination. All of these tend to push patients into isolation, resulting in psychological ill-being. Patients with cancer can receive supportive care by means of different strategies, including educational techniques, behavioral or skills training, social support, and psychotherapy. Taken together, cancer patients could benefit from genetic testing, conventional and novel treatments/drugs, as well as psychological care to move toward a successful elimination of tumor.

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Chapter 19
Alternative or Complementary Medicine: History and Legacy

Heinz Schott

Abstract Alternative respectively complementary medicine is an important sector of the present health care market. It is mostly viewed as a counter part of academic medicine and seems often to be incompatible with it. This article outlines the historical origins of the healing concepts of alternative medicine and its relation to modern academic (Western) medicine.

Keywords Alternative medicine · Complementary medicine · Healing power of nature · Medical pluralism · Naturopathy · Religious medicine

19.1 Introduction

Complementary medicine is an established term for a variety of so-called alternative healing methods, implying that they can support and enhance academic (i.e., scientific) medicine or biomedicine in a constructive manner. Certain Asian healing concepts, such as Traditional Chinese Medicine (TCM) or Indian *Ayurveda* medicine, are thus widely appreciated today as approaches which complement modern Western medicine. However, a form of what could be termed Traditional Western Medicine also exists, which is based on historical concepts of European medicine extending from antiquity to the 19th century. As a German medical historian with an interest in modern naturopathy (*Naturheilkunde*)—an approach which currently represents the most important form of alternative medicine in this part of the world—the main focus of my work is the situation in German speaking countries. These nations were the main birthplace of the naturopathy movement as the genuine forerunner of contemporary alternative medicine. This was probably due to the strong impact of romantic natural philosophy on German medicine and science, which occurred around 1800, as exemplified by the influence of Schelling's natural philosophy within the context of German idealism. However, I will not deal with

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this interesting aspect of the history of philosophy here. Rather, I will outline those basic historical concepts which are essential in order to understand the current panorama of alternative and complementary medicine approaches.

Many terms are used to describe alternative or complementary medicine. However, these terms are not synonyms; rather, their meanings differ to varying extents. Available terms include "Fringe Medicine" (German: *Außenseitermedizin*); "unconventional medical approaches" (*unkonventionelle medizinische Richtungen*); "unorthodox therapeutic methods"; "holistic medicine" (*ganzheitliche Medizin*); "soft medicine"; "lay medicine"; and last, but not least, naturopathy (*Naturheilkunde*). These concepts contrast with high tech medicine (*Apparatemedizin*), respectively "(natural) scientific" or "academic medicine" (*Schulmedizin*).

The term complementary or alternative medicine comprises very heterogeneous healing concepts, whose principles are in some cases entirely contradictory (cf. Jütte 1996). For example, the underlying principles of phytotherapy and homeopathy are radically different. Similarly, hydrotherapy like the Kneipp therapy (*Kneippkur*) adhere to principles that are incompatible with those of spiritual healing. However, the various complementary healing concepts have a common ideological link: the appreciation of the omnipotent concept of the "healing power of nature" (*Naturheilkraft*; Greek: *physis*; Latin: *vis medicatrix naturae*), which implies the existence of religious and magical elements. Examination of the dichotomy between academic medicine and alternative medicine, and of the diversity of the various alternative methods, reveals how difficult it is to identify a common basis. Those who have witnessed the tendency of academic authorities to ridicule homeopathy as representing nothing more than a placebo, and the vehement resistance of homeopaths to this psychological hypothesis, are aware of the irreconcilable gap between the respective medical cultures, which are thus sometimes much more "alternative" than "complementary" in nature.

In recent decades, complementary medicine has become established alongside academic medicine (now often termed biomedicine) as an additional branch of medical therapeutics. However, the boundaries are in a constant state of flux: what was rejected in the past may be more accepted in the present, such as the classical methods of naturopathy, anthroposophical medicine, homeopathy, and Traditional Indian (*Ayurveda*) and Chinese Medicine, in particular acupuncture. In Europe, the extent to which alternative medicine should be accepted by the responsible regulatory authorities, and the degree to which it differs from miracle healing and charlatanism (cf. Heyll 2016), are issues that remain controversial. Notably, many of the long-established forms of magic and religious medicine to have originated from European culture and science are excluded from the "complementary medicine" category. This is true for various methods of spiritual healing (German: *Geistheilung*) or magic healing, for example the use of incantations to charm away warts, which remains popular in some rural districts of Germany and in other European countries.

19.2 Academic Medicine Versus Alternative Medicine: The Historical Schism

Natural science based modern medicine was established in the second half of the 19th century. Although they continued to be discussed and appreciated by proponents of "Romantic medicine" and its natural philosophy, from around 1800, magical and religious concepts became the subject of increasing skepticism in the medical scientific community. By the end of the century, they had become associated with "occultism", and excluded from the academic medical world. However, these now suspect concepts continued to flourish outside scientifically-based medicine, a field now represented mainly by bacteriology (Louis Pasteur, Robert Koch) and cellular pathology (Rudolf Virchow). At this point, the schism between academic and alternative medicine became obvious, particularly as regards the practices of scientifically educated academic physicians and "naturopaths" (*Naturarzt*, *Physiater*) or lay healers. However, the boundary between these two medical cultures was not always clear-cut. For practical reasons, agents of heterogeneous concepts could also cooperate. Thus at the end of the 19th century, university psychiatrists had no objection to the use of hydrotherapy for patients with hysteria, and the famous French neurologist Jean-Martin Charcot experimented with the use of metals and magnets to treat nervous disorders performing public demonstrations, for example of the transfer of anesthesia from one individual to another. Nevertheless, the boundary between scientific medicine and "occultism", "charlatanism", or "quackery" (German: *Kurpfuscherei*) became increasingly enforced by proponents of academic medicine. With the rise of biomedical research into histology, bacteriology, and biochemistry, alternative methods of healing began to be viewed as discredited relics of a superstitious past, which had been superseded by the scientific and technological progress of academic medicine. In contrast, naturopaths criticized academic medicine for being insufficient and poisonous, and for ignoring the healing power of nature.

This historical dichotomy or schism first became evident in the Renaissance, although during the early modern period, religion and magic continued to flourish within the context of medical alchemy. The latter was promoted in particular by Paracelsus (i.e., Theophrastus Bombast von Hohenheim; 1493–1541), and followers such as the ingenious laboratory researcher Johan Baptist van Helmont (1579–1644). The crucial turn occurred in the 18th century, the period of so called enlightenment, when physical and mechanical principles were assigned to the human organism. According to the famous materialistic treatise *"L'Homme Machine"* (de La Mettrie 1748), the human organism was constructed like a machine. In consequence, magical practices, as taught by Paracelsian and other scholars, were judged to represent a superstitious clinging to "occultism". Ultimately, progress in the 19th century of the (natural) sciences and biology led to the foundation of modern medicine. Besides bacteriology and cellular pathology, the impact of Darwinism was of great importance. This stimulated "Social Darwinism", and the later concept of eugenics. From the scientific viewpoint, the

advances in immunology, human genetics, and molecular medicine that had occurred over the preceding 100 years appeared to render alternative medicine superfluous and obsolete. However, many patients—particularly those with chronic disease—continued to require alternative methods to relieve symptoms that were unresponsive to the therapies offered by academic medicine.

19.3 The Healing Power of Nature: The Common Basic Principle

In 1926, Max Neuburger offered a profound analysis of the concept of the healing power of nature in his classical monograph *Die Lehre von der Heilkraft der Natur im Wandel der Zeiten* (The doctrine of the healing power of nature over the course of time). This concept first emerged in antiquity, within the context of the Hippocratic writings. In the sixth book on "Epidemic Diseases", the term *physis* (Latin: *natura*, i.e., nature) appears: "Educated is the physis and produces by herself the necessary, without having it learned" (Hippocrates, Epidem. VI 5,1). Despite its fundamental relevance to medical history throughout the ages, this concept is no longer referred to in modern encyclopedias or medical handbooks.

Hippocratic physicians in ancient Greece assumed that the human organism had a tendency towards self-healing, and they illustrated this using the paradigm of acute febrile disease. Here, the *physis* would transmute the evil mixture of the humors and the crude (pathogenic) substances through an increase in vital heat. In the best case scenario, the *physis* would result in the excretion of these pathogenic matter through natural pathways (intestine, bladder, skin), or via hemorrhage [nosebleed; hemorrhoids (in males); menstruation (in females)]. However, in severe disease, the *physis* would be insufficient. In such cases, the intervention of a physician was necessary. The physician was expected to apply his skill to support and consolidate the natural self-healing process of the organism. Therefore, to determine the point at which his intervention had become necessary, the physician was required to differentiate between the symptoms of the self-healing power of the *physis,* and the symptoms of its defeat, i.e., symptoms which would aggravate the disease state. The Hippocratic physician was thus required to act as a "servant of nature" (Greek: *tes physeos hyperetes*).

From this traditional perspective, the medical treatment appeared to be merely an imitation of nature, since its sole task was to accomplish those natural processes which were impaired due to a weakness in the healing power of nature. Here, the medical treatment is conceptualized as an adherence to the principles of nature. According to this view, all natural healing methods (German: *Naturheilverfahren*) provide only a secondary strengthening of the healing power of nature, which represents the primary source of resistance to disease. This way of thinking was especially appreciated by authors who conceptualized medicine as being based on

natural philosophy. One of the most prominent of these was the afore-mentioned Paracelsus, who promoted medical magic and alchemy. However, the other side of the coin must also be considered, namely the religious dimension of healing.

19.4 Divine Power: Religious Medicine

Either implicitly or explicitly, the so-called healing power of nature (*Heilkraft der Natur*) assumes a religious dimension. This is illustrated by the fact that in all periods of cultural history, the healing power of nature has been associated with light, fire, glamour, or beneficial beams. The visualization of this power is particularly pronounced in the traditions of the Jewish Kabbala, the Christian mysticism of the Middle Ages, the mystic experiences of the theosophists and paracelsians, and certain esoteric movements within alternative medicine. In religious art, the halo (German: *Heiligenschein*) was commonly used as a motif of divine power. Interestingly, light imagery also emerges in contexts with no overt religious connotations. For example, the emitting of powerful beams by the "Eye of God" was an image used by Franz Anton Mesmer as a symbol for the magnetic "*fluidum*", as showing an illustration in one of his later published works (Mesmer 1814). The symbol is reminiscent of emblems used by alchemists, hermetic natural philosophers, and the Freemasons to denote their search for divine nature, or *Gott-Natur,* to use the term once highlighted by Johann Wolfgang von Goethe.

The legacy of religious medicine continues to exert a remarkable influence, even in Europe. Thus, despite the enlightenment and the foundation of modern medicine in the 18th and 19th centuries, medical demonology did not disappear, and remains a topic of fascination to the wider public. The concepts of demonic possession and exorcism, which go back to antiquity, had a strong impact on the Christian tradition of spiritual healing. The latter remains active not only in the realm of the Roman Catholic church (the famous exorcist priest Gabriele Amorth died recently in Rome),[1] but also in some Protestant evangelical circles. In 1976, German student Anneliese Michel underwent exorcism by two Catholic priests, and her subsequent death provoked a heated debate in Germany.[2] The general conclusion was that cases of "demonic possession" should be treated by psychiatrists, not priests. However, exorcism according to clerical rites is still practiced, especially in Italy. Other methods of spiritual healing are also used, such as healing through prayer; the laying on of hands (palm healing); the adoration of saints; and pilgrimage (e.g., Lourdes). Religious healing is often practiced independently of any given clerical framework, and frequently merges with forms of magic healing popular in folk traditions, such as the application of amulets.

[1] https://en.wikipedia.org/wiki/Gabriele_Amorth (14.11.2016).

[2] https://en.wikipedia.org/wiki/Anneliese_Michel (14.11.2016).

19.5 Natural Magic: The Impact of Philosophy

In general, magical medicine is based on natural philosophy. The most important origin of early modern magical medicine in Europe was the teaching of Paracelsus, which was based on a specific concept of natural philosophy. Paracelsus conceptualized the inner nature of man as a "microcosm" (small world) implicating all things of the "macrocosm" (large world), which would impress the former through the influence of the stars ("astral impression"). Invisible "seeds" (German: *Samen*) would produce diseases originating from the macrocosm, and induce the specific visible disease within the human organism. The task of the physician was to seek remedies that were as strong as the disease. This search was motivated by the concept of the healing power of nature. The doctor, as a "philosopher" (*philosophus* according to Paracelsus), had to work "in accordance with nature" (*im Lichte der Natur*). The paracelsian metaphor of Nature as the first or "inward physician" (*inwendig Arzt*) remains popular in the discourse of naturopathy. In his book *Paragranum* (1530), Paracelsus wrote: "Primarily, a physician should know where Nature tends to go. Because she (Nature) is the first physician, man the second. Where Nature begins, there the physician should help, that she can go out from this location. Nature is a better physician than man […]. Because the disease emanates from Nature, the remedy emanates from Nature and not from the physician […]. Nature teaches the physician and not man" (Paracelsus, Ed. Peuckert, vol. 1, p. 503; transl. H.S.).

The practical guideline is clear: The "outward" physician must be subordinate to the "inward" physician and must cope with him. The *Labyrinthus medicorum errantium—Vom Irrgang der Aerzte* (1537/38) states: "Man is born to fall over. Now, he has two in the light of nature which raise him: the inward physician with the inward remedy, which are born with him and given to him at his conception […]. But the outward physician only begins when the inborn one succumbs and wilts, tired, and renders his office to the outward physician" (Paracelsus, Ed. Sudhoff, vol. 11, p. 198 seq.). Paracelsus advocates the strengthening of the healing power of nature within man; potentiating it via the use of drugs, and distilling it from natural materials using alchemical procedures. A number of terms are applied to demonstrate this (*archeus*, *arcanum*, *vulcanus*, virtue, inner balm, mummy (*mumia*)), all of which allude to the elusive (spiritual) healing power of nature.

This natural philosophy perspective led to the emergence of the art of magic or "magnetic" healing. Within the context of mesmerism or animal magnetism, which emerged at the end of the 18th century, the healing power of nature was viewed as a so-called magnetic *fluidum*, which pervaded the cosmos like ether, and which could be transferred to the human nervous system via certain therapeutic techniques. The term *fluidum* was first introduced by the Viennese physician Franz Anton Mesmer (1734–1815), and was viewed as a very subtle physical power. This harmonized with the idea of enlightenment, in particular the recently introduced phenomenon of artificial electric light, whose vital sparks symbolized divine Nature. During the period of Romantic natural philosophy in the early 19th century, mesmerism was

transformed into a spiritual or psychological concept, whereby magnetizing (mesmerizing) scholars assumed the presence of an unconscious realm within the (autonomous) nervous system. This so-called ganglion system (*Ganglien system*) was located in the abdomen (*hypochondrium*), and corresponded directly with the near and distant environment or macrocosm. Proponents of this theory assumed that during "magnetic sleep" or "artificial somnambulism" (later termed "hypnotic" sleep), patients could obtain insights into their disease and possible therapy, since they came into closer contact with the healing power of Nature during this state than was possible in a sober state of consciousness. Paradigmatic of this approach was a famous case history published by the Swabian doctor and poet Justinus Kerner (1786–1862), *Die Seherin von Prevorst* (1829), which was subsequently translated into English (*The Seeress of Prevorst*, 1846). The "seeress" was Friederike Hauffe, a critically ill young woman, who reported a wide variety of paranormal perceptions and visions. In later decades, she would have been classified as either a great medium by parapsychology or as a psychotic patient by psychiatrists. While in a somnambulistic state, she imagined and suggested remedies, therapeutic devices, and healing procedures both for herself and for her fellow patients. The impact of this specific natural philosophy tradition remains evident today, for example in Sigmund Freud's (1856–1939) theory of the "unconscious" and its creative powers. This relates in particular to the concept of "dream work" (*Traumarbeit*), which Freud introduced in his opus magnum *The Interpretation of Dreams* (*Die Traumdeutung*, 1900).

19.6 Vitalism and Naturopathy: The Foundation of Modern Alternative Medicine

In the 19th century, the healing power of nature was conceptualized as the principle of life or life force (or vital force; German: *Lebenskraft*; Latin: *vis vitalis*), a concept which included all tendencies of organic life. Especially influential in the early 19th century in terms of the development of public health and naturopathy was the viewpoint of the famous physician Christoph Wilhelm Hufeland (1762–1836). He first practiced in Weimar, where he was associated with Goethe and his circle. In 1810, he became the first dean of the medical faculty of the newly founded University of Berlin. His treatise *System der praktischen Heilkunde* (System of Practical Medicine) included a chapter on the therapeutics of nature (*Therapeutik der Natur*). The concept of the healing power of nature was a cornerstone of his doctrine. This approach became a model for many concepts of naturopathy (also called "*Physiatrie*" in its pure form), and was also held in high regard by renowned representatives of clinical medicine. According to Hufeland's definition: "That which we term the healing power of nature is not a specific power of its own, but the same vital force (life force; *Lebenskraft*) of the organic nature, conforming to the whole body and enlightening it and making it alive, related to the object of

disease and the healing thereof" (Hufeland 1818; translation H.S.). Insofar, all "healing operations of nature" could be deduced from the "basic principles of the organism", or the "principles of natural cures", e.g., the laws of excitement or the laws of sympathy, or the principle of antagonism, when affection status in one organ produces the opposite affection status in other organs.

With regard to these considerations, Hufeland raised the question of, "whether it would thereforebe unreasonable to leave all diseases to nature, and consequently abstain from the (healing) art". His answer is clear-cut: The abnormal and artificial human lifestyle—being related to "an increase in luxury, refinement, and immorality"—requires the art of medicine. Hufeland based his medical ethics on his plea to strengthen the vital force through the adoption of a natural lifestyle. In his influential book *Die Kunst, das menschliche Leben zu verlängern* (1796), which was later published under the general title *Makrobiotik* in many editions. Hufeland described the practical aspects of his "natural therapeutics" (*Naturtherapeutik*). The first edition was translated immediately into English (Hufeland 1797).

In particular, Hufeland promoted the rise of naturopathy, including homeopathy, an approach first introduced by Samuel Hahnemann (1755–1843) at the beginning of the 19th century. Hahemann's main work, *Organon der rationellen Heilkunde,* was published in 1810. Hufeland was acquainted with Hahnemann, and in 1796, he published an important article by Hahnemann in his *Journal der practische Arzneykunde* …. This article introduced the homeopathic law of similars (*similia similibus curentur*; literally: likes may be cured by likes). Although persistently refuted by academic medicine, homeopathy has continued to spread, and is now a well-established healing method in many regions of the world, particularly in India and South America (Dinges 2010). In contrast to homeopathy, the aim of "pure"naturopathy (also termed *Physiatrie* in the German literature) is to cure disease without the use of drugs. Pure naturopathy was introduced in the mid 19th century by the Bavarian military doctor Lorenz Gleich (1798–1865), and involved the use of natural resources, such as cold or heat, fresh air, fresh water, and a natural diet. These "physiatrists" rejected not only the "allopathic" drugs of academic medicine, but also homeopathic, chemically modified substances. Hydrotherapy—in particular with cold, fresh water—played a central role in the naturopathy movement, and diverse hydrotherapy healing concepts were introduced. In the early and late 19th century respectively, these included those of the peasant farmer Vinzenz Prießnitz (1790–1851) from Gräfenberg (Austrian Silesia), and the catholic priest Sebastian Kneipp (1821–1897) from Wörishofen (Bavaria).

Other lay doctors advocated the health benefits of raw foods. Proponents of this approach included the vegan and fruitarian Gustav Schlickeysen (1843–1893), who proposed the use of "fruit medicine". Schlickeysen viewed raw fruits as "sunlight nutrition", in anticipation of the vegan movement.

An alternative approach was advocated by the Swiss pioneer Arnold Rikli (1823–1906), who proposed exposure of the body to light and air as a remedy for all forms of weakness and dysfunction. At the end of the 19th century, the lay medicine movement was socially influential and well organized, and had created its own "temples of health" in the form of specialized sanatoriums. These

developments were embedded within a broader social movement in industrialized European countries and the United States, i.e., the life reform movement. Nevertheless, most forms of classical naturopathy (hydrotherapy, dietetics) were administered by registered physicians.

No absolute separation has ever existed between academic and alternative medicine. What is more, they have an ideological overlap: The doctrine of the "life force"—the so-called vitalism—which prevents the organism from falling ill or which heals the diseased body. Although the concept of vitalism has been subject to mounting rejection by academic medicine, the concept of the healing power of nature has remained popular, even among the luminaries of academic medicine. In a lecture entitled "On the healing powers of the organism", the famous pathologist Rudolf Virchow, who was a leading contemporary figure in scientific medicine, stated that: "Physiocrats were those physicians who assumed that healing powers lay within the physiological order of the organism, while technocrats were those who were considered able to identify the healing powers with means and impacts originating from outside of the patient and which must be applied to him". (Virchow 1875; transl. H.S.) He referred to Paracelsus, who had assumed a threefold power play: "the disease, the healthy rest of the body, and the specific force rulingit". The respective interaction would perform the "struggle for healing". On the one hand the essence of the disease, on the other hand the healing intervention. Virchow did not criticize the model per se; rather, speculative personifications of this power play. In his opinion, the presupposed force had to correspond with a real organism. "In this way, the human organism can be viewed as a composite of independent parts, the simple parts of it, i.e., the cells, can be viewed as independent, since they are self-living and self-acting and their force emerges from their own fabric, their Physis". Virchow's view exemplifies the intensive interest in the traditional concept of the healing power of nature, which persisted even after the scientific revolution of academic medicine in the mid 19th century (cf. Pagel 1931). Insofar, academic- and alternative medicine have always had a key concept in common, despite major controversies concerning methodological and practical issues.

19.7 Medical Pluralism: What Does It Mean?

The situation in the contemporary Western world is a paradox: On the one hand, a sophisticated and well-organized medical system exists, which is supervised by state authorities and regulated by the respective laws. On the other, an extensive, and more or less unofficial, market exists for alternative healing methods. Some alternative methods, such as hydrotherapy, are widely accepted in academic medicine, and have become more or less assimilated into it. Others are firmly excluded, such as certain concepts of spiritual healing. In the case of homeopathy, no consensus has been reached. Some representatives of academic medicine label homeopathy as a pure placebo cure or suggestive therapy, or even as a form of

fraud, whereas many general practitioners (at least in Germany) administer homeopathic drugs. This situation may be characterized by the term "medical pluralism": Different concepts act simulateously, and can be selected by the patient or the client according to current circumstances. This is much more evident in certain Asian, African, and Latin American countries, where Western biomedicine co-exists with other traditional healing concepts. For example, in Tanzania (East Africa) three concepts play an important role in healthcare alongside western high tech medicine: (1) *Ayurveda* medicine, from India; (2) *Yunani* medicine, which is of Greek-Arabic origin; and (3) traditional folk medicine, which is based on demonology and magic. This complex situation arose due to historical events in the colonial and post-colonial periods. A detailed investigation of this was undertaken by Bruchhausen (2006).

What is the current state-of-the-art for alternative healing? To answer this, we must consider both the social and the economic context. The wide variety of approaches available today may represent the reactions of people made anxious by socioeconomic imbalances, and who are uncomfortable with the impersonal approach of contemporary high-tech biomedicine. These approaches include the ecological movement, with its fine sense for a healthy environment; the rituals of traditional folk beliefs (e.g., miraculous healing and pilgrimage); new magical (pagan) healing procedures, which are associated with witchcraft (e.g., *Wicca*); and a flourishing market for alternative cures. A wide variety of approaches are even placed on show at (para) medical fairs. Often, the respective methods are not explained within their historical context, but are praised instead as singular innovative methods. In certain cases, the methods are declared to be thousands of years old and thus most efficient. This claim is evident in advertisements for certain forms of Traditional Chinese and Indian (*Ayurveda*) Medicine. However, these Oriental concepts are probably no older than the Occidental tradition of Greek medicine, which is in turn based on much older scientific cultures, such as those of ancient Egypt and Mesopotamia. In contrast, that which we might term Traditional Western Medicine (e.g., humoral pathology, dietetics, magical-sympathetic medicine), was rejected by Western academic medicine during the 19th century as unscientific speculation and "occultism". Thus Western historical sources and origins were overturned in the name of scientific progress. (Whether this was good or bad is not for me to judge.) In other regions of the world, this effect was only partial: There, heterogeneous healing systems were established and coexisted, as in India, which probably has more homeopathic practitioners than all European countries combined.

What is really new regarding alternative medicine? One could claim: All healing methods practiced in the field of alternative medicine were once "scientifically" acknowledged by medical schools, either directly or as practiced in a modified form; they belonged to the respective contemporary field of academic medicine. This was the case, for example, with early modern astrological and herbal medicine (phytotherapy). Even in the 19th century, universities chairs were established for animal magnetism and homeopathy. What was lege artis in former times is viewed

as odd in the present. Thus the application by Paracelus of a magnetic stone to relocate a displaced uterus would be denounced as unscientific by contemporary academic physicians.

Nevertheless, academic medicine includes elements of certain traditional healing rites, and is not as "rational" as it claims. Certain elements of the doctor-patient-relationship remind us of phenomena—one could also speak of "magical elements"—that are common to all healing methods: "transference-love" (Übertragungsliebe, S. Freud), placebo effect, confidence, or faith. Throughout the cultural history of mankind, the brain, heart, stomach, kidneys, and other organs have been associated with emotions, and this cannot be eradicated simply because a "rational", "scientific" explanation has been offered (e.g., "the heart is merely a pump"). The controversial debate on the concept of brain death reveals the contrast between scientific definition and emotionally experienced reality.

The popular statement "the doctor is the drug" (or: the drug "doctor"), which was coined by the psychoanalyst Michael Balint (1836–1970), cannot be defined and measured by means of natural science alone. Nevertheless, ill-defined and unconscious magical and religious elements are inherent to the technical procedures of modern medicine. One may therefore concur with a statement made by Sigmund Freud prior to his founding of psychoanalysis is regarding the popular attitude of patients towards the use of naturopathy: "If we have any reason to blame the pious expectation of the sick person, we must not be so ungrateful as to forget that the same power also continuously supports our own medical work" (Freud 1961, p. 300; transl. H.S.). Indeed, the miraculous "placebo effect", which was first introduced into medical terminology as late as the 1950s, is a potent healing factor beyond the traditional separation of academic versus alternative medicine. One can also speak of the "healing effect of suggestion", which was the title of a work by Hippolyte Bernheim, the great French pioneer of psychotherapy (Bernheim 1888; translated into German by Sigmund Freud). Medical pluralism does not necessarily imply the clash of medical cultures and the obstruction of Western bio-medicine. Rather, it can also represent the basis of an effective form of assistance to sick persons in distress. In this spirit, many patients all over the world receive the benefits of multidisciplinary treatment.

19.8 Conclusion

Alternative or complementary medicine is a fuzzy term comprehending very different concepts of healing methods. They originate from certain periods of medical history and different regions of the world. Today, they are understood as the counterpart or opposite pole of the academic Western medicine in a negative or positive way. On the one hand, academic medicine tends to refuse alternative medicine as "unscientific" speculation, although health funds (at least in Germany) accept more and more certain concepts of "complementary medicine". On the other hand, the adherers of alternative medicine stress its usefulness either as a

complement or—particularly in the scope of lay medicine—as a substitute of academic medicine. This area of conflict reflects as well ideological as financial interests of both sides. What has to be done? It is the task of all academic, social, and political institutions concerning the health care system to focus the approach of medical pluralism evaluate its possible benefit. This can only be achieved when the history of medicine and culture is globally appreciated as a common source of the healing art. Especially cross-cultural communication and research is necessary to foster global health. The marvel of the so-called placebo effect striking all medical respectively therapeutic interventions is a common challenge for all the rivals of the health care market. It should rather join than separate them.

19.9 Summary

The term complementary or alternative medicine comprises very heterogeneous healing concepts, whose principles are in some cases entirely contradictory. This article tries to outline fundamental traits from a historical perspective. First of all, the historical schism of academic versus alternative medicine is described. It became only actually relevant, when the scientifically-based medicine was fully established in the second half of the 19th century. Simultaneously to the exclusion of traditional, "occult" concepts from the academic medicine these now suspect concepts continued to flourish outside academia. There is a common basic principle of all the diverse traditional concepts at least in regard to traditional European medicine arising from Greek antiquity: the idea of the healing power of nature. It had a strong impact on renaissance and early modern medicine, when natural philosophy, alchemy and magic became dominant doctrines stimulating early modern natural science. They provided alternative medicine with historical concepts which are still effectual in a more or less modified form. Apart from that, the tradition of religious medicine produced spiritual healing methods which are also an important element of alternative medicine, especially of popular medicine with its religious and magical rites. Modern alternative medicine developed in Germany and other European countries in the 19th century, when vitalism and naturopathy blossomed out as a counter movement to the upcoming natural scientific medicine. Also Asian healing concepts, mainly from India and China, were adapted to the Western health care market and broadly accepted as an element of complementary medicine. At the present time one should be aware of the possibilities of a beneficial cooperation between academic and alternative medicine as well as the advantages of medical pluralism within the scope of global health.

Appendix

Overview of concepts and practical methods of alternative medicine with varying degrees of popularity in Europe, in particular Germany

Based on *Die Bilanz des 20. Jahrhunderts* [The balance of the 20th century], ed. by Bodo Harenberg, Dortmund 1991, p. 189; with essential modifications.

Alternative or complementary medicine is a poorly defined discipline or field of disciplines. Many overlapping doctrines and therapeutic methods exist. This complex situation cannot be compressed into an unambiguous historical and systematical framework. *The following scheme provides a preliminary overview, and is far from being complete or systematically elaborate* (Table 19.1).

Table 19.1 An overview on methods of alternative medicine

Healing method	Principle	Application
Naturopathy (*Naturheilkunde*); biological medicine	Regulation of disturbed physiological functions and biological rhythms regarding respiration, heat regulation, secretion etc.; treatment of biological imbalances	Functional, psychosomatic disorders; stress conditions; methods of preventive medicine; not used in emergency medical aid
Naturopathy in the narrow sense: treatment by natural means (light, air, heat, soil, water)	Healing stimulation by natural means empowering weakened physiological functions (hardening against softness); treatment of symptoms by opposing agents (traditional principle: *contraria contrariis,* e.g., fever is lowered by a cold compress)	Chronic diseases, neurasthenia, psychosomatic disorders; hydrotherapy (Kneipp Cure, steam baths, sauna, drinking cure with healing waters); sun and light therapy; cataplasms: mud, moor, fango
Therapy via nervous system including regulation of the disease herd, neural therapy, chirotherapy, acupuncture, reflexology therapy	Reflex arcs between organs, muscles, connective tissue, and skin areas supplied by the same nerve roots; stimulation of the body surface is thought to affect the corresponding organs and tissues of the same segment	Pain syndromes: headache, strain traumas, renal colic; exploration of "interference fields" in chronic diseases
Dietetics, nutrition teaching, fasting cures (e.g., Bircher-Benner, M. O. Bruker, Waerland), food combining, macrobiotic food	Care of the organs of digestion and excretion; regulation of the excretion of pathogenic substances from the body	Rheumatism; muscle bracing and muscle pain; circulation disorders; obesity; digestive disorders
Chirotherapy (manual medicine)—included within academic medicine	Disorders of the motion apparatus are thought to affect general health via the nervous system	Therapy of disorders of the musculoskeletal system, in particular the spine (back pain)

(continued)

Table 19.1 (continued)

Healing method	Principle	Application
Osteopathy; Osteopathic medicine practiced by physicians in the US only; elsewhere it is considered a form of alternative medicine	The bone as the starting point of disorders of certain organs, holistic anthropological model, diversity of different concepts and doctrines	Relaxation of muscle bracing, alleviation of pain, bone setting, cure of vertical subluxation causing irritation of respective nerve roots
Chiropractic; no strict demarcation from chirotherapy and osteopathy	Vertebral subluxation causes a variety of disorders, overlapping with principles of manual and osteopathic medicine	Manipulation of the spine to cure especially (low) back and neck pain, bone setting, special methods of manipulations (different schools)
Phytotherapy (herbal medicine); Established within ancient (Greek) medicine, fundamental for the history of Occidental medicine; also basic for traditional Chinese, Japanese (*Kampo*) and Indian (*Ayurvedic*) medicine	Medicinal plants as drugs for interior and exterior application (e.g., plant fluids, tea blends); complex ingredients, no mono preparations; therefor very difficult to standardize	All types of functional disorders of specific organ systems (cardiovascular, respiratory, dermal etc.), chronic diseases; also acute illnesses (e.g., common cold) and stress symptoms (e.g., insomnia)
Homeopathy (high and low potencies); one of the most controversial concepts of alternative medicine; academic medicine experts criticize the lack of research evidence; largely discredited as pure placebo therapy	Based on the principle of Samuel Hahnemann (1755–1843): *Similia similibus curentur* (like cures like)—a substance that causes disease symptoms in healthy persons could cure similar symptoms in sick persons; substances (herbal, animal, mineral) are prepared by dilution and succession ("potentization")	Mainly applied in chronic diseases which cannot be treated effectively by academic medicine (e.g. neuro-dermatitis, asthma, vegetative dystonia); pain therapy (eventually additionally); veterinary homeopathy also widely used (homeopaths argue that its effectiveness here proves that homeopathy is not merely a placebo)
Anthroposophical medicine; A concept of complementary medicine acknowledged in Germany and Switzerland	Based on anthroposophy, the philosophical doctrine of Rudolf Steiner (1861–1925) focuses on holistic, spiritual, and esoteric anthropology; diseases originate from disharmony of the "essential members" of an individual	Harmonizing therapeutic methods enabling the patient to recover from his/her imbalances, such as herbal medicine and homeopathic substances, curative eurythmy [sic], chromotherapy (color therapy), art and painting therapy, music therapy
Folk or popular medicine; Increasing popularity worldwide corresponding to regional traditions of healing; historically important in Europe	Based on ideas of natural magic, demonology, healing rites, religious practices	Amulets against evil (demonic) influences; regional traditions of healing (e.g., charming away warts); household remedies

(continued)

Table 19.1 (continued)

Healing method	Principle	Application
Traditional Chinese Medicine (TCM); influenced traditional Japanese (*Kampo*) and Korean medicine; established in Europe in the late 20th century, now spreading worldwide	Central is the conception of *"Qi"*, the subtle life energy flowing within the organism via the "meridians"; imbalance between *Yin* (dark, cold female) and *Yang* (light, hot, male) produces disorders	All cases of non-surgical treatment; therapeutic objective is to correct the imbalances directing the *Qi* by certain techniques: acupuncture, moxibustion, specific drug therapy, movement therapy (*Qigong*), massage (*Shiatsu* in Japan)
Ayurveda (Traditional Indian Medicine) Increasing popularity in Europe over recent decades	The Sanskrit term means "life knowledge"; the imbalance between the three *doshas* (elemental substances) *Vata*, *Pitta,* and *Kapha*) results in disease; holistic approach of harmonizing	In Europe, Ayurveda practices are integrated within general wellness applications; medical use focuses on good digestion and excretion applying yoga, meditation, herbal medicine, special (*Sattvic*) diet
Kundalini yoga; derived from Indian Sanskrit scriptures and corresponds to various schools of yoga	*Kundalini*, a primal energy located at the base of the spine, can be awakened by meditation ascending to and enlightening the other six superior *chakras* (energy nodes of the subtle body)	Yoga exercises have become a part of the Western life style and a form of "ersatz religion" since the end of the 20th century; yoga techniques are thought to be helpful in all psychological and physical disorders
Spiritual healing; "Healing through the Spirit"; Faith healing (e.g., Christian Science)	Assumption of a divine healing power which can be mobilized through prayers, religious rites, pilgrimage (e.g., Lourdes), (clerical) exorcism	All possible disorders may be treated; "miraculous healing" of severe diseases as a provocation of academic medicine
Esoteric healing methods; unclear boundaries, heterogeneous concepts	Very different principles: "white magic", Wicca (contemporary neopagan witchcraft), natural religion doctrines, reincarnation therapy	No specific indication, all possible disorders may be treated; healing rites are embedded in a specific religious environment (e.g., pagan mythology of the Celts)
Magnetopathy, mesmerism, radionics, medical dowsing	Assumption of occult cosmic rays ("*fluidum*", "magnetism" etc.) which can be perceived by sensitive people and used for diagnostics and healing	Chronic diseases and disabilities; specific techniques: magnetopathic cures (e.g., laying on of hands), devices for detection of (pathogenic) "earth rays"
Lay medicine, support or self-help groups	Concept of self-treatment: patients should become their own doctors, often in cooperation with academic medicine and health institutions	Chronic diseases and disabilities: e. g Alcoholics Anonymous; diabetes support groups; encounter groups for cancer patients

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Chapter 20
Pharmacotherapy of Cancer from the Perspective of Traditional Persian Medicine

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Abstract Cancer has always been considered as a life-threatening disease with an etiology which is not yet completely understood. Currently, there are a wide variety of chemotherapeutic and biological agents in the market which are used for the treatment of different types of cancer under specific guidelines. A numerous number of these chemotherapeutic agents like taxanes, vinca alkaloids, and camptothecin derivatives have been isolated from natural sources; thus, nature provides an endless source of new phytochemicals as lead compounds for the generation of future anticancer agents. To find better candidates as anticancer agents, folklore and traditional medicine of countries all over the world can be a reliable guide. In addition to purification of anticancer compounds, complementary and alternative medicine (CAM) can be considered as an adjuvant therapy in line with the standard chemotherapy protocols (Farzaei et al. in Curr Pharm Des 22:4201–4218, 2016a). To achieve this goal, enough evidence must be provided regarding the safety and efficacy of these natural agents. Traditional Persian medicine (TPM) is one of the

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most ancient categories of CAM which is globally well-known due to the unique medical texts like Canon of medicine by Avicenna written in 1025 A.D and Razi's "continens" (Alhavi) written around 960 A.D and its specific doctrine based on temperaments and humors. This chapter aims to introduce the most important anticancer agents used in TPM and summarizing current evidence on the anticancer properties of these natural medicines.

Keywords ■■■

Abbreviations

Bax	Bcl-2-associated X protein
Bcl-2	B-cell lymphoma 2
CAM	Complementary and alternative medicine
Casp	Caspase
CAT	Catalase
CD	Cluster of differentiation
CDK	Cyclin-dependent kinase
COX	Cyclooxygenase
CXCR4	C-X-C chemokine receptor type 4
GSH	Glutathione
HIF	Hypoxia inducible factor
JNK	C-Jun N-terminal kinase
IFN	Interferon
iNOS	Inducible nitric oxide synthase
IL	Interleukin
ROS	Reactive oxygen species
SOD	Superoxide dismutase
NF-κB	Nuclear factor kappa B
MAPK	Mitogen-activated protein kinases
MMP	Matrix metalloproteinase
ODC	Ornithine decarboxylase
PARP	Poly (ADP-ribose) polymerase
PKC	Protein kinase
POLD1	Polymerase delta catalytic subunit gene 1
RBC	Red blood cell
RANKL	Receptor activator of nuclear factor kappa-B ligand
mRNA	Messenger RNA
STAT	Signal transducer and activator of transcription
TPM	Traditional Persian medicine
TGF	Transforming growth factor
TNF	Tumor necrosis factor
TCF-4	T-cell factor 4
VEGFR	Vascular endothelial growth factor receptor

20.1 Introduction

Without any doubt, chemotherapy is the main approach in the treatment of cancer. Chemotherapeutic agents including alkylating agents, antimetabolites, aromatase inhibitors, anthracyclines and monoclonal antibodies, are considered as specified treatment regimens for different types of cancer. Despite the acceptable efficacy of current treatments, there are still cases which are not completely responsive to chemotherapeutic agents. On the other hand, current pharmacotherapy of cancer is associated with a wide spectrum of neurological, gastrointestinal, hematological or cardiac adverse events which are difficult to manage and complicate the treatment process of malignancies. As a result, investigations are widely performed to find new therapeutic agents with fewer side effects and higher efficacy (Perrino et al. 2014; Verstappen et al. 2003; Farzaei et al. 2016a).

Traditional Persian medicine (TPM) as one of the most ancient systems of medicine has been in use since Zoroastrian era about 550 B.C during Achaemenid empire. The religious Zoroastrian manuscript, "Avesta," contains details like classifications of physicians, use of medicinal plants, and spiritual psychotherapy which shows the significance of ancient Persian knowledge about medicine (Zargaran et al. 2012). Later, this knowledge was developed in Parthian (247 B.C–224 A.D) and Sassanid empires (224–637A.D) which are evident from manuscripts like "Bondahesh," a manuscript of Sassanid era, in which several important medicinal plants like olive, castor, and hemp are described (Hamedi et al. 2013). After the entrance of Islam religion, several Persian physicians further developed TPM amongst which Razi (865–925 A.D) and Avicenna (980–1037 A.D) were the most well-known pioneers due to their important manuscripts including Razi's "continens" (Alhavi), and Avicenna's "Canon of medicine" as well as "Kitab-Al-Shifa" which is referred to as "the magnum opus of Avicenna" (Hamedi et al. 2013; Evans 2009). It is worthy to mention that some terms currently used in medical terminology originate from TPM, such as "bezoar" which is derived from the ancient word "Pāt-zahr" (antidote), and the meaning has been changed during centuries (Zargaran et al. 2013).

TPM suggests a specific approach of pharmacotherapy in the treatment of different disorders which is based on the use of simple natural drugs originated from plants, animals or minerals (called "Mofradeh") at the first step. If the simple drugs were not able to effectively manage the disease, then a multicomponent preparation (called "Morakkabeh") would be chosen for the treatment process. In other words, pharmacotherapy in TPM should include as few drugs as possible which shows the importance of considering toxicity and side effects in the selection of different drugs. Since cancer is considered as a complex and severe disease, most of the treatment options contain more than one simple drug. Surgery and pharmacotherapy are the main treatment strategies for cancer in TPM; however, in cancers involved main vessels or nerves and in metastatic cancers, surgery can increase the risk of invasion and decrease life expectancy; whereas pharmacotherapy can improve longevity (Azam Khan 1869). In this chapter, we introduce the main simple drugs

which have repeatedly been mentioned in ancient text books of TPM and describe recent evidence regarding their anticancer properties in cellular and animal models.

20.2 Cellular and Sub-cellular Targets of Medicinal Plants for Antineoplastic Activities

Programmed cell death or apoptosis is a complex cascade of events leading to the elimination of the damaged or abnormal cells. Any interruption in the order or sequence of the involved events can result in abnormal cell proliferation, known as cancer. Apoptosis occurs via intrinsic (mitochondrial or endoplasmic reticulum) and extrinsic (death receptors) pathways which include several molecular mechanisms considered as potential targets for antineoplastic agents (Lowe and Lin 2000). In the intrinsic pathway of apoptosis, pro-apoptotic proteins like BAX and BID as well as anti-apoptotic ones, e.g. BCL-2, regulate the process of cell death via downstream events including mitochondrial membrane permeation, cytochrome c release and formation of apoptosomes by the help of caspase-9 (Casp-9) which results in activation of Casp-3 and Casp-7. Caspases are a group of cysteine-rich protease enzymes which are involved in both intrinsic and extrinsic pathways of apoptosis. Amongst different types, Casp-3 and Casp-9 are the most studied ones in cellular studies on anticancer drugs. The imbalance between the anti-apoptotic and pro-apoptotic proteins can lead to inactivation of Casp-3 and Casp-9 enzymes and prevent apoptosis which results in abnormal cell proliferation and tumor formation (Fiandalo and Kyprianou 2012). Extrinsic pathway involves other factors like tumor necrosis factor α (TNF-α). Downstream signaling of TNF-α results in the formation of complex I which induces pro-survival nuclear factor-κB (NF-κB) pathway and complex II that activates apoptosis (Fiandalo and Kyprianou 2012).

NF-κB family consists of a group of transcriptional factors present in an inactive state in normal cells. In response to their stimulators like reactive oxygen species (ROS) or TNF-α, these molecules rapidly become activated by IκB kinase and enter the nucleus which finally results in the prevention of cell death. Pathologic levels of NF-κB activators, like free radicals and pro-inflammatory cytokines, causes irregular expression of other pro-survival genes like cyclins D and B, c-Myc and survivin, and uncontrolled prevention of cell death which has a main contribution in the pathogenesis of cancer (Grivennikov and Karin 2010). Survivin expression would also be affected by PI3K/AKT pathway, activated by epidermal growth factor receptor (EGFR) that increase hypoxia-inducible factor 1 (HIF-1), which can directly elevate survivin (Kanwar et al. 2011).

The same as NF-κB, signal transducer and activator of transcription (STAT) factors have an anti-apoptotic activity. As a result of STAT3 activation in response to its stimulators, like interleukin (IL) -6, IL-10 and vascular endothelial growth factor (VEGF) in malignant cells, expression of proto-oncogenes like K-Ras and

further phosphorylation of STAT3 occurs which results in abnormal proliferation (Grivennikov and Karin 2010).

Poly (ADP-ribose) polymerases (PARP) form a family of enzymes responsible for the protection of DNA integrity. Overexpression of PARP has been observed in several cancerous cells; thus, PARP inhibitors are capable of inducing cytotoxic activity. This effect is due to the increase in un-repaired DNA breaks which finally results in tumor cell death, known as synthetic lethality (Helleday 2011).

Another well-known participant of the disrupted apoptosis is p53. This protein is an important tumor suppressor whose mutations cause its disturbed activity that promotes cell survival—i.e. via activation of the NF-κB pathway—and results in malignancies (Muller and Vousden 2014).

The chemokine receptor 4 (CXCR4) is a G-protein-coupled seven-transmembrane receptor with the CXCR12 as its ligand. Following the binding of CXCR12 to the receptor, protein kinase B (AKT)/mitogen-activated protein kinases (MAPK) cascade would be activated which results in cell growth and migration, a crucial event in embryonic development. Nevertheless, the over-activation of the above-mentioned cascade in tumor cells, resulting in the higher rate of metastasis and tumor growth, has been observed in several studies and that is why the inhibitors of CXCR4 are considered as antineoplastic agents (Domanska et al. 2013).

Matrix metalloproteinases (MMPs) are another group of enzymes involved in the invasion and metastasis of tumor cells as they have proteolytic activity on the extracellular matrix, decrease tumor cell adherence to the original tumor site and increase tumor progression (Gialeli et al. 2011).

There are several other contributors identified as molecular targets of anticancer natural agents. Having a better knowledge of cellular pathways involved in the anticancer properties of natural drugs, especially medicinal plants, can help scientists to design and develope new generations of anticancer drugs or to consider natural agents as adjuvant treatments along with the conventional anticancer therapies (Farzaei et al. 2016a).

Here we represent natural anticancer medicines based on TPM along with their underlying mechanism of action.

20.3 Traditional Persian Medicine for the Treatment of Cancer

20.3.1 Animal and Mineral Based-Simple Drugs

Minerals have mostly been used in combination with medicinal plants and were usually applied in topical form. The most important mineral drugs for cancer therapy in TPM include silver salts, zinc oxide, copper oxide, lead oxide, mercuric sulfide, and tin. Some of these introduced simple drugs like silver salts are currently

well-known for their anticancer properties (Banti and Hadjikakou 2013); while some others considered as toxic agents (e.g. lead derivatives) which have demonstrated harmful effects on human health. In such cases, TPM suggested the medicine be applied topically to the location of the tumor, or it has been mixed with other drugs to reduce toxicity.

There are also some simple drugs with animal origin like crab and tortoiseshell which were used in multicomponent formulations for the treatment of cancer; though, they are not as extensive as medicinal plants (Aghili 2009; Azam Khan 1869).

20.3.2 Herbal Simple Drugs for the Treatment of Cancer in TPM

20.3.2.1 Autumn Crocus

Colchicum autumnale or autumn crocus is a plant from the family Colchicaceae (www.itis.gov) with large corms which are the main medicinal part of the plant. The plant is called "Sourenjan" in TPM with a hot and dry nature and was mostly famous for its application in gout, an indication which is confirmed in modern medicine, as well. It was also used together with saffron for the treatment of tumors (Aghili 2009). All parts of the plant contain an isoquinoline alkaloid, colchicine, which can cause toxic effects for both human and animal (Brvar et al. 2004) but is also the major active component of the plant. Colchicine derivatives are identified in several other species of the genus *Colchicum*, as well.

Colchicinoids from *C. brachyphyllum*, *C. crocifolium*, *C. hierosolymitanum* and *C. tunicatum* exhibited antineoplastic activity in MCF-7 human breast carcinoma, NCI-H460 human large cell lung carcinoma, and SF-268 human astrocytoma cell cultures (Alali et al. 2005, 2006, 2010). The main antineoplastic mechanism of colchicine is the inhibition of microtubule formation which blocks mitosis in G2/M phase (Kumar et al. 2017; Johnson et al. 2017). Colchicine could also activate anti-proliferative genes, AKAP12, TGFB2 and MX1 in human hepatocellular carcinoma as well as cancer-associated fibroblast cell lines (Lin et al. 2013). A synthetic analog of colchicine, 4o (N-[(7S)-1,2,3-trimethoxy-9-oxo-10-[3-(trifluoromethyl)-4-chlorophenylamino]-5,6,7,9-tetrahydrobenzo[a]heptalen-7-yl]acetamide), could demonstrate cytotoxic effects in pancreatic cancer cells through induction of mitochondrial and endoplasmic reticulum dysfunction via elevation of reactive oxygen species (ROS) (Kumar et al. 2016a). The same mechanism was also observed for tetramethoxyallocolchicine, another synthetic derivative; however, this compound did not seem to have the classic antimitotic properties of colchicine (Larocque et al. 2014).

20.3.2.2 Black Nightshade

One of the common medicinal plants in TPM for cancer is *Solanum nigrum* fruit which is well-known as black nightshade. The plant belongs to Solanacease family (www.itis.gov) and has a cold and dry nature. It was called "Enab-Al-Salab" in TPM and was used for inflammatory and hot gastrointestinal disorders as well as cancers with exudative lesions (Aghili 2009).

Black nightshade is a toxic plant due to its steroidal alkaloids like solanine and solamargine (Ding et al. 2012). The leaves also contain a variety of phenolic acids and flavonoids (Li et al. 2007a). Polysaccharides and glycoproteins are other identified active ingredients of *S. nigrum* (Heo et al. 2004a).

A group of steroidal glycoalkaloids from *S. nigrum* were assessed for their anticancer activities in human gastric cancer MGC-803 cells which could induce apoptosis via regulation of Bax, Bcl-2, p53 and activation of Casp-3 (Ding et al. 2013). Solanine showed anticancer activity in HepG2 cells by inhibiting Bcl2 protein expression (Ji et al. 2008). Solamargine decreased migration and invasion of HepG2 cells by suppression of MMP-2 and -9 (Sani et al. 2015). Also, Uttroside B, a saponin from *S. nigrum*, showed anticancer effects in HepG2 cells through suppression of MAPK and mTOR signaling (Nath et al. 2016). In human hepatoma SMMC-7721 cells, solamargine induced G2/M cell cycle arrest and increased the level of Casp3 (Ding et al. 2012). Solamargine also caused damage in mitochondrial membrane potential and increased mitochondrial cytochrome c release as well as the intracellular Ca2+ concentration in human K562 leukemia cells (Sun et al. 2010). Furthermore, solamargine decreased phosphorylation of STAT3 and increased p38MAPK in NSCLC cultures (Zhou et al. 2014). Aqueous extract of the fruit increased E-cadherin and reduced ZEB1, N-cadherin, and vimentin in MCF-7 human breast adenocarcinoma cells (Lai et al. 2016). The anticancer effect of ripe fruits was also observed in MCF-7 cells (Son et al. 2003). In 12-O-tetra decanoylphorbol-13-acetate (TPA) induced-MCF-7 cell culture (which does not have estrogen receptors), a glycoprotein isolated from *S. nigrum* showed antineoplastic activity by suppressing NF-κB and AP-1 as well as the elevation of NO production (Heo et al. 2004a). The same effect of *S. nigrum* glycoprotein on NF-κB was also observed in HT-29 colon adenocarcinoma culture treated with glucose oxidase; an enzyme stimulates the production of hydroxyl radicals (Heo et al. 2004b). Additionally, in HT-29 cells, glycine- and proline-rich glycoprotein of *S. nigrum* increased the activity of Casp-3 via mitochondrial cytochrome c (Lee et al. 2005). In AU565 human breast cancer cells, *S. nigrum* leaf extract showed cytotoxic effects by inducing autophagy and apoptosis (Huang et al. 2010).

The plant also showed antineoplastic properties in animal models of cancer. In tumor-bearing mice model of cervical cancer (U14), polysaccharides of *S. nigrum* induced apoptosis via elevation of Bax and reduction of Bcl2 and mutant p53 expression, along with a significant decrease in the pro-inflammatory cytokine, TNF-α (Li et al. 2007a, b) as well as G2/M phase cell cycle arrest (Li et al. 2009).

Polysaccharides also suppressed the invasion of tumor cells to the thymus and restored T-lymphocytes CD4+/CD8+ ratio in peripheral blood (Li et al. 2010). Also, in tumor-bearing S180 and H22 mice, these polysaccharides could improve RBCs membrane fluidity (Yuan et al. 2014), a parameter related to malignancies (Kolanjiappan et al. 2002). In a nude mouse model of HeLa cells tumor, total alkaloids of the plant could significantly inhibit tumor formation (Li et al. 2008b). In breast tumor-bearing mice (4T1 cells), the number of inflammatory cells like T-cells, NK cells and macrophages was increased as a result of tumor disruption. The level of TNF-α, IFN-γ, and IL-4 were increased; while there was a reduction in IL-6 (Razali et al. 2016). The aqueous extract of the plant also inhibited metastasis in primary mouse xenograft and lung metastasis of melanoma together with a decrease in MMP-9 and Akt activity as well as NF-κB, Ras, and PKCα expression (Wang et al. 2010).

20.3.2.3 China Root

Smilax china or China root is a plant from Liliaceae family (www.itis.gov), and the saponin-rich rhizome is used in traditional medicine of countries all over the world (Wu et al. 2010). In TPM, *S. china* is called "Chub-e-chini" and has a hot and wet nature. It was used for the treatment of phlegm and melancholy accumulation and related disorders. China root was also administered in different types of cancer, especially solid tumors (Aghili 2009).

As mentioned, the rhizomes of *S. china* and other species of the genus *Smilax* are rich in steroidal saponins like furostanol saponins (Xu et al. 2014) as well as polyphenolic compounds like kaempferol derivatives (Xu et al. 2008) and phenylpropanoids including smilasides and heloniosides (Kuo et al. 2005).

In SMMC-7721 human hepatocellular carcinoma cell line, *S. china* extract induced cell cycle arrest in S phase and suppressed POLD1 (Cao et al. 2013), a gene encoding human DNA polymerase δ subunit (Zahng et al. 2016). Another study reported the antineoplastic activity of the extract in human ovarian carcinoma A2780 cells via inhibition of proliferation and induction of apoptosis by G2/M phase cell cycle arrest, activation of Casp-3, PARP, and Bax, suppression of Bcl-2 and NF-κB. Also, the extract increased the sensitivity of cancerous cells to cisplatin and adriamycin (Hu et al. 2015). Li et al. (2007b) reported significant cytotoxic activity of the flavonoid-rich fraction of China root in HeLa cervical carcinoma cell line (Li et al. 2007a, b). Kaempferol-7-O-beta-D-glucoside, a flavone glycoside from *S. china* reduced cell proliferation in HeLa cells by G2/M cell cycle arrest, reduction of Cyclin B1 and Cdk1 which was independent of p53, as well as modulation of Bax/Bcl2 and NF-κB nuclear translocation (Xu et al. 2008). Also, in human breast cancer MDA-MB-231 cells, China root decreased metastasis via inhibition of extracellular matrix degradation and elevation of tissue inhibitors of metalloproteinases (Nho et al. 2015).

Other species of the genus *Smilax* also showed anticancer activity. In human colon carcinoma cell line HT-29, breast cancer cell line MCF7, and gastric cancer

cell line BGC-823, *S. glabra* showed antineoplastic activity via an increase in mitochondrial membrane permeability and ROS production, elevation of intracellular calcium, cytochrome-c relocation and Casp-3 activation. These effects were also observed in human adenocarcinoma cells in Balb/c nude mice and murine hepatoma H22 cells in ICR mice (Gao et al. 2011). In SGC7901 cells gastric cancer cells, *S. glabra* inhibited Akt phosphorylation, Akt(p-Thr308)/Bad and Akt (p-Thr308)/MMPs pathways and reduced cell invasion (Hao et al. 2016). In addition, in HepG2 and Hep3B human hepatoma cultures, *S. glabra* exhibited cytotoxic effects via activation of p38, JNK, and MAPK/ERK signaling (Sa et al. 2008).

20.3.2.4 Coriander

Coriandrum sativum is a member of Umbelliferae (Apiaceae) family (www.itis.gov) with a vast application in food and medicine. The fresh or dried aerial parts, as well as the aromatic fruits, are used in culinary and for some therapeutic purposes like carminative effects. In TPM, the plant is called "Geshniz" or "Kozborah" and has a cold and dry nature. Coriander was used for the treatment of gastrointestinal or other disorders related to the accumulation of bile or blood humours. *C. sativum* was also used for tumors with exudative and non-exudative lesions (Aghili 2009).

Coriander contains a variety of terpenes and terpenoids in the essential oil as well as phenolic compounds (Laribi et al. 2015).

In 1,2-dimethyl hydrazine induced colon cancer in rats, coriander decreased cholesterol and cholesterol to phospholipid ratio in tissues (Chithra and Leelamma 2000). Tang et al. (2013) reported the anticancer effects of coriander root in MCF-7 breast cancer cell line via inhibition of H_2O_2-induced cell migration, G2/M phase cell cycle arrest, activation of Casp-3, Casp-8, and Casp-9. The plant also significantly reduced ROS formation in *Helicobacter pylori*-infected human gastric adenocarcinoma cells (Zaidi et al. 2012).

20.3.2.5 Doder

Cuscuta epithymum or clover doder is a parasitic plant from the family Convolvulaceae (www.itis.gov) which grows yellow to orange strands around the host and is the parasite of several herbs like marigold and basil (Stefanović and Olmstead 2004; Behbahani 2014). The plant is known as "Afteimoun" in TPM and has a hot and dry nature. It was used for neuropsychological and melancholic diseases as well as tumors with exudative lesions (Aghili 2009).

Several phytochemicals have been identified in this plant including eugenol, lupeol and different flavonoids like luteolin which are usually present in the host plant, as well (Behbahani 2014). Different species of the genus *Cuscuta* demonstrated antineoplastic activity in cellular models.

In MDA-MB-468 cell line (human breast carcinoma), HT29 cell line (human colorectal adenocarcinoma) and Hela cells (human uterine cervical carcinoma),

aerial parts of *C. chinensis* and *C. epithymum* showed antineoplastic activity (Jafarian et al. 2014). *C. reflexa* aqueous extract showed proapoptotic properties in human hepatoma Hep3B cell cultures which were mediated by NF-κB, elevation of BAX and p53 as well as reduction of Bcl-2 and survivin (Suresh et al. 2011). Phenolic components isolated from *C. reflexa* was also assessed in HCT116 colorectal cells amongst which 1-O-p-hydroxycinnamoylglucose could show considerable anticancer activity (Riaz et al. 2017). In contrary, a macrocyclic glycolipid lactone, cuscutic resinoside A, could induce the proliferation of cancerous cells in MCF-7 human breast cancer cells (Umehara et al. 2004).

20.3.2.6 Myrrh (Guggul)

Different species of the genus *Commiphora* (Burseraceae) under the common name of myrrh or guggul (www.itis.gov) has been widely used in traditional medicine of countries all over the world, including TPM. *C. molmol*, *C. myrrha* and *C. mukul* (Synonym *C. wightii*) are the most popular species of the trees producing guggul gum resin (Sairkar et al. 2016). This resinous exudate is known as "Moghl" in TPM and has a hot and dry nature. Guggul gum has been used for gynecological diseases like amenorrhea, gastrointestinal disorders, and some types of tumors like tumors of the liver (Aghili 2009).

A large body of investigation have been made to identify the active components of guggul gum. Different types of terpenoids including furanosesquiterpenoids, cycloartane triterpenoids, as well as guggulsterones with steroidal structure constitute major phytochemicals of these species (Shen et al. 2012a).

Shishodia et al. (2007) reported anticancer activity of guggulsterone in a wide variety of cancerous cells including human head and neck carcinoma, lung carcinoma, melanoma, breast and ovarian carcinoma, multiple myeloma and leukemia. Also, guggulsterone was also active against drug-resistant cancer cell cultures. The anticancer activity was mediated by the activation of Casp-8 and Casp-9, poly (ADP-ribose) polymerase (PARP) cleavage and c-Jun N-terminal kinases (JNK) as well as downregulation of Akt and S-phase cell cycle arrest (Shishodia et al. 2007). Inhibition of receptor activator of NF-κB ligand RANKL-activated NF-κB and inhibition of IκBα- pathways involved in bone remodeling and osteoclastogenesis with a crucial role in specific types of cancer like multiple myeloma- was also suppressed by guggulsterones (Ichikawa and Aggarwal 2006). In human triple-negative (MDA-MB-231) breast cancer as well as estrogen receptor-positive (MCF-7) cells, guggulipid (standardized guggul extract) showed anticancer activity via cytoplasmic histone-associated DNA damage as well as suppression of β-Catenin signaling and Casp-3 activity which was explained due to the high guggulsterone content. In cultures of human head and neck squamous cell carcinoma, guggulsterone treatment inhibited invasion and induced apoptosis both in vitro and in vivo by decreasing STAT-3 and HIF-1 (Leeman-Neill et al. 2009). Guggulsterone was also effective for the prevention of angiogenesis in human prostate cancer cells (Xiao and Singh 2008). Topical application of guggulsterone

on 12-O-tetradecanoylphorbol-13-acetate (TPA) tumorigenesis in SENCAR mice could significantly reduce biomarkers of skin inflammation and damage including cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), MAPK and NF-κB (Sarfaraz et al. 2008).

In addition to guggulsterone, new steroids from *C. mukul* also showed antiproliferative activity via apoptosis and G2/S cell cycle arrest in prostate cancer PC3 cells (Shen et al. 2012a, c). Some sesquiterpenoids also demonstrated antineoplastic properties in prostate cancer cell cultures by decreasing androgen receptor expression (Wang et al. 2011). A cycloartane triterpenoid from *C. myrrha* exhibited anticancer effects against human prostatic cancer PC-3 cell line by induction of apoptosis via regulation of Bax, Bcl-2, p53 and Casp-3 (Gao et al. 2015).

20.3.2.7 Mustard

Brassica nigra or black mustard (www.itis.gov) is one of the most studied anticancer medicinal plants with a large body of evidence regarding its antineoplastic activity (Mazumder et al. 2016). The plant is called "Khardal" in TPM, and the seeds have a very hot and dry nature and were used for the treatment of chronic disorders related to phlegm and melancholy humours accumulation as well as specific types of neoplasms (Aghili 2009).

Mustard is a member of the family Brassicaceae (Cruciferae) and is rich in glucosinolates like sinigrin which will turn into isothiocyanates in the presence of water and the plant hydrolyzing enzyme, myrosinase. The most significant anticancer components of the seeds are the allyl isothiocyanates (Bhattacharya et al. 2010b; Anubhuti et al. 2016).

In rats with colon cancer induced by azoxymethane, dietary mustard oil with high content of ω3 polyunsaturated fatty acids (PUFA) had more potent effect toward the reduction of tumor incidence in comparison to fish oil; however, the serum and colonic tissue levels of ω3 PUFA was higher in the fish oil treated animals which suggest active components other than these fatty acids be responsible for mustard oil chemopreventive activity (Dwivedi et al. 2003). Mustard oil also caused a significant reduction in tumor growth and vasculature in mice with Ehrlich ascites tumor (EAT) cells (Kumar et al. 2009). It is recently revealed that isothiocyanates specifically accumulate in bladder cancer cells (Bhattacharya et al. 2010a). The allyl isothiocyanates released from hydrated black mustard powder as well as hydrolysed sinigrin not only decreased tumor growth and G2/M phase arrest in bladder cancer cell line and orthotopic rat bladder cancer model but also totally prevented tumor muscle invasion. These effects were observed as a result of cyclin B1, VEGF and Casp-3 inhibition (Bhattacharya et al. 2010b). Also, in wild type as well as mutated bladder cancer cell lines, allyl isothiocyanates could affect the gene expression of factors involved in cell apoptosis including S100P, BAX, and BCL2 (Savio et al. 2015). Another species of mustard, *B. compestris*, was assessed in an animal model of benzo(a)pyrene [B(a)P]-induced forestomach tumorigenesis and

3-methylcholantrene (MCA)-induced uterine cervix tumorigenesis. The addition of 2.5–5% mustard seed powder as a dietary supplement could significantly reduce tumor burden and lipid peroxidation in the animals and improved endogenous antioxidant defense mechanisms including reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) (Gagandeep et al. 2005). In animal model of carcinogen-induced hepatocarcinogenesis, sinigrin showed antitumor activity via elevation of p53, inhibition of Bcl-2 family and different types of caspases (Casp), and induction of cell cycle arrest in G0/G1 phase (Jie et al. 2014).

In a clinical study in 14 healthy volunteers, three-day supplementation with 25 mg mustard isothiocyanates caused a significant decrease in DNA damage which suggests an antigenotoxic activity for mustard, even in short-term administration (Lamy et al. 2012).

Despite all these studies regarding the antineoplastic activity of mustard, there are studies reporting possible tumorigenic properties of these agents (Shukla and Arora 2003); thus, the clinical application of mustard preparations should be well-monitored.

20.3.2.8 Olibanum

Different species of the genus *Boswellia* (from the family Burseraceae) including *B. serrata* and *B. carteri* (www.itis.gov) produce oleo-gum-resin exudates which are known for the anticancer activity. *Boswellia* spp. is called as "Kondor" in TPM and has a hot and dry nature. It was used for the improvement of memory, treatment of gastrointestinal complications related to the excess accumulation of phlegm humour, and several types of inflammation (Aghili 2009). It was also considered as one of the main medicines for the treatment of tumors (Azam Khan 1869).

Current evidence supports the anticancer activity of *Boswellia* spp. The main active ingredients of olibanum or frankincense—*Boswellia* resin—are a group of pentacyclic triterpene compounds called boswellic acids (Huang et al. 2000).

Triterpene-rich fractions of *Boswellia* sp. as well as the isolated boswellic acids exhibited antitumor and antineoplastic activity in several cell lines. Topical application of olibanum extract in 7,12-dimethylbenz[a]anthracene (DMBA)-initiated mice significantly reduced skin tissue cells proliferation and tumor promotion. Also, DNA synthesis was inhibited by the extract in human leukemia HL-60 cells (Huang et al. 2000). The extract was also able to show antineoplastic effects in two brain tumor cell lines as well as five types of leukemia cell cultures (Hostanska et al. 2002). In human breast cancer cells, *B. thurifera* extract demonstrated cytotoxic activity via induction of p53, a pro-apoptotic factor (Yazdanpanahi et al. 2014). Acetyl-11-keto-beta-boswellic acid, one of the well-established anticancer components of *Boswellia* species (Khan et al. 2016), could reduce metastasis of pancreatic cancer cells by decrease in cysteine x cysteine (CXC) chemokine receptor 4 (CXCR4) expression, a principle factor involved in tumor metastasis (Park et al. 2011a). This inhibitory effect on CXCR4 as well as COX-2, MMP-9, and VEGF was also observed in an orthotopic nude mouse model of pancreatic cancer (Park

et al. 2011b). Acetyl-11-keto-beta-boswellic acid also suppressed tumor angiogenesis via inhibition of vascular endothelial growth factor receptor 2 (VEGFR2) kinase in human prostate tumor xenograft mice (Pang et al. 2009). In highly metastatic human fibrosarcoma and mouse melanoma cells, a mixture of alpha- and beta-boswellic acids inhibited topoisomerase II activity, a key enzyme in DNA synthesis, and induced cell differentiation (Zhao et al. 2003). In a mouse model of colon cancer induced by azoxymethane + dextran sodium sulfate, *B. serrata* resin extract showed antitumor activity through inhibiting Akt phosphorylation and suppression of cyclin D1. The extract also reduced glycogen synthase kinase 3β activity, one of the enzymes which have a dual action both as tumor suppressor and proliferation inducer (Beurel et al. 2015).

20.3.2.9 Stinging Nettle

Urtica dioica is a perennial plant from Urticaceae family which is commonly known as stinging nettle (www.itis.gov). It has been named upon its fluffy leaves and stems which cause skin irritation due to the release of formic acid and histamine (Fu et al. 2006). The plant is called "Anjorah" in TPM and has a hot and dry nature. It has been used to treat accumulation of viscous humours and different types of cancers (Aghili 2009).

U. dioica is rich in flavonoids, and phenolic acids and different extracts of the aerial parts have been assessed as in vitro antineoplastic agents (Kavtaradze et al. 2001).

In tissues obtained from patients with localized prostate cancer, aqueous extract of *U. dioica* exerted an inhibitory effect on the activity of adenosine deaminase, an important enzyme responsible for DNA turn-over (Durak et al. 2004). Nettle extract also showed antineoplastic activity in PC3 human prostate cancer cells by induction of apoptosis via elevation of Casp-3 and Casp-9 as well as Bcl2 expression (Mohammadi et al. 2016a). In MCF-7 human breast cancer cells, the plant suppressed cell proliferation through Casp-3 and Casp-9 activation, elevation of calpain 1 and calpastatin levels and increase in DNA fragmentation (Fattahi et al. 2013). Also, in MDA-MB-468 human breast cancer cell cultures, the same results were obtained regarding the antiproliferative activity of the extract in cancerous cells (Mohammadi et al. 2016b).

20.4 Conclusions

There are numerous simple drugs introduced in TPM for the treatment of cancer. Here we represented the most important medicines in the pharmacotherapy of cancer. Most of the plants used in TPM for the treatment of cancer have been investigated by scientists and cellular and–in some cases–preclinical evidences are available regarding their anticancer activity; however, no clinical study was found.

Thus, a long path still lies ahead of the clinical application of these components. Some of these agents have been recently attracted the attention of researchers and has not yet been widely studied.

There are assessments on the pharmacological evidences of TPM for the treatment of several diseases like peptic ulcer, uterine bleeding, different types of wounds and rheumatoid disorders (Farzaei et al. 2013, 2014, 2016b; Mobli et al. 2015) which provides modern scientific support regarding the efficacy of drugs introduced in TPM.

Medicinal plants could act via several cellular mechanisms including induction of cell cycle arrest at different phases of cancer cell cycle, activation of Casp enzymes, induction of pro-apoptotic proteins like Bax and reduction of anti-apoptotic mediators like Bcl2 which leads to a higher rate of apoptosis in tumor cells, reduction of tumor vascularization, decrease in tumor cells invasion and metastasis as well as mitochondrial damage in neoplasms (Table 20.1).

TPM has an individualized approach to treatment; meaning there are not same prescriptions for the same disease in different patients since patients show various reactions to both the disease and the treatment. In other words, TPM considers individual genetic differences amongst patients to choose medicines with suitable potencies and designate the most appropriate treatment regimen (Jafari et al. 2014). Most of the represented simple drugs for the treatment of cancer have a hot nature. Since TPM is categorized as allopathic medicine, the treatment options are chosen based on the opposite nature of the disease. Thus, we can conclude that in most cases, cancers have a cold nature; although, we can find exceptions like black nightshade and coriander with cold nature which have been widely used as a part of cancer treatment in TPM.

Various experimental and preclinical studies support the effectiveness of medicinal plants traditionally used in TPM for the prevention and treatment of cancer. Further studies regarding the safety and efficacy of medicinal plants introduced in TPM are essential to pave the way for the clinical application of these natural drugs.

20.5 Summary

Current chapter aimed to introduce TPM approaches for the pharmacotherapy of cancer. The most important medicines for systemic administration in cancer are medicinal plants as simple or multicomponent preparations; however, natural remedies with animal or mineral origin have also been used. Screening the modern literature to find scientific evidence for anticancer effects of the introduced natural remedies could support beneficial effects of several types of these natural medicines; however, most of the current researches are limited to in vitro or in vivo studies which provide a lower level of evidence in comparison to clinical studies. As cancer is a complicated disease with high mortality rate, it is conceivable that clinical investigation of anticancer drugs cannot be easily performed. Thus, more

Table 20.1 In vitro and in vivo studies on medicinal plants used in TPM for management of cancer

Scientific name(s)	Part	Active components	Assessed models		Mechanisms
			in vitro	in vivo	
Boswellia serrata, *B. carteri*, *B. ovalifoliolata*, *B. thurifera*, *B. sacra*	Oleo-gum-resin exudates of the tree and its essential oil	Boswellic acids, lupeolic acids, 3α, 24-dihydroxyurs-12-ene, 3α, 24-dihydroxyolean-12-ene, cembrene A	Human leukemia HL-60, K 562, U937, MOLT-4, THP-1 cell culture (Huang et al. 2000; Hostanska et al. 2002), human brain tumor (Hostanska et al. 2002), human colorectal cancer (Shen et al. 2012b), cervical cancer cells (Bhushan et al. 2009), prostate cancer cells (Pang et al. 2009), mouse melanoma and human fibrosarcoma cells (Zhao et al. 2003), meningioma cells (Park et al. 2002)	DMBA-initiated mice (Huang et al. 2000), mouse colorectal cancer tumors (Yadav et al. 2012), heterotopic (subcutaneous) human pancreatic cancer xenograft nude mouse (Ni et al. 2012), Ehrlich ascitic tumor, Ehrlich ascitic carcinoma and sarcoma- 180 tumor model (Qurishi et al. 2013), orthotopic pancreatic cancer nude mouse (Park et al. 2011b), azoxymethane/dextran sodium sulfate-induced colon cancer (Chou et al. 2017)	Inhibition of DNA synthesis (Huang et al. 2000), induction of apoptosis through Casp activation, increased Bax expression, NF-κB down regulation and induction of PARP cleavage (Khan et al. 2016), increase in p53 gene specific mRNA (Yazdanpanahi et al. 2014), decrease invasion and metastasis via downregulation of COX-2, MMP-9, CXCR4, and VEGF (Park, 2011a, b), suppression of VEGFR2 signaling pathways and angiogenesis (Pang et al. 2009), induction of differentiation and inhibition of topoisomerase II (Zhao et al. 2003), inhibition of Akt phosphorylation, GSKβ, and cyclin D1 (Chou et al. 2017)

(continued)

Table 20.1 (continued)

Scientific name(s)	Part	Active components	Assessed models		Mechanisms
Brassica nigra *B. juncea* *B. compestris* *B. carinata*	Seed powder and essential oil	Allyl isothiocyanate, sinigrin, nitrogen mustard oil glycosides	Bladder cancer (Bhattacharya et al. 2010b), wild-type and mutated bladder cancer cells (Savio et al. 2015)	Orthotopic rat bladder cancer model (Bhattacharya et al. 2010b), azoxymethane-induced colon cancer in rat (Dwivedi et al. 2003), chemically induced murine uterine cervix and forestomach tumor (Gagandeep et al. 2005), carcinogen-induced hepatotoxicity in rat (Jie et al. 2014), Ehrlich-solid-tumor-bearing mice (Kumar et al. 2009), CT in healthy volunteers (Lamy et al. 2012), preneoplastic hepatic foci development in rat (Shukla and Arora 2003),	Inhibition of cyclin B1, VEGF and Casp 3, inhibition of tumor growth and muscle invasion (Bhattacharya et al. 2010a, b), elevation of GSH, CAT, SOD (Gagandeep et al. 2005), induction of apoptosis via up-regulation of p53 and down-regulation of Bcl-2 family members and cell cycle arrest in G0/G1 phase (Jie et al. 2014), antiangiogenic activity (Kumar et al. 2009), decrease in DNA damage and micronucleus formation (Lamy et al. 2012), involvement of Bax/Bcl2, ANLN and S100P pathways (Savio et al. 2015), risk of tumorigenesis (Shukla and Arora 2003)

(continued)

Table 20.1 (continued)

Scientific name(s)	Part	Active components	Assessed models		Mechanisms
Colchicum autumnale *C. brachyphyllum* *C. crocifolium* *C. hierosolymitanum* *C. tunicatum*	Corm extract	Colchicine and colchicinoids	Human breast carcinoma, human large cell lung carcinoma and human astrocytoma (Alali et al. 2006, 2010), pancreatic cancer (Kumar et al. 2016a; Larocque et al. 2014), colon cancer (Kumar et al. 2016b), leukemia cells (Larocque et al. 2014), human hepatocellular carcinoma and human cancer-associated fibroblast (Lin et al. 2013)	Nude mice (Lin et al. 2013)	Antimitotic activity via inhibition of tubulin polymerization (Bhattacharyya, et al. 2008), endoplasmic reticulum stress and mitochondrial dysfunction by production of ROS (Kumar et al. 2016a; Larocque et al. 2014), up-regulation of MX-1, AKAP12 and TGFβ2 genes (Lin 2013)
Commiphora mukul, *C. africanum* *C. molmol* *C. myrrha* *C. opobalsamum* *C. wightii* *C. guidotti*	Oleo-gum-resin exudates of the tree and its essential oil	Guggulsterones, myrrhanones, furanosesquiterpenoids, cycloartane triterpenoids	Human prostate cancer (Gao et al. 2015), leukemia, multiple myeloma, lung carcinoma, melanoma, breast carcinoma, and ovarian carcinoma, drug-resistant cancer cell lines including dexamethasone-resistant multiple myeloma, gleevac-resistant leukemia, and	Xenograft model of HNSCC (Leeman-Neill et al. 2009), topical application in SENCAR mouse skin tumorigenesis model (Sarfaraz et al. 2008), human breast cancer and drug-resistant breast cancer xenograft mice model (Xu et al. 2014)	Induction of apoptosis via regulation of Bax, Bcl-2, p53 and Casp-3 (Gao et al. 2015), S-phase cell cycle arrest, activation of Casp-8, Casp-9, PARP cleavage and JNK, downregulation of Akt (Shishodia et al. 2007), suppression of RANKL-activated NF-κB and inhibition of

(continued)

Table 20.1 (continued)

Scientific name(s)	Part	Active components	Assessed models		Mechanisms
			doxorubicin-resistant breast cancer (Shishodia et al. 2007), monocytes co-incubated with melanoma and breast cancer cell lines (Ichikawa and Aggarwal 2006), human estrogen receptor-positive and triple-negative breast cancer cells (Jiang et al. 2013), HNSCC (Leeman-Neill, et al. 2009), androgen-sensitive human prostate adenocarcinoma (Wang et al. 2011)		IκBα, inhibition of monocytes differentiation to osteoclasts (Ichikawa and Aggarwal 2006), elevation of histone-associated DNA fragmentation, reduction of beta-Catenin related gene expression like cyclin D1, C-myc and survivin) and suppression of TCF-4 (Jiang et al. 2013), reduction of invasion, HIF-1 and STAT3 (Leeman-Neill et al. 2009), decrease in MMP-9 (Noh et al. 2013), decrease in tumor incidence, tumor body burden and delay in the latency period for tumor appearance, induction of ODC, iNOS, modulation of MAPK and NF-κB (Sarfaraz et al. 2008), inhibition of angiogenesis and

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Table 20.1 (continued)

Scientific name(s)	Part	Active components	Assessed models		Mechanisms
					VEGFR2 (Xiao and Singh 2008), cell cycle arrest in the G2/M phase (Shen et al. 2012), decrease in androgen receptor expression and translocation into the nucleus (Wang et al. 2011), reversal of doxorubicin resistance (Xu et al. 2014)
Coriandrum sativum	Aerial parts, Roots, Seeds	Phenolic compounds	Human breast adenocarcinoma (Tang et al. 2013), human gastric adenocarcinoma cells infected with *H. pylori*	Chemically induced colon cancer in rat (Chithra and Leelamma 2000)	Modulation of lipid metabolism (Chithra and Leelamma 2000), DNA protective effects in normal cells, inhibition of H_2O_2-induced cell migration, G2/M phase cell cycle arrest, activation of Casp-3, Casp-8 and Casp-9 (Tang et al. 2013), decrease ROS formation (Zaidi et al. 2012)
Cuscuta epithymum *C. campestris* *C. chinensis* *C. reflexa*	Aerial parts, seeds	Lutein and other flavonoids, lupeol, eugenol and their epoxide forms	Human breast carcinoma cell line, human colorectal adenocarcinoma cell line, human uterine	–	Regulation of NF-κB, elevation of BAX and p53, reduction of Bcl-2 and survivin (Suresh et al. 2011)

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Table 20.1 (continued)

Scientific name(s)	Part	Active components	Assessed models		Mechanisms
			cervical carcinoma (Jafarian et al. 2014), human hepatoma (Suresh et al. 2011), colorectal cancer (Riaz et al. 2016)		
Smilax china *S. glabra* *S. riparia* *S. scobinicaulis*	Rhizome, Roots, Tubers	Smilaxin, phenylpropanoids, smiglasides, heloniosides	Human hepatocarcinoma (Cao et al. 2013), human breast cancer, gastric cancer and colon carcinoma (Gao et al. 2011), gastric cancer (Hao et al. 2016), ovarian cancer (Hu et al. 2015), human melanoma and leukemia (Li et al. 2007b), human breast cancer (Nho et al. 2015), human hepatocellular carcinoma (Sa et al. 2008), human cervix carcinoma (Xu et al. 2008)	Human adenocarcinoma cells in Balb/c nude mice and murine hepatoma cells in ICR mice (Gao et al. 2011)	S phase cell cycle arrest, inhibition of POLD1 gene expression (Cao et al. 2013), increased mitochondrial membrane permeability and ROS production, elevation of intracellular calcium, cytochrome-c relocation, Casp-3 activation (Gao et al. 2011), inhibition of Akt (p-Thr308)/Bad and Akt (p-Thr308)/MMPs pathways, reduction of invasion (Hao et al. 2016), inhibition of proliferation and induction of apoptosis via G2/M phase cell cycle arrest, activation of PARP and Bax, suppression of Bcl-2 and NF-κB (Hu et al. 2015), G1 phase cell cycle arrest

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Table 20.1 (continued)

Scientific name(s)	Part	Active components	Assessed models		Mechanisms
					and DNA fragmentation (Li et al. 2007b), reduction of metastasis by inhibition of extracellular matrix degradation and increase in tissue inhibitors of metalloproteinases (Nho et al. 2015), activation of p38, JNK, and MAPK/ERK (Sa et al. 2008), S phase cell cycle arrest, autophagy and apoptosis via decrease in glutathione and involvement of MAPK1 signaling (She et al. 2015), G2/M cell cycle arrest, reduction of Cyclin B1 and Cdk1 independent from p53 (Xu et al. 2008)
Solanum nigrum	Leaves, Fruits	Physalins, steroidal glycoalkaloids, glycoprotein, polyphenols, polysaccharides	Human gastric cancer (Ding et al. 2013), human hepatoma cells (Ding et al. 2012), human breast cancer (Heo et al. 2004a; Huang et al. 2010), human colon	Tumor-bearing mice model of cervical cancer (Li et al. 2007a), nude mice nodel of HeLa cell tumor (Li et al. 2008b), breast tumor bearing-mice (Razali et al. 2016), primary mouse	Induction of apoptosis via regulation of Bax, Bcl-2, p53 and Casp-3 activation (Ding et al. 2013), decrease in cell migration and invasion via reduction of MMP-2

(continued)

Table 20.1 (continued)

Scientific name(s)	Part	Active components	Assessed models		Mechanisms
			adenocarcinoma (Heo et al. 2004b), human liver carcinoma (Ji et al. 2008), human prostate cancer (Nawab et al. 2012), human leukemia cells (Sun et al. 2010), NSCLC (Zhou et al. 2014), human cervical carcinoma (Li et al. 2008a)	xenograft and lung metastasis of melanoma, hepatoma and sarcoma tumor bearing mice (Yuan et al. 2014)	and -9 (Sani et al. 2015), G2/M phase cell cycle arrest (Ding et al. 2012), damage to mitochondrial membrane potential and elevation of intracellular Ca2 + (Sun et al. 2010), suppression of NF-κB and AP-1, increased NO (Heo et al. 2004a), autophagy (Huang et al. 2010), decrease in Bcl2 (Ji et al. 2008), increase in E-cadherin, decrease in ZEB1, N-cadherin, and vimentin (Lai et al. 2016), increase in mitochondrial cytochrome-c release, Casp activation and PARP cleavage (Lee et al. 2005), restoration of CD4+/CD8+, prevention of thymus invasion (Li et al. 2010), disruption of tumor cells morphology, decrease in tumor size, elevation of TNF-α, IFN-γ, IL-4 and

(continued)

Table 20.1 (continued)

Scientific name(s)	Part	Active components	Assessed models		Mechanisms
					reduction of IL-6 (Razali et al. 2016; Li et al. 2009), decrease in Ras and PKCα expression (Wang et al. 2010), improvement of RBC membrane fluidity (Yuan et al. 2014), elevation of p38, MAPK and involvement of STAT3 signaling (Zhou et al. 2014), involvement of mTOR pathway (Nath et al. 2016)
Urtica dioica, U. urens	Aerial parts, Roots	Not well-identified	Prostate cancer cell from patients (Durak et al. 2004), human breast cancer (Fattahi et al. 2013), prostate cancer cell line (Mohammadi et al. 2016a),	–	Inhibition of adenosine deaminase (Durak et al. 2004), induction of apoptosis via Casp-3 and -9 activation, elevation of calpain 1, calpastatin and DNA fragmentation (Fattahi et al. 2013)

Abbreviations *Casp* caspase; *Bax* Bcl-2-associated X protein; *Bcl-2* B-cell lymphoma 2; *STAT* signal transducer and activator of transcription; *IL* interleukin; *iNOS* inducible nitric oxide synthase; *ROS* reactive oxygen species; *GSH* glutathione; *CAT* catalase; *SOD* superoxide dismutase; *NF-κB* nuclear factor kappa B; *CDK* Cyclin-dependent kinase; *PARP* Poly (ADP-ribose) polymerase; *TNF* tumor necrosis factor; *MAPK* Mitogen-activated protein kinases; *IFN* interferon; *CD* cluster of differentiation; *PKC* protein kinase; *RBC* red blood cell; *CXCR4* C-X-C chemokine receptor type 4; *VEGFR* vascular endothelial growth factor receptor; *MMP* matrix metalloproteinase; *ODC* ornithine decarboxylase; *HIF* hypoxia inducible factor; *JNK* c-Jun N-terminal kinase; *RANKL* Receptor activator of nuclear factor kappa-B ligand; *COX* cyclooxygenase; *mRNA* messenger RNA; *TGF* transforming growth factor; *TCF-4* T-cell factor 4; *POLD1* polymerase delta catalytic subunit gene 1; *GSK3β* glycogen synthase kinase 3β

mechanistic studies regarding the safety and efficacy of the newly introduced drugs should be considered to provide better support for the clinical evaluation of these agents. Furthermore, isolated phytochemicals from natural sources can be assessed as new backbones for chemical modifications to design antineoplastic agents with higher efficacy and fewer adverse effects.

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Chapter 21
Nutrigenomics, Epigenetics and Pain in Cancer

Saeed Pirouzpanah

Abstract Pain is inevitable experience taken in everyone life, utmost dreadful, and knowledge takes long way chronicle stories to gift heals. Cancer in terms of solid tumors is associated with infiltrating malignant cells in stroma, normal tissue with secreting malignant broth containing inflammatory soup, pound of growth factors, acidic motif combined with toxic oxidants. Terminal dendrites of pain sensory nerves within normal tissue can be activated by caner-induced neuropathy synergize in different ranges with these malignant materials. Recent advances in molecular medicine have speculated the dynamic fact of genetic reprogramming explained in terms of epigenetic. Aberrant events within epigenetic landscape is a well-established theory to figure out every episodes of tumorogenesis, through which some neurologic alterations corresponding to cancer growth and propagation can potentiate noxious stimulus to reach a threshold of irritating pain sense which is nociception. Related receptors as nociceptors and circuits of stimulating mediators might be considered as targets of therapeutic approaches. This chapter will review the known mechanisms of pain sensory nerves and concerning epigenetic process might regulate persistent pain states and finally will review the advances of nutrition in association with genomic alterations (nutrigenomics) might be applicable in the management of chronic pain. Current results showed that neuropathy and reasons for permanent activation of nociceptive nerve fibers may cause to pathologic plasticity of nociception lead less threshold of pain signals to secondary nerve fibers. Therefore pain can persist over longer time and causes wind-up phenomenon. Very complex epigenetic reprogramming are the principle actor in neuronal plasticity. Nutrigeneomics is an active field of research explore benefits of dietary manipulation or nutraceutical components on the transcriptomics, which is being detailed in this review to elucidate the related molecular mechanisms underlie modulation of noxious stimuli and nociceptive pathways.

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Abbreviations

AD	Alzheimer's disorder
APP	Amyloid-beta precursor proteins
AR	Adenosine receptor
A1R	Adenosine A1 receptor
ARE	Antioxidant response element
A1R	Adenosine A1 receptor
ASICs	Acid sensing ion channels
BDNF	Brain-derived neurotropic factor
cAMP	Cyclic adenosine monophosphate
CCT5	Chaperonin containing TCP1 subunit-5
CFA	Complete Freund's adjuvant
CIBP	Cancer-induced bone pain
Cl^-	Chloride ion
CLEC3B	Tetranectin or C-type lecithin domain family 3 member B
CNS	Central nervous system
CpG	Cytosine-phosphate-guanine
CWP	Chronic widespread pain
COMT	Catechol-O-methyl transferase
COX	Cyclo-oxygenase
CREB	cAMP response element-binding protein
CWP	Chronic widespread pain
CX3CL1	Chemokine fractalkine
CX3CR1	Fractalkine receptor
CYT-P450	Cytochrome-P450
DADS	Diallyl disulfide
DAG	Diacylglycerol
DHA	Docosahexanoic acid
DRG	Dorsal root ganglia
DNMT	DNA methyltransferase
ecRNAs	Extra-coding RNAs
EET	Epoxyeicosatrienoic acid
EPA	Eicosapentaenoic acid
EPSCs	Excitatory postsynaptic currents
ER	Estrogen receptor
ERK/MAPK	Extracellular regulated kinase/mitogen-activated protein kinase
EZH2	Enhancer of zeste homolog 2
FOXO3A	Forkhead box O3
GAD	Glutamic acid decarboxylase
GLO1	Glyoxalase-1

GRM2	Glutamate receptor 2 gene
GWAS	Genome-wide association
HAT	Histone acetyl transferase
HDAC	Histone deacetylases
HETE	Hydroxyeicosatetraenoic acid
HPETE	Hydroperoxyeicosatetraenoic acid
HINT1	Histidine triad nucleotide binding protein-1
HTM	High threshold mechanical nociceptor
IL	Interleukins
iNOS	Inducible nitric oxide synthase
Kcna2	Gene encodes "potassium voltage-gated channel subfamily A member 2"
Keap1	Kelch-like ECH-associated protein-1
Kv1.2	Potassium (K) voltage-gated channel subfamily A member 2
LOX	Lipoxygenase
LT	Leukotriene
Lys	Lysine residue
MAPK	Mitogen-activated protein kinase
MKP	Mitogen-activated protein kinase phosphatase
mTOR	Mammalian target of rapamycin
NADPH	Nicotinamide adenine dinucleotide phosphate
MeCPs	Methyl-CpG binding proteins
NAT15	NatF catalytic subunit
NatF	N-terminal acetyltransferase
NFkB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NGF	Neural growth factor
NMDA	Glutamate *N*-methyl-D-aspartate
nNOS	Neural nitric oxide synthase
NRF2 or NFEDL2	Nuclear factor (erythroid derived-2) like-2
NSAID	None steroid anti-inflammatory
OPRM1	Mu-ospioid receptor gene
PDGF	Platelet derived growth factor receptors
PE	Prostaglandin E2 receptor
PG	Prostaglandin
PI3K	Phosphatidil-inositol-3-kinase
PKA	Protein kinase A
PKC	Protein kinase C
PLA2	Phospholipase A2
PLC	Phospholipase C
PPAR-γ	Peroxisome proliferator-activated receptor-gamma
PS1	Presenilin-1
RNS	Reactive nitrogenous species
sn-2	Stereo-chemical numbering
ROS	Reactive oxygen species

SAH	*S*-adenosyl homocysteine
SAHA	Suberoylanilide hydroxamic acid
SAM	*S*-adenosyl-L-methionine
SFN	Sulforaphane
Src	Proto-oncogene tyrosine-protein kinase Src
STAT	Signal transducer and activator of transcription
Txinp	Thioredoxin-interacting protein
TLR	Toll-like receptors
TNF-alpha	Tumor necrosis factor-alpha
TRPA1	Transient receptor potential ankyrin 1
TRPM8	Transient receptor potential cation channel subfamily M member 8
TRPV1	Transient receptor potential vaniloid 1
TrkA	Tyrosine kinase A
TX	Thromboxane
VDR	Vitamin D receptor
VHL	Von Hippel-Lindau

21.1 Introduction

Pain in medical diagnosis is a symptom. Based on pain definition provided by the International Association for the Study of Pain's is sensory and emotional experiences based on the physiologic dysfunctioning system, involving anatomic site, intervals and pattern of pain occurrence, intensity, duration and the etiology.

Pain is a hallmark of cancer development experienced in transitory acute pain, known as breakthrough pain, often becomes persistent and influencing the quality of life of cancer survivors (Reyes-Gibby et al. 2016). Reasons for suffering pain in malignant patients are different and depend mainly on the anatomic sites of developing tumors. The infiltrated tumor or advanced levels of carcinogenesis as metastasis can potently make pathologic changes in ground tissue cause experiencing unpleasantness and severe pain (Rahman et al. 2013). Tumoral induced aggregating inflammatory motives is a drawback of extreme synthesis of eicosanoids and numerous cytokines synergistically supposed to raise the intensity of pain. Although, chronic pain is estimated as the third most common health concerning problem worldwide (Sharif-Naeini and Basbaum 2011), generally, unraveling the mechanistic pathways behind pain in cancer patients still remained as a complicated research topics, particularly in case of being resistant to pharmacologic therapies and specially in cancer patients (Scarpi et al. 2014).

21.1.1 History of Analgesia and Pain

There are many references have been addressed in history for using anesthesia, analgesia and relieving pain in different nations where civilizations had been flourished. Persian and Greek scripts remained to unravel very earlier techniques used by human being to leave surprises for their ages and era. Persian surgeons (near 3000 B.C.) used anesthesia in operating plastic nasal surgery, cataract surgery and so forth evidently proved by studied biofacts explored by archeologist in Shahr Sukhteh historical site (bronz age urban settlement), Zabol, Iran (Dabbagh et al. 2014). There is also an evidence of surgery on skull on a patient may had diagnosed with posthumously with chronic hydrocephalus (Dabbagh et al. 2014). Iranian poet Ferdowsi around 1000 A.D. narrated remedies used to heal wounds in pre-historic era (Dabbagh et al. 2014). He described using folk medicine (combination of cannabis and camphor) in a historic operation of giving birth to a child (Dabbagh et al. 2010, 2014). Caesarian delivery is a term used alternatively in later centuries (Dabbagh et al. 2010).

Chinese Nei Ching (2600 B.C.) considered heart as an organ generate streams of Yin and Yang as powers but the role of brain was ignored (Perl 2011). Alcmaeon (450–500 B.C.) indicated sensations felt by the body and transferred to brain as an earlier evidence of showing the role of brain (Perl 2011). Although Aristotole (384–322 B.C.) provide information on different senses, believed in central role of heart in realizing senses (Perl 2011). Even Hippocrates believed in that pain originated from imbalance body fluid (Dallenbach 1939). For the first time a realistic theory near to recent definitions of pain remarked in Canon of Medicine wrote by Persian philosopher and medicinal researcher Avicenna, who lived at 10th and 11th centuries, introduced pain as a feeling sense along with other senses like titillation and touch with different apparatus sensory circuits (Dallenbach 1939). He substantiated that nervous system is the origin of sensation and specified pain distinctively from touch (Perl 2011). Accordingly, Descartes provided resemble developed theory about pain as a specific sensation independent of other senses (Gonzalez 1973; Bonica 1990). Jorjani also provide evidence of artery-nerve interactions in concerning with trigeminal neuralgia (Dabbagh et al. 2014). He also detailed obstetrical pain and the complication of childbirth (Dabbagh et al. 2014). Very lately, Mahwah brought up issues of thin nerves may transfer pain signals and thicker diameter nerves may sensory links of the injury to dorsal horn of spinal cord, whereas thicker nerve fiber can carry inhibitory signals cause less pain to be perceived (Mahwah 2004).

In very recent definition provided by Woolf as new insights on pain suggests a system which is actively evolved by physiologic stimuli fall into one of following categories of (1) inflammatory pain which is associated with hypersensitivity of potential or real tissue damage and the infiltration of immune cells, (2) nociceptive

pain alerts damaging or noxious stimuli as an early-warning protective apparatus, or (3) pathological pain in terms of abnormal functioning of the nervous system or its abnormal function such as fibromyalgia, irritable bowel syndrome and so forth (Woolf 2010).

21.1.2 Cellular Sources of Pain

Sensory nerve fibers so-called as nociceptors can stimulated transiently or long-lasting to noxious stimuli depends on the intensity of exposure. Based on the mode of noxious subtype, different classifications can be sorted as thermal (heat or cold), mechanical (crushing, scratching, stabbing and others) and chemical (alcohol on a cut or local secretion of prostaglandin E2) stimuli. The presence of a single or combination of these modalities can make nociceptive responses. There is important determinant of pain which is the intensity of presentation of stimuli when reaches the range of noxious thresholds (Julius and Basbaum 2001). Neurons of nociceptor cells locates in dorsal root ganglia (DRG) for the sensory of signals originated of body and the trigeminal ganglion for sensory derived from face (Basbaum et al. 2009).

Nociceptors falls into two main subcategory which are (1) medium diameter myelinated A_delta and (2) small diameter unmyelinated C fibers (Perl 2007). A-delta neuronal afferents presented acute and earlier or rapid sense of pain which functions differently from A-beta fibers responds to mechanical stimuli. In addition, A-delta neural fiber was also subdivided into two other main subtypes. Type I A-delta (high threshold mechanical nociceptor) which is generally responded to mechanical and chemical stimuli and also sensitized to response to weak stimuli if become exposed to noxious heat (>50 °C) for long time. Type II A-delta nociceptors response to mechanical sensations and a very low heat temperature (Perl 2007, 2011).

Small diameter unmyelinated C fibers are sensible and react to slow pain and classified in heterogeneous subsets to exert in multimodal manner. Hence, all C fibers are not recognized as nociceptors. For example peptidergic C fibers secrete neuropeptides, substance P and calcitonin-related gene peptide that they express TrkA neurotrophin receptor (nerve growth factor receptors) in response to neural growth factor (Basbaum et al. 2009). In other hand, the nonpeptidenergic C nociceptor interacts via c-Ret neurotrophic receptor that accepts ligands as glial-derived neurotrophic factor, neurturin and artemin (Basbaum et al. 2009; Perl 2011).

The heterogeneous profile of nociceptors is also attributed to feel specifically the senses. Indeed, diversity of ion channels in which confer to make sensible to hot temperature (transient receptor potential vaniloid 1, TRPV1), cold temperature (transient receptor potential cation channel subfamily M member 8; TRPM8), acid by multiple H+ sensors (acid sensing ion channels; ASICs), and chemical activators (transient receptor potential ankyrin 1, TRPA1) (Basbaum et al. 2009; Gouin et al.

2017). The TRPV1 and TRPA1 ion channels express in non-sensory cells occurring in keratinocyte, mast cells, dendritic cells and endothelial cells where can activate the inflammatory process and introduce neuro-immune axis (Gouin et al. 2017).

21.1.3 Anatomy and Physiology of Pain

21.1.3.1 Sensory Nerves

Chronic pain can be divided into nociceptive and neuropathic pains. Polymodal nociceptors are primary nociceptive sensory neurons responsible for pain sensation, composed of small and medium-sized neurons placed in DRGs (Woolf 2010). There are three main subtypes of nociceptors based on the noxious stimuli those are responding mechanical (touch, scratch, stabbing and pressure), thermal (cold and heat) and chemical activators (Julius and Basbaum 2001; Perl 2011). The nociceptors will be discussed in detail in the section of "cellular mechanism of acute pain" within this chapter.

Neuropathic pain is a persistent pain usually results from nerve injury of nociceptors and associated with mechanical and thermal hypersensitivity (hyperexcitability and abnormal ectopic firing) (Rice and Hill 2006). Potassium (K) voltage-gated channel subfamily A member 2 (known as Kv1.2) is a transmembrane channel that control resting membrane potentials involved in neural excitability and neuropathic pain genesis. The encoding gene, which is responsible for the expression of Kv1.2, is *Kcna2* that could be down-regulated because of peripheral nerve injury and epigenetic events through which can increase pain sensation (Zhao et al. 2017). A-type K channels (A-channels) are functioned transiently and inactivated rapidly (subtypes in mammals: Kv1.4, Kv3.4, Kv4.1, Kv4.2, and Kv4.3). Despite Kv3.4 is activated by high-voltage, the other four are functional at low-voltage (Zhao et al. 2017).

21.1.3.2 Spinal Pathways

Afferent axons of peripheral sensory nerves split into branches where one of them goes to spinal cord and another one placed in periphery (Fig. 21.1). Hence, the "first order" sensory nerve cells (nociceptors) are psudounipolar neuron (Basbaum et al. 2009). The body of sensory neurons that they responsible to transmit signals from the body are placed in DRG, whereas periphery nerves of face transmit to trigeminal ganglion (Marchand 2015).

Two forms of afferent axons exist to transfer pain signals from sensory neurons to spinal cord which are (1) A-delta and (2) C fibers. A-delta takes part in the feeling of quick and sharp pains reasoned out for carrying spiking signals faster because of presence of myelinated sheath on A-delta axons. C-fibers showing dull feelings of pain because carry signals through unmyelinated fibers (Marchand 2015).

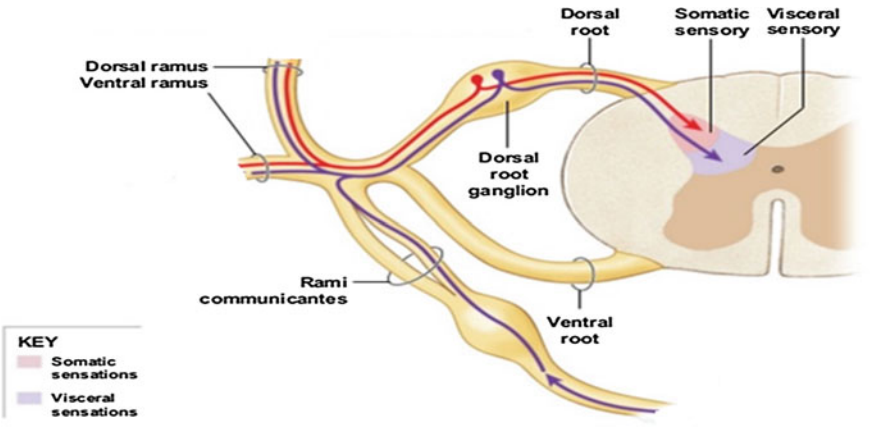

Fig. 21.1 The position of DRG and its psuedounipolar form neuron is shown. This figure was taken from the homepage of Wikipedia.org, a free encyclopedia

Axons of the first-order fibers link by synapses to "second-order" neurons where they place in the central gelatinous substance of the spinal cord (substantia gelantosa) as lamina II and III of the dorsal horns). The location of gelatinous substance in spinal cord demonstrates in Fig. 21.2. A-delta nociceptor fibers project to lamina I and also to lamina IV as deeper dorsal horn root. The low threshold noxious stimuli travel through A-beta afferent fibers projects to deep lamina III, IV, and V. However, C fibers projects to lamina I and II (Braz et al. 2005; Basbaum et al. 2009). Albeit, considering most peptidnergic C afferents project to lamina I and lamina II connected mostly to dorsal region, non-peptidenergic C fibers terminate within lamina II. The ventral lamina II as interneuron of spinal dorsal horn expresses protein kinase C (isoform gamma). Related phosphorylation signaling pathway can be reacted in pathologic pain and persistent tissue injury (Neumann et al. 2008). The wide dynamic range neurons are a bundle of neurons entitled for indirect polysynaptic C fiber, which responds to different intensities of noxious stimulus originated visceral and deep somatic pain (Basbaum 2006; Sykes et al. 2008). Visceral structure of pain is associated quite often with a source of inflammation, ischemia or mechanical tension in a visceral tissue and cause a diffuse and poorly-localized pain (Sykes et al. 2008). Deep somatic inputs of pain can be evoked by nociceptors in ligaments, bones, blood vessels and muscles and being felt like poorly-centralized and dull pain. This is induced as the secondary events to an injury affecting these somatic tissues (Sykes et al. 2008).

Nerve fibers (A-delta and C) locate in gelatinous substance in the middle of spinal cord (anterior white commissure) and carry pain sensation within axons of these nerve fibers through spinothalmic tract to reach thalamus or brain stem. The lateral spinothalamic tract named neospinothalamic tract transferred signals of A-delta fibers to thalamus and connect to "third order" neurons exist in somatosensory cortex (Skevington 1995; Basbaum et al. 2009). This line provides distinguishing sense of pain to be experienced from which place it is originated and

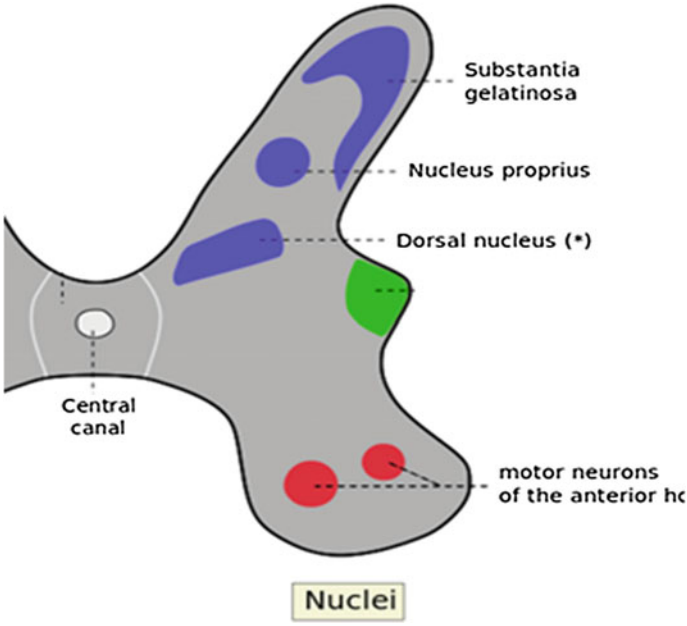

Fig. 21.2 Section of spinal cord shows the location of gelatinous substance (taken from Wikipedia.org, the free encyclopedia)

what intensity it has. The medial part of afferent spinoreticulathalamic tract which ended to brain stem called paleospinothalamic tract which mostly carry pain sensation travel in C fiber neurons to feel poorly localized and distributed sense of pain. Paleospinothalamic neurons also have some branches in thalamus, medulla, pons and periaqueductal gray matter (Skevington 1995). Pain sensation to be perceived in discrimination to other sensory feeling like itch must be distributed to cortical structure of insular cortex and anterior cingulate cortex (Craig 2003; Apkarian et al. 2005). Several lines of imaging evidence also provide insights that sensing pain can activate prefrontal cortical region and also other parts unknown to have role in pain processing where they are basal ganglia and cerebellum (Basbaum et al. 2009; Perl 2011). Basal ganglia and cerebellum might be involved in pain procedure because of the person's response to stimulus (Basbaum et al. 2009).

21.2 Cellular Mechanism of Acute Pain

There is difference between warmth and hot to make painful feeling. The thermal activation threshold of sensory nerve fibers to the hurt and make nociceptive pain is reaching or exceeding approximately above 43 °C consider as noxious heat (Zimmermann et al. 2007). There is non-selective cation channel known as TRPV1

that depolarize certain subsets of A-delta and C fibers to parallel heat sensitivity at both subsets of afferent C and A-delta fibers. Although exceeded heat can take as noxious stimulus to affect TRPV1, other chemical activators are capsaicin, resiniferatoxin and allyl isothiocianate make similar irritation through this pathway and even agonize the activity of TRPV1 (Basbaum 2006; Khairatkar-Joshi and Szallasi 2009). The sensitivity of TRPV1 to heat can be enhanced by inflammatory factors such as prostaglandins, bradykinin, neutrophins and extracellular protons (Caterina et al. 2000). Some metabolites of linoleic acid (13-hydroxy-9,11-octadecadienoic acid and 9-hydroxy-10,12-octadecadienoic acid and keto-analogs) suggested to have potential to mediate enhanced sensitivity of pain sensing neurons in animal models (Patwardhan et al. 2009; Green et al. 2016). N-arachidonyl-dopamine is an earlier identified endogenous compound synthesized in mammals activates TRPV1 with comparable potency to capsaicin (Huang et al. 2002). N-oleoyldopamine is another capsaicin-like endogenous lipid derivative agonized TRPV1 through entourage effects (Chu et al. 2003). N-palmitoylethanolamine is another conjugated fatty acid derivative suggested to have capability to enhance the antiproliferative effects on breast cancer cell lines beside showing agonistic effects on vanilloid receptor-1(Chu et al. 2003).

TRPM8, also known as cold and menthol receptor 1, functions as cold and menthol-sensitive channel to transduce cold somatosensation (Andersen et al. 2014). Likewise knockout animal models for TRPV1 can not sense the noxious heat, TRPM8-deficient mice show significant loss of menthol and cold-evoked sensations over a wide range of temperatures from 30 to 10 °C (Basbaum et al. 2009; Andersen et al. 2014).

Mechanical stimuli can be detected by somatosensory structure to discriminate the intensity of diverse stimuli. A-beta fibers are sensitive to innocuous mechanosensations such as gentle touch, whereas C and A-delta sensory cells carry information of noxious mechanical stimuli (Basbaum 2006). Studying the mechanism in which a transducer introduce the mechanical sensation to nerve system had been primarily postulated in nematode *Caenorhabditis elegans* (*C. elegans*) to show mec-4 and mec-10 as members of the degenerin/eoithelial Na+ channel families (Hill et al. 2017). Accordingly, studies suggested that the mammalian orthologues of ASICs act as mechanotransducer. Indeed, ASICs can also response to increasing protons (H+) in the microenvironment of nociceptive sensory neurons and acts as acid-sensitive ion channels. Increasing the extracellular protons is a condition observed often in ischemia and acidosis.

The microenvironment of solid tumors is highly acidic and cause local acidosis. The high proliferation rate of tumoral cells highly demands faster metabolism in an anaerobic and failures in metabolic conditions, which aggregate metabolic acid equivalents (H+) as metabolic byproducts (Stock and Pedersen 2016). Malignant cells by using abundant circulating glucose to survive for cellular proliferative purpose, in return they release proton and lactate in the metabolic mode of lack or less availability to circulating oxygen (Stock and Pedersen 2016). There is cellular membrane transporter known as Na+/H+ exchange isoform 1 (NHE1), which is ubiquitously expressed in normal cells become overregulated in malignant cells and

can take part in aggravating proliferation, invasiveness and mobility of tumoral cells (Stock and Pedersen 2016). The role of NHE1 inhibition in diminishing noxious nociception in concern with inflammatory and neuropathic pains have been suggested (Torres-López 2013). However, to the best of our knowledge, there is no study to reveal the connection between NHE1 overexpression and ASIC-related pain signaling.

TRPV2 is another ion channel responds to heat and also osmotic stretch and distributed often in smooth muscle cells to detect osmotic stimuli (Muraki et al. 2003). However, this role has not being addressed for somatosensory mechanotransduction in animal models. The role of TRPV4 in serving as mechanotransducer seems unlikely to take place but it responds to injury related pain hypersensitivity (Basbaum 2006). TRPA1 has also been suggested to take part as transducer of mechanical stimuli but TRPA1-deficient mice presented weak defects on mechanosensation (Basbaum 2006). Allyl isothiocyanate (found in wasabi) and allicin (found in garlic) as chemical noxious substances are electrophiles permeable within plasma membrane and make covalent modification with cysteine residues of amino-terminus of cytoplasmic domain of TRPA1 to provide heat sensation (Macpherson et al. 2007).

21.3 Cellular Mechanism of Persistent Pain

Chronic pain is defined as an unpleasant feeling to continue above 12 weeks or three months. Chronic or persistent pain is attributed to injury or disorders related to tumor growth, inflammation, ischemia (diabetes) and so forth. Persistent pain associates with histological alteration or damage to peripheral nerve fibers (Rice and Hill 2006; Basbaum et al. 2009). Persistent presentation of noxious substances to nociceptors causes to transmit continuous pain signals to dorsal horn. This permanent activation of nociceptive nerve fibers may cause to pathologic transformation in nociception to lead less threshold of pain signals to secondary nerve fibers. Moreover, C fibers are responsible to nociception which holds slow rate of conductivity in pain signals in afferent nerve fiber. Therefore, pain can persist over a long period of time. This is so-called as a term of pain wind-up phenomenon.

21.3.1 Chemical Stimuli of Inflammation

Raising the inflammatory factors in association with recruited immune cells to the injured tissue which initially proceeded by paracrine eicosanoids in the locus of injury can change the chemistry around nerve fibers (Umar et al. 2016). This chemical inflammatory change enhance sensitivity in the environment of neurons is implicated to the reduced threshold of pain (Umar et al. 2016).

Eicosanoid is a collective term referred to different subtypes of leukotrienes, prostaglandins, thromboxanes, lipoxins and resolvins, all of which are oxygenated metabolites of unsaturated fatty acids, produced in the case of exposing to mechanical or physical perturbation, collagen breakage, raising the ratio of adenosine-diphosphate to adenosine triphosphate, ischemia, fibrinogen, external pathogens and chemotactic mediators (Funk 2001). This physical or chemical stimulants activate phospholipase A2 (PLA2) to isolate hydrolytically the fatty acid (stereo-chemical numbering position 2) esterified in phospholipids of plasma membrane. Cyclooxygenase (COX-1 and COX-2) catalyzes the oxidation of fatty acids such as arachidonate (C20:4n6) to produce initial forms of prostaglandin (PGG2 and PGH2) and supports later derivatives of prostaglandins (PGD2, PGF2, PGE2 and prostacyclin) and thromboxanes (TXA2 and TXB2) (Fig. 21.3). Prostaglandins in series-2 derived from C20:4n-6 only have two double bonds in their circular structure and show very potent pro-inflammatory effects in the same region of synthesis and secretion (autocrine hormones). They play active role in the initiating stages of inflammation when encounter to stimuli and can postpone inflammation by their chemotactic effects on enhancing cellular immunity and perturbing the function of immune system depends on the ratio of cellular to humoral immunity and inducing cytokine-dependent (1) chemotaxis and (2) transcriptional over-regulation of COX (Cha and DuBois 2007; Rao 2007). One of the hallmark inflammatory stimuli plays important role in acute and even persistent pain is raising the concentration of PGE2 in sensory nerve moiety. Malignant cells experience an induced over-expression of

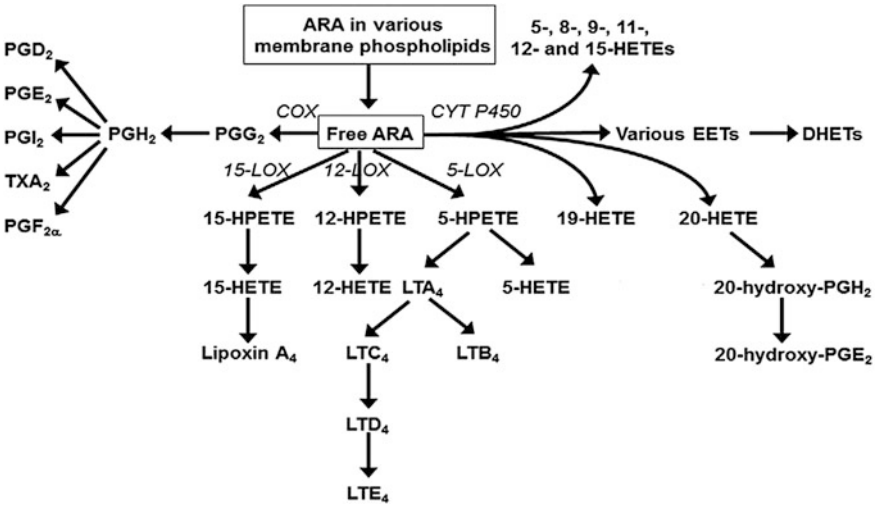

Fig. 21.3 The pathway of eicosanoid synthesis from arachidonic acid (ARA, C20:4n-6).Taken from review provided by Calder (2015). *COX* cyclooxygenase; *CYT-P450* cytochrome-P450; *DAG* diacylglycerol; *DHET* dihydroxyeicosatrienoic acid; *HETE* hydroxyeicosatetraenoic acid; *HPETE* hydroperoxyeicosatetraenoic acid; *EET* epoxyeicosatrienoic acid; *LOX* lipoxygenase; *LT* leukotriene; *PE* phosphatidylethanolamine; *PG* prostaglandin; *TX* thromboxane

COX-2 and subsequent elevations in PGE2 levels (Harris et al. 2014). Inflammatory circumstances raised prominently in states of pathological transformations, which encompass metaplasia, dysplasia and ultimate appearing as malignant cells (Harris et al. 2014; Pang et al. 2016). Sustained exposure to high levels of proinflammatory mediators in the pathologic lesions and leading the transition from acute inflammation to persistent condition can tightly linked to the promotion of tumorogenesis from initiation to progressively to become advanced propagated stages and being resistance to adjuvant therapies such as chemotherapy and radiotherapy (Cha and DuBois 2007; Pang et al. 2016). COX-2 is an inducible enzyme highly can be upregulated in response to proinflammatory activators such as cytokines, growth factors and mitogens can reason out the elements to describe pain mechanism in malignancies (Cha and DuBois 2007; Pang et al. 2016).

None steroid anti-inflammatory (NSAID) drugs such as aspirin are known to make inhibition potently on the activity of COX-1 and COX-2. Aspirin also has been shown to modulate the activity of COX-2, NFkB (nuclear factor kappa-light-chain-enhancer of activated B cells) and signal transducer and activator of transcription 3 (STAT3) pathways (Kim et al. 2016a, b). There are also evidence suggesting that diclofenac and celecoxib can put inhibitions on COX-2 and subsequently can repress cellular proliferation through intervening in WNT/beta-catenin signaling pathway which is important in growth of glioblastoma cells (Sareddy et al. 2013).

Active form of vitamin D or 1,25-dihydroxy-cholecalciferol have been frequently suggested to have anti-inflammatory effects and cellular immune stimulation and make regulation in the transcription of cytokines such as interleukins (IL-6 and IL-8) and tumor necrosis factor-alpha (TNF-alpha) (Gao et al. 2013; Batai et al. 2016). Vitamin D is a ligand for vitamin D receptor (VDR) as a nuclear receptor, and upregulate the transcription of mitogen-activated protein kinase phosphatase (MKP) and related regulatory miRNA. This vitamin D/VDR complex and activated miRNA can inhibit the expression of COX-2 and NFkB in cancerous cells (Gao et al. 2013). Epigenetic regulations mostly mediated by methylation and acetylation on target genes and also proteins such as NFkB can affect the inflammatory intensity (Pirouzpanah et al. 2010), and subsequently bring up promising hypothesis to shape up mechanisms behind inflammatorydependent sensitized pains.

Leukotrienes (LTs) are also derivatives of eicosanoids mediate proinflammatory process and take part in the etiology of allergy, asthma, cancer and chronic inflammatory disorders (Fig. 21.3) (Moore and Pidgeon 2017). Lipoxygenase (5-LOX) is responsible to catalyze the oxidation of poly-unsaturated fatty acids such as C20:4n-6 to inflammatory mediators such as 5-hydroxyeicosatetraenoic acid (5-HETE), 5-hydroperoxyeicosatetraenoic acid (5-HPETE) and LTs. Dihydroxy acid LTB4 (cysteinyl-leukotrienes, CysLT1), LTC4, LTD4 and LTE4 are referred to slow reacting substance of anaphylaxis (Funk 2001; Haeggström et al. 2010). Zileuton is a selective-inhibitor of 5-LOX and other antagonists of CysLT1 are involved in therapeutic approach for asthma to avoid joining LT to its receptor that proceed message in G-protein related intracellular signaling in target cells (Haeggström et al. 2010).

Large number of experimental evidence showed that damaged tissue lead to releasing and accumulation of endogenous inflammatory factors, which are known as inflammatory soup, including eicosanoids (PGs, TXs, LTs, endocanabinoids), peptides (such as substance P and bradykinin), neutrophins, chemokines (CXC, CC, CX3C and XC), protease, protons and several cytokines. Involving cells to provide inflammatory motif in association with tissue damage are granulocytes, neutrophils, macrophages, platelets, endothelial cells and fibroblasts (Basbaum et al. 2009). Inflammatory factors are potently capable to sensitize nociceptive nerve cells because they also have some receptors to detect inflammatory messengers to respond and provide inflammatory signalings (Basbaum et al. 2009). Therefore the excitability of sensory neurons increased (Basbaum et al. 2009). Given, using NSAID, as COX inhibitor, can quite helpful to relieve inflammatory pain respondent to PGE2 synthesis.

In central neurons, neural growth factor (NGF) is an important neural regulator to keep surviving, differentiation and maintenance of nerve cells. In periphery, NGF or neurotrophin is an activating factor of peptidergic C fiber afferents, to express two distinct subclasses of glycosylated receptors: (1) NGF receptor TrkA (tyrosine kinase, TrkA+ neurons) with high-affinity to NGF to transfer the signaling of proliferation and survival of the target neuron, and (2) neurotrophin receptor (P75) with low affinity to NGF when binds to pro-NGF lead to apoptosis whereas NGF acts as a survival signal for neurons (Lee et al. 2001; Basbaum 2006). When NGF binds to TrkA activates downstream signaling pathway involving phospholipase C (PLC), mitogen-activated protein kinase (MAPK), and phosphoinositoide-3-kinase (PI3K) to provide diacylglycerol and inositol-4,5-bisphosphate as intercellular messengers and phosphorylation of target proteins at the peripheral nociceptor terminal (TRPV1) (Chuang et al. 2001). In addition, NGF is also capable to act as transcription factor within nociceptor to promote the transcription of nociceptive proteins including substance P, TRPV1 and the Nav1.8 voltage-gated sodium channel subunit. The expression of substance P when comes along with the existence of excitatory neurotransmitter glutamate in neuron respond to painful stimulation (Donkin et al. 2007). When substance P releases from the peripheral sensory nerve fibers, neurogenic inflammation can rise mostly in response to exposure to pathogens or tissue injury (Donkin et al. 2007). Injury also accompany with raising plenty amounts of different cytokines enhance the existing sensitized inflammatory pain (Ritner et al. 2009). The interaction of inflammation on pain mechanism is of great interests of researchers to introduce the potent with acceptable efficacy of proalgesic agents for persistent pain.

Platelet derived growth factor (PDGF) is actively expressed not only in platelets, but also transcribed in CNS (myelinated and unmyelinated primary sensory neurons and spinal cord) (Narita et al. 2005). Recently, there are supporting evidence showing up the neuroprotective effects of PDGF receptors-beta (PDGFR-beta) in animal models and suggesting the importance of PDGF signaling to play an important role in neuropathic pain induced by nerve injury (Narita et al. 2005; Andrae et al. 2008). Presentation of thrombin to CNS can result hyperalgesia and allodynia showing that PDGF is a mediator for neuropathic pain-like state (Narita et al. 2005).

The role of TRVP in inflammatory pain significantly associates with hypersensitivity in polymodal nociception (Brain 2011). Patients who suffer from neuralgia, arthritis or bone-related malignancies experience severe pain that could not tranquilized thereby makes a physiologic pain to psychologically debilitating (Basbaum et al. 2009). Cancer-induced bone pain (CIBP) is the most common cause of complaining for pain is linked directly to the metastatic colony growth (Scarpi et al. 2014). Migrant malignant tumor cells resides in bone cause a great deals of secretion of eicosanoids and cytokines those induce local aberrant super-activation of osteoblasts and osteoclasts in bone. Transcription of COX is highly up-regulated in tumoral cells and cause burden release of prostaglandins such as PGE2 (Funk 2001). Growing tumor and pathologic changes bring out the worst in damaged bones. Tissue acidosis and accumulating inflammatory factors (Caterina et al. 2000), NGF and eicosanoids produced by malignant cells can sensitize TRPA1 (Basbaum et al. 2009). TRPA1 plays important role in neurogenic and inflammatory pains in response to oxidative stress induced by increasing the endogenous production levels of reactive oxygen species (ROS) and reactive nitrogenous species (RNS) while raised beside inflammation in burden tumor (i.e., CIBP). It has been shown that neurotrophins such as NGF and brain-derived neurotropic factor (BDNF) are highly responsible to lead nociceptive sensitization (Obata and Noguchi 2006; Lozano-Ondoua et al. 2013).

Bone metastasis can be evoked in many types of cancer with frequent occurrence primarily in malignancies of breast, prostate, colon and lung (Delaney et al. 2008; Lozano-Ondoua et al. 2013). Growing tumor cells in bone can also cause pathologic fractures and microfractures those activate sensory nerve terminals (Scarpi et al. 2014). Persistent pain is experienced often in CIBP and exacerbated at night.

Tissue injury and metabolic stress can cause accumulation of extracellular protons and subsequent pH changes. The microenviroment of tumors and metastatic tumors are highly prone to exhibit pH change and cancer-induced acidity within the host tissue (Lozano-Ondoua et al. 2013). As noted above, ASIC channels are categorized into subtypes with characteristic of homotetramers or hetertetramers channel, which show distinct sensitivity for sensing proton levels in pain process (Basbaum et al. 2009). Channels of ASIC also can be expressed by osteoblast and osteoclast through which can be implicated in the process of severe pain perception, when osteolytic (pathologic resorption of bone matrix) and osteoblastic lesions (over-activity of osteoblasts to build bone) are being activated in CIBP (Lozano-Ondoua et al. 2013). Extracellular lactate makes chelate with extracellular free calcium ion, thereby modulate the activity of ASIC1a and ASIC3 and subsequently enhancing the sensitivity of ASICs activity (Immke and McCleskey 2003; Basbaum et al. 2009). Indeed, it seems that calcium ion can compete with protons to link to active site of ASIC and reason out for potentiating channel in lower threshold of proton concentration to be nociceptive (Immke and McCleskey 2003; Ohbuchi et al. 2010). When local hypoxia generated in osmotic motif, there would be a proton gradient in response to the production of lactate in focal ischemia concerning to insufficiency of blood flow to certain fragments of tumor (Basbaum et al. 2009; Ohbuchi et al. 2010).

21.3.2 Glutamate/N-Methyl-D-Aspartate (NMDA) Receptor

Glutamate *N*-methyl-D-aspartate (NMDA) receptor is an ionotropic receptor (form ion channel pore). Activated NMDA can interfere in transferring the sensory signals to the next neurons when this channel remained open by means of the interactive effects of glycine or glutamate (Li et al. 2011). Inhibition of NMDA receptor by potent noncompetitive antagonist such as ketamine is a promising therapy in psychological depression (Li et al. 2011). Li and colleagues showed that NMDA antagonists can increase the density and activity of synapses of neurons in spine to allow transferring the electrical current to afferent prefrontal cortex through mediating of mammalian target of rapamycin (mTOR) signaling, where expressed in the neural dendrites to regulate the level of protein synthesis using in synaptic plasticity which is occurred in hippocampal long-term potentiation (Li et al. 2011; Jafarinia et al. 2016). When glutamate detached from NMDA in central terminals of nociceptors cause excitatory postsynaptic currents (EPSCs) in the following second order neurons of dorsal horn (Basbaum et al. 2009). In the setting of tissue injury, nociception can lead signal to postsynaptic neurons after depolarizing them to trigger quiescent NMDA receptor to become activated (Basbaum et al. 2009). In subsequent neurons NMDA-mediated cytosolic calcium influx can reinforce signaling between nociceptors and dorsal horn later neurons with exacerbating nociception and generating EPSCs (hyperalgesia) (Basbaum 2006; Basbaum et al. 2009). Along with ionotropic receptor activity of NMDA, the activation of metabotropic receptor related to intracellular G-protein signaling to transfer NMDA and substance P receptors on the postsynaptic neuron can exacerbate influx of calcium to cause EPSCs. Downstream molecular pathway metabortropic receptors actively involved phosphorylations performed by certain series of enzymes such as MAPK, PI3K and protooncogene tyrosineprotein kinase Src (c-Src). The upregulation of MAPK and PI3K are considered in G-protein linked nociception and enhance the excitability of these neurons (Latremoliere and Woolf 2009). In other hand, the down regulation of mediators associated in DNMR-metabotropic signaling can be a research target to relive pain.

21.3.3 GABAergic and Glycinergic Controls

GABAergic is an inhibitory synapse in neural process functions, when gamma-amino butyric acid (GABA) secrets in the synaptic neural junction. The presentation of GABA in synapse is driving force to move negatively charged chloride ions out of the cell (GABA causes depolarization), flows chloride net into neuron cells lead to hyperpolarizing (GABA cause inhibition and more negative voltage) and moves positively charged potassium ions out of the cell across ion channels (Szabadics et al. 2006). Actually, GABA is a main inhibitory neurotransmitter in vertebrate. It reduces the neuron excitability in neural process through

signaling via $GABA_A$ receptor subtype (a section of ligand gated ion channel complex) in the postsynaptic plasma membrane of central neurons (Banks et al. 2005). Neural subpopulations express neurokinin 1 receptor in lamina I and II are sensitive to the inhibitory functions of GABA (Takazawa et al. 2017).

Glycine receptor is an ionotropic receptor cause chloride current in response to the glycine (amino acid) as a neurotransmitter. There are four subtypes of glycine receptor alpha and one beta subtype have been identified (Imlach 2017). The frequently distributed isoforms are heterodimers of alpha-1-beta and alpha-3-beta glycine receptor in the spinal cord dorsal horn, where highly active to control pain signaling (Imlach 2017). However, glycine receptor alpha3 is an isotype actively involved in inflammatory pain signaling induced by proinflammatory PGs such as PGE2 binds principally to certain receptor, which is PE (G-protein coupled receptors). Activated PE induces cAMP-dependent protein kinase A (PKA)-mediated phosphorylation of glycine receptor, which in turn leads to reduction of glycinergic signaling and consequently disinhibit and takes out this controlling mechanism of pain circuits (Ahmadi et al. 2002; Imlach 2017). Therefore taking into consideration the ways to potentiate the function of glycine receptor alpha-1-beta and alpha-3-beta receptors in favor of reversing the loss of function in glycine receptor is a promising therapy as analgesics (Imlach 2017).

Recently it is evidently shown that some natural bioactive components can promote the activity of glycine receptor to stimulate inhibitory intermediate neurons to control persistent pain. Balansa et al. showed that some active component of an algae (*Irciniidae sponges*), which are glycine lactam sesterterpenes (geranyl/farnesyl pyrophosphate) can potentiate the activity of glycine receptor alpha-1 and aplpha-3 to intensify negative voltage of inhibitory synapse (Balansa et al. 2010). This is an issue suggested possibly for treating pain and might be used as analgesic in the future (Imlach 2017). Gelsemine, an indole alkaloid isolated from *Gelsemium*, has been shown to lead inhibition on glycinergic neurotransmission which subsequently may involve in increasing spinal excitability. In a study by Lara and colleagues indicated that gelsemine can apparently mediate changes in the function of ion channels independent to gate voltages in synapse (Lara et al. 2016; Imlach 2017). Gelsemine can inhibit glycine receptor containing homomeric alpha-2 or alpha-3 and heteromeric alpha-1/beta, whereas potentiate the activity of homomeric alpha-1 glycine receptor (Imlach 2017). There is another study showed that using popofol derivative 2,6-di-tert-butylphenol (2,6-DTBP) can act as antagonist to relieve inflammatory pain by interfering in the glycinergic actions of alpha/beta glycine receptor to reverse its disinhibition (Acuña et al. 2016; Imlach 2017). Accordingly some studies observed that antagonists of PE receptors can mimic its intracellular phosphorylative functions of PKA to suppress the activity of glycine receptor and its regulating effects on pain signaling (Wada et al. 2013; Imlach 2017). Other ligands of glycine receptor are hypotaurine and taurine (Nguyen et al. 2013; Imlach 2017). Taurine can function as a target molecule to inhibit nociceptive transmission because it can activate glycinergic neurons (Nguyen et al. 2013; Oh et al. 2016). Likewise glycine, administration of

hypotaurine (sulfinic acid), as an intermediate metabolite of taurine biosynthesis of L-cysteine, can function in inhibition of nociception via interacting with glycine receptor (alpha-2 subunit) (Brand et al. 1998; Oh et al. 2016).

Earlier studies showed that gene silencing of protein kinase C gamma (PKCγ) can substantially decrease injury-induced persistent mechanical hypersensitivity in nociception, Which can explain the role of PKCγ expressing neurons in dis-inhibitory process (Imlach 2017). The projection neurons in lamina I and III are potently suppressed by glycine and this subpopulation specifically expressed PKCγ and so forth (Takazawa et al. 2017).

There is a surge of attentions through large number of studies highlighted the important role of adenosine in controlling the nociception (Sawynok 2016). Preclinical studies showed that endogenous adenosine can exert analgesic effects by means of interfering in the activity of G-protein coupled adenosine receptor (AR). Adenosine A1 receptor (A1R) signaling inhibits hyperalgesia and nociception, and is another underlying mechanism postulated by presynaptic Ca^{2+}-dependent inhibition and K^{+}-dependent post-synaptic hyperpolarization, decreased releasing levels of neuropeptide and excitatory amino acids from afferent neurons, interaction with the PLC/IP3/DAG pathway and perceived raise in pain stimulus over time (pain wind-up). Although preliminary and preclinical studies provide numerous findings support the analgesic effects of adenine by treating the function of A1Rs, the analogous agonist could not sufficiently conclusive to prove the effectiveness of adenosine in human trials (Baratloo et al. 2016; Takazawa et al. 2017). Caffeine, the most widely used dietary agent in the world, is a nonselective antagonist of A_1R and $A_{2A}R$ and inhibits antinociceptions (Sawynok 2016). Recently numerous evidence underline the effect of caffeine (alkaloid: 3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione) as a competitive antagonist of glycine receptor Cl^{-} (chloride ion) channel complex through which shows the excitatory effects of caffeine as a methylxanthine in the central hypersensitization and hyperalgesia and allodynia (Baratloo et al. 2016; Uneyama et al. 1993). Jagla and colleagues have shown that using methylnaltrexone in patients, who administered opioid receptor blockade led to significant increase in intravenous morphine dose to acquire analgesia (Viet et al. 2017). Methylnaltrexone is a drug prescribed for cancer patients to antagonize opioid side effects such as constipation of morphine without affecting its analgestic effects (Stein and Lang 2009). Thereby, opioid receptor is a target for opioid and its inactivation can cause its antagonists to become unresponsiveness. Viet et al. (2017) showed that epigenetic silencing of the mu-opioid receptor gene (*OPRM1*) in cancer cells can intensify cancer pain such as CIBP and reactivating the *OPRM1* can lead to antinociception.

Another approach could be planned to increase glycinergic signaling in dorsal horn is to reduce the uptake of glycine from synaptic moiety of glycinergic neurons by glycine transporter-2 (GlyT2) to enhance the availability and maintain high glycine levels at the synapse (Imlach 2017). It is revealed that GlyT2 is highly transcribed in glycinergic neurons and located in presynaptic terminals of neurons (Imlach 2017). The isoforms of GlyT1 and GlyT2 belong to the superfamily of sodium-dependent solute carrier family 6 transporters (neurotransmitter symporter)

and their inhibition is important in controlling neuropathic and inflammatory pain by delaying the clearance of glycine from synaptic moiety (Harvey and Yee 2013; Imlach 2017). Provided evidence by Wiles et al. in animal models showed that using endocannabinoids such as N-arachydonyl-glycine (NAGly) can potently and selectively inhibit GlyT2 and mimics the inflammatory and neuropathic pain (Wiles et al. 2006). Other researches have shown that oleoyl-L-carnitine and N-oleoyl-glycine are synthetic lipid derivatives can show better specificity and irreversible inhibition on GlyT2 (Carland et al. 2014; Vandenberg et al. 2014). Given these lipid derivatives can exert effective beneficial for targeting GlyT2 to control persistent pathological pain.

In the function of nociceptive sensory neuron transmitted to superficial dorsal horn GABAergic or glycinergic inhibitory neurons present the basis of the long standing gate control theory of pain (Imlach 2017). When inhibitory interneurons display strong defects on disinhibition of pain signal can result in promotion of pain. Accordingly, Moore et al., suggested that the peripheral nerve damage-induced death of GABAergic interneurons result consequently enhancing depolarization and excitability of project neurons (Moore et al. 2002). Zeilhofer and colleagues reviewed carefully endogenous mechanisms leading to diminished spinal pain control and suggested that lower inhibitory pain control in the spinal dorsal horn can be reasoned out by interactions in the synaptic levels of inhibitory neurotransmitter that could be depend on the synaptic clearance levels of inhibitors or the integrity of receptors to function appropriately, and also alteration on chloride homeostasis (Zeilhofer et al. 2012).

21.3.4 Interactions of Glial Cell (Neuroglia) and Neurons

Glial cells notably consisted of microglia, astrocytes and satellite glial cells or Schwann cells can contribute to pain signaling and take part in nociception in the setting of injury (Basbaum et al. 2009). Microglial are resident macrophages specifically located in neural tissue and infiltrated homogenously within the gray matter of spinal cord and CNS to generate innate immune system response (Basbaum et al. 2009; Gosselin et al. 2010). The accumulation of microglial and satellite glial cells occurs in superficial dorsal horn and inner part of the termination afferent fibers of injured peripheral nerve fibers (Basbaum et al. 2009), release a large number of signaling mediators such as various cytokines (as a part of immune system), sensitizing neuropathic pain and triggering persistent pain condition, which, in turn, refer to neuroglia (Basbaum et al. 2009). The microglial activation can cause pain to become long lasting and persistent in the model of neuropathy with transitory initiation of central nervous derived chronic pain, while astrocyte activation may cause the maintenance of this initiated long-lasting pain (Gosselin et al. 2010).

Physical reason of nerve injury in periphery afferent prompt some insights about the potential signals such as adenosine-5′-tri-phosphate (ATP) could be secreted

synaptic terminals to be significantly detected by microglia through targeting microglial P2 types of purinergic receptors (purinoceptors) (Basbaum 2006; Basbaum et al. 2009). There are two main subtypes of ATP receptors which are P2X and P2Y receptors exert their impact in pain signaling through ligand-gate ion channels (ionotropic, P2X) and G-protein coupled receptors (metabotropic, P2Y), respectively (Gosselin et al. 2010). Inhibiting purinergic receptors can prevent or reverse neuropathic induced allodynia (Basbaum et al. 2009). However, the activated ATP/P2X4 subtype has been shown to mediate disinhibition and releasing BDNF from microglial, which in turn activates TrK-beta receptors on the projection neurons (lamina I) to change the Cl^- ion gradients and subsequently depolarize GABAergic inhibitory signaling to make excitability in interneurons (Coull et al. 2003; Basbaum et al. 2009). Given the activation of microglial can enhance the sensitivity of lamina I neurons to transmit nociceptive information or indirect signals from A-beta fibers (Basbaum et al. 2009).

Chemokine fractalkine (CX3CL1) is expressed by both primary afferents and spinal cord neurons which shows adhesive and chemoattractant functions, whereas, CX3CR1 is the receptor for fractalkine could be also transcribed by microglial cells and overregulated, when peripheral neural tissue injury occurred and subsequently can be detected as marker of central sensitization (Basbaum et al. 2009; Shin et al. 2015). Another mechanism plays positively in pain circuits is of cross-talk between injured neural fibers and microglial cells through which cleavage of fractalkine from neural surface by cathepsin S is occurred, as a microglial derived protease, to finally amplify the pain signals (Clark et al. 2007). In case of knockout animal models the activity of cathepsin S to sensitize pain remains inactive (Clark et al. 2007). Given inactivation of CX3CR1 can prevent pain initiation and getting long lasting after nerve injury, hence synergize the neuropathic allodynia and hyperalgesia (Basbaum et al. 2009).

Toll-like receptors (TLRs) are transmembrane receptors to translate immune stimuli (pathogens, endogenous ligands such as mRNAs and heat shock proteins) to molecular signaling in the recognition process occurred in innate immune system. The underlying activity of TLR4 in microglial cells of CNS can significantly contribute in inducing the production of proinflammatory chemoattractant by microglial as a transformed macrophage. Thus the activated inflammation related to TLR-4 can attributed to intracellular signaling of inflammatory pain generated following to the nerve tissue injury (Basbaum et al. 2009; Jia et al. 2016). Taken together, these observations could suppose that either antagonists of TLR-4 lead to repress its functionality (pharmacologic intervention) or transcriptional factors downregulate its protein levels (genetic inhibition) can be considered importantly in alleviating microglial activity, reducing hyperalgesia, and generally neuropathic pain associates with inflammation (Basbaum et al. 2009).

Peroxisome proliferator-activated receptor-gamma (PPAR-γ) is a transcriptional factors takes part actively in transcription of proinflammatory mediators, when heterodimerized with NFkB (Genolet et al. 2004). However, there is limited number of studies showing the attributed role of PPAR-γ antagonists (e.g. pioglitazone) on neuropathic pain associated with inflammation (Jia et al. 2016).

21.4 Epigenetic Mechanisms and Pain

The pathology behind the persistent neuropathic pain is complex and the underlying mechanism are limited to physiologic variables, which hardly postulated the molecular backgrounds. However, the obstacles have not been over passed by the pathways where have been enlightened. The transcriptomics of pain-related genes are suggested to be involved in initiation and becoming persistent of neuropathic pain (Wang et al. 2016a, b, c).

Epigenetic which means "outside conventional genetics" is an heritable molecular process contribute in the regulation of transcriptions of genes involved as tumor suppressor genes, controlling cell cycle arrest, cellular apoptotic domains, DNA repairing system and putative nuclear receptor, all of which are independent on the alterations in the sequences of DNA in terms of genetic mutations (Jaenisch and Bird 2003; Pirouzpanah et al. 2010; Mehdipour et al. 2015; Kim et al. 2016a, b). The aberrant methylations at the promoter regions of genes in DNA and acetylation/deacetylation of histones are major epigenetic phenomenon can silence or activate the transcription of genes (Pirouzpanah et al. 2010; Kim et al. 2016a, b). There is highly demands to know how far epigenetic factors can potently regulate transcription and expression of pro-nociceptive or anti-nociceptive genes. Even it is appealing to understand that epigenetic change may be one of the major contributors in neuropathic pain.

Human methyl-cytosine-phosphate-guanine (CpG) binding proteins (MeCPs) binds to the site of methylated CpG and retract the transcription machinery to work out. Following a complete Freund's adjuvant (CFA, the process of stimulating cell-mediated immunity)-induced in ankle join inflammation and nerve injury can lead to the phosphorylation of MeCP2 and consequently results in dissociation and detachments of MeCP2 from methylated DNA (Géranton et al. 2007). It is frequently addressed that nerve injury can induce alterations and variations abnormally in the activity of nuclear methyltransferase that causes aberrant DNA methylation. Other accumulating studies also provide strong evidence that histone modifying enzymes react in the acetylation and/or deacetylation of amino acid residues of histone proteins in chromatin structure can transit euchromatin to heterochromatin (chromatin remodeling) forms after injury of the spinal cord (Mandal et al. 2011; Tochiki et al. 2012; Singh and Thakur 2017). However, observations lacked consensus and containing no convincing evidence on the profiling of transcriptional changes, may be because of unknown molecular details in defining nociceptive pathways.

21.4.1 DNA Methylation

DNA methylation is an important characteristic of epigenetic paradigm in mammals to possibly initiate silencing of nociceptive and anti-nociceptive genes (Jaenisch

and Bird 2003). Region-specific DNA methylation is a certain event of epigenetic leads to certain gene silencing, whereas reduced methylation density of CpG islands in the regulatory sequence of a gene can proceed the upregulation of transcription (Pfeifer 2016). The CpG island is a region of DNA containing a frequent expansions of CpG dinucleotides in short stretch of DNA. DNA methylation is mediated by DNA methylatransferases (DNMTs) catalyze the transferring of methyl group using S-adenosyl-L-methionine (SAM) as a global methyl donor in biologic reactions to cytosine base in the dinucleotide sequence of CpG of DNA (Jaenisch and Bird 2003; Pirouzpanah et al. 2010). There are two important subclasses of DNMTs functionally involve in the maintenance of methylation of DNA (i.e. DNMT1) and de novo methylation of DNA (i.e. DNMT3a and DNMT3b) (Yacqub-Usman et al. 2012). Human methyl-CpG binding proteins (MeCPs and MBDs) can place specifically over the methylated CpGs in DNA and repress transcription of methylated genes (Derecki et al. 2013; Yacqub-Usman et al. 2012).

The limitation of availability of dietary methyl donors (folate, pyridoxine, cobalamin, choline, riboflavin, betaine, serine and methionine) in association with other modifiers necessary for single–carbon metabolism can influence the epigenetic controls on the expression of genes important for biology of neurons (Pirouzpanah et al. 2015; Rubio et al. 2017). There is a growing body of evidence showing that the methylation of cytosine comprising CpG dinucleotides locates in specific regulatory region of DNA plays crucial role in regulating the transcription of gene (Herman and Baylin 2003). It is widely discussed by many studies that the methylation of bases in DNA can promote or inhibit binding of protein in the structure of chromatin (Christman 2003). Overall, hypomethylation are frequently reported to be observed in repeated sequence (such as satellite DNA, single-copy repeated sequences and retroposons), chronic dietary folate deficiency and selective loss of specific-region of DNA, all of which contributed actively in genomic instability, failure in DNA repairing and activation of genes (Christman 2003; Herman and Baylin 2003; Pirouzpanah et al. 2015).

Gene hypomethylation can be influenced in part by depletion of SAM and elevation of intracellular S-adenosyl homocysteine (SAH) as a potent inhibitor of DNMTs. Actually, when the ratio of SAM:SAH reduced within the cell can cause SAH to competitively inhibit SAM-dependent DNA methylation catalyzed by DNMTs (Pirouzpanah et al. 2014a, b; Liu et al. 2016). Despite the overall loss of methylation density of DNA, aberrant hypermethylation at specific DNA regions can occur primarily in CG rich regions (CpG islands) and associates with inactivating the transcription of regulatory genes (Herman and Baylin 2003). There is ample evidence for epigenetically silencing of genes in the progression of neurologic disorders (Kim et al. 2016a, b). Dietary deficiency of methyl donors can lead to accumulation of SAH and when the intracellular levels of adenosine are sufficiently exist to support SAH levels by catalytic activity of SAH hydrolase. Elevated SAH can potently inhibit methyltransferase to methylate a wide variety of biologically important substrates such as DNA (Christman 2003). Accordingly, if this transitory inhibition of DNMTs occurred frequently as course of events in

nucleus might reason out the aberrant hypermethylation at certain region of DNA specifically on regulatory parts (Pirouzpanah et al. 2010, 2015).

Epigenetic mechanisms such as DNA methylation are critical procedures regulate different biological pathways need to sustain the survival of neurons, memory function, integrity of neurons and cellular signaling to transmit electrical current (Savell et al. 2016). DNA methylation is a dynamic paradigm necessary to regulate genes involved in the developing of brain activity which in turn can be also reconditioned by neural activity and behavioral experiences (Savell et al. 2016). However, it is still remained to be uncovered how the methylation procedure can be controlled by upstream pathways.

Recently, Savell et al. have suggested that extra-coding RNAs (ecRNAs) can interfere in the activity of DNMTs to possibly regulate keeping or rearranging the neural DNA methylation (Savell et al. 2016). It is interesting to know that the transcriptional regulations on ecRNAs are also can be altered by promoter hypomethylation under the influence of neural activity. Additionally, Savell et al. showed that animal models underwent knockdown for the locus of Fos ecRNA induced hypermethylation status and gene silencing, where hippocampal transcription of Fos ecRNA is necessary for the formation of long-term fear memory deposition in rats (Savell et al. 2016). Thereby, this is a new promising clue to understand the underlying mechanism and explaining controls on methylation procedure of neuronal tissue.

In Alzheimer disease, recently Liu et al. have indicated another regulator of methylation attributed to pathogenesis of Alzheimer initiation and progression (Liu et al. 2016). Amyloid-beta oligomers repress DNMT activity in neural cells, subsequently increase an integral membrane protein (PS1, presenilin-1) and amyloid-beta precursor proteins (APP) needed for development of Alzheimer and decrease cell viability (Liu et al. 2016). They suggested that folic acid supplementation may reverse the methylation by activating DNMT function in order to promote hypermethylation on upstream regulatory sequences of APP and PS1 and suppress the transcription of these genes to enhance finally the viability of neurons in AD transgenic mice (Liu et al. 2016).

Although studies have made their endeavors to unravel the potent genetic biomarkers susceptible to become aberrantly methylated in diagnosis of neurologic disorder, some results were not sufficiently being supported in the scope of power of analysis, the heterogenesity of nociceptive phenotype, and inadequacy of study design (Burri et al. 2016; Zhang et al. 2016). Recently, a meta-analysis out of genome-wide association (GWAS) has supported an acceptable main effects went for the susceptibility of methylation in chaperonin containing TCP1 subunit-5 (CCT5) and family with sequence similarity 173 member B, which both suggested to have roles in nociceptive regulation of chronic widespread pain (CWP) (Peters et al. 2013; Burri et al. 2016). In another genome-wide methylation study, Minizes and colleagues have shown that there are some differentially methylated sites in DNA linked with biological relevance to fibromyalgia (Menzies et al. 2013). The susceptible gene to be methylated are *BDNF*, *N-terminal acetyltransferase 60* (*NatF*), *NatF catalytic subunit* (*NAT15*) and *PRKCA* (Menzies et al. 2013). Burri et al. have additionally reported

other new genes, which are predisposed to methylation and gene silencing and subsequently contribute in pain regulation (Burri et al. 2016). They found out that CpGs in the DNA sequences of malate dehydrogenase, tetranectin (C-type lecithin domain family 3 member B) and heat shock protein-beta6 are those genes incorporated in pain and candidates to be methylated in CWP (Burri et al. 2016). Another recent evidence based on genome-wide methylation analysis on monozygotic twins reported by Gombert et al., suggested that the methylation of TRPA1 is a nociceptive marker can associate inversely with the threshold of sensing heat and mechanical-related pain by TRPA1 (Gombert et al. 2017). The study subject (healthy volunteers), who experienced low threshold of heat-evoked pain had hypermethylation in the promoter CpG islands of TRPA1 and also the intensity of methylation was remarkable in female participants, who had experienced high pressure pain sensitivities rather than male did. They finally provide evidence to show the contribution of epigenetic regulation of TRPA1 on mediating the sensitivity of thermal and mechanical pain (Gombert et al. 2017).

Catechol-O-methyl transferase (COMT) is an intracellular catalytic enzyme located in post-synaptic neurons to mediate the catabolism of catecholamine neurotransmitters such as dopamine, norepinephrine and epinephrine (Nielsen et al. 2017). Researchers have brought up variants of COMTs associated with nociception (Anand et al. 2016). Inhibition of COMT have shown in increasing mechanical and thermal pain as long as the inhibitor exposed to animal models (Ciszek et al. 2016; Hartung et al. 2016). Variability in the COMT, as a nonopioid system, can influence the efficacy of morphine in cancerous patients to relieve pain (Nielsen et al. 2017). Some potent intercorrelated variables affect the COMT-dependent pain sensitivity are pro-inflammatory cytokines, TNF-alpha, phosphorylated MAPK-P38, and ERK (Hartung et al. 2016; Ciszek et al. 2016). Zhao et al. have pronounced that DNMT3a mediates association between *Kcna2* methylation and related down regulation in the injured DRG after peripheral injury, which partly takes part in neuropathic pain (Zhao et al. 2017).

Ineffective pain management in malignancies is of inadequate mechanistic information regarding to cancer pain and opioid tolerance. The down-regulation of the OPRM1 on the neural cell membrane can be one of the reasons for opioid tolerance in cancer patients (Garzón et al. 2015). The OPRM protein is a G-protein-coupled receptor, which is activated by morphine and its derivatives to induce antinociception in supra-spinal controlling centers of pain. The antinociceptive activity of OPRM can be inhibited by NMDAR function, where both locate in midbrain periaqueductal grey neurons and have cross-talk to desensitize antinociception. This effect is the outcome of contribution of NMDAR within the pathway of NMDAR/neural nitric oxide synthase (nNOS)/calcium and calmodulin-dependent kinase II (Garzón et al. 2015). In addition, it has been revealed that OPRM-associated histidine triad nucleotide binding protein-1 (HINT1) binds to NMDAR and negatively impresses its nociceptive sensory activity and acts as a scaffold protein to hold the cross-talk between OPRM and NMDAR (Vicente-Sánchez et al. 2013). The down-regulation of HINT1 may associate with resistant to opioid antinociceptions and increasing the

hypersensitivity of NMDAR to come up with allodynia that accompanies with neuropathies (Vicente-Sánchez et al. 2013; Garzón et al. 2015). Viet et al. (2017) demonstrated with strong evidence that epigenetic silencing of *OPRM1* gene in cancer cells is an event increase the likelihood of opioid tolerance and exacerbating cancer pain. Tyrosine hydroxylase or tyrosine mono-3-oxygenase is the catalytic enzyme to convert L-tyrosine to L-3,4-dihydroxyphenilalanine (L-dopa) which is the precursor of dopamine. The expression of tyrosine hydroxylase can be down-regulated, when the CpG methylation at the promoter increased which consequently causes dopamine reduction which in turn can decrease related neurotransmitters of epinephrine and norepinephrine (Wang et al. 2016a, b, c). In addition to DNA methylation in controlling the production of dopamine, there is a methyltransferase G9 as an enzyme leads to methylation of H3k9 (histone 3 lysine 9) that partially controls dopaminergic transmission in peripheral nerve injury-induced neuropathic pain (Wang et al. 2016a, b, c).

DNMT3a is one of the prominent contributor in setting the aberrant hypermethylation in numerous genes for instance OPRM1, by which has been suggested that DNMT3a can trigger epigenetic repression on OPRM1 expression in DRG neurons. Herein, one can suppose the promising role of DNMT3a inhibition to possibly manage the neuropathic pain (Sun et al. 2017). In other hand, Jiang et al. showed that peripheral nerve injury downregulates DNMT3b, which in turn may have role in demethylation of CXCR3 promoter and subsequent bindings of enhancer transcription factors with CXCR3 promoter can play a role partially in overregulation of CXCR3 in spinal neurons to contribute and manifest neuropathic pain by central sensitization (Jiang et al. 2017).

Overall, region-specific DNA methylation have been shown as an underlying mechanism to actively inhibit the antinociception and decrease analgesia and pain relief, whereas the hypomethylation or global DNA demethylation can increase the expression levels of nociceptive genes in spite of the concurrent hypermethylation at certain parts of DNA.

21.4.2 Histone Modifications

Post-translation histone modification actively involved in an important part of epigenetic mechanism to regulate transcription levels and genome organization (Zhang et al. 2016). Nucleosomes composed of histones as octameric core proteins and DNA that organize the bases of supercoiling as an essential element incorporates in chromatin formation to regulate the structural integrity of DNA (chromosomal stability), DNA repair and transcriptions. The rate of supercoiling and consequently regulating chromosome dynamic can be ensured by the rate of histone acetylation, which catalyzed actively by histone acetyltransferases (HAT) on lysine residue of N-terminus of histone protein (Robert et al. 2011; Kim et al. 2016a, b). Acetylation is the process that uses acetyl-coenzyme A as substrate. Acetylation change the electronegativity of histones (removing positive charge) and make loose

in the interaction of histones with DNA, which have negative charges attributed to the phosphodiester links between nucleotides (Singh and Thakur 2017). Thereby, the condensed chromatin takes an alteration toward become more relaxed structure (euchromatin) through which can potently associate with overregulated gene transcription. Contrariwise, deacetylation is another epigenetic reactions removing acetyl groups from histones to reverse the relaxation of chromatin structure, increasing the supercoiling of chromatin, which is referred to heterochromatin with less transcriptional active form of DNA. Deacetylation mainly catalyzed by histone deacetylases (HDACs) (Singh and Thakur 2017). When the process of deactylation contributed in DNA methylation, they synergize the condensation of chromatin, in consequence tensely increase the supercoiling and effectively repress the gene expression (Robert et al. 2011). Inhibition of HDAC is a promising therapeutic approach in cancer and studies showed that valproate as an inhibitor of HDACI and HDACII may associate with reversing the gene silencing (Robert et al. 2011), which could be effective strategy to repress hypersensitivity related to pain.

Histone methylation is another step of histone-related modifications plays important role beside acetylation-related changes on histone proteins to regulate gene expression. Methyl group can be transferred by enzymes including SAM-dependent histone lysine (Lys) methyltransferase and protein arginine methyltransferase (Zhang et al. 2016). As a rule of thumb, histone methylation at lysine residues of Lys9 or Lys27 on histone-3 (H3K9 or H3K27, respectively), or at Lys20 of histone-4 (H4K20) associates with transcriptional inhibition, whereas methylation at H3K4, H3K36 and H3K79 induce enhancement in transcription. Overall, histone methylation depend on the Lys-related position and intensity of methylation can affect the transcriptional activation (Zhang et al. 2016). The downstream of IL-17 signaling includes signal transducer and activator of transcription (STAT5), which is important in recruiting enhancer of zeste homolog 2 (EZH2: a histone-lysine N-methyltransferase) to bind to tandem or multiple GAS motif, and subsequently this correlation across the proliferating pro-B cells revealed that STAT5 likely functions as a epigenetic repressor affected regulatory regions of genes (Mandal et al. 2011). Genome-wide analysis provided insights of association between STAT5 tetrameric binding motif and epigenetic repression (Mandal et al. 2011).

21.5 Nutritional Epigenetics and Pain

Histone acetylation is an important modality in chromatin remodeling and incorporates in epigenetic regulation of gene expression (Christman 2003). Inhibitors of HDAC are utmost supposed to be used as epigenetic-based drugs for relieving persistent pain models in studies. Several studies showed that HDAC inhibitors or what reagent can activate HAT in nucleous of nociceptive neural routs can relieve hyperalgesia (Harrison and Dexter 2013). The rat brainstem nucleus raphe magnus is important in central sensitization of chronic pain, persistent inflammatory and

neuropathic pain (Zhang et al. 2011). A study by Zhang et al. showed that inducing pain by intraplantar CFA administration, as an inflammatory agent, in animal models can decrease the acetylation rate at H3 regulate the promoter of glutamic acid decarboxylase (GAD2 gene) in the nucleous of raphe magnus of the brain to downregulate GAD65, which results in impaired GABAergic neuron inhibition in brainstem (Bai et al. 2010; Zhang et al. 2011). Suberoylanilide hydroxamic acid (SAHA; vorinostat) is an inhibitor of HDAC indicating a broad epigenetic-related changes in transcriptome. Studies showed that SAHA can reverse the expression of GAD65 to attenuate the hypersensitivity and show analgesic effects (Zhang et al. 2011; Sharma and Taliyan 2016). Another study also showed that the administration of SAHA to inhibit visceral hypersensitivity induced by 17b-estradiole mediates hyperacetylation at H3 and enhancing the binding of H3K9ac to the regulatory DNA sequence of metabotropic glutamate receptor 2 gene (GRM2) in conjunction with binding of activated estrogen receptor-alpha in the spinal cord to provide analgesia (Cao et al. 2015). The given findings of studies suggest that GAD65 and HDAC are potential markers as therapeutic targets of persistent pain (Zhang et al. 2011; Sharma et al. 2015).

Although, chronic opioid use and injuries can induce hypersensitivity and once become resistance to HDAC inhibitors, administrating the HAT inhibitors such as anacardic acid and curcumin can attenuate hypersensitivity in this model, showing that increasing rate of the hyperacetylation seems as an acceptable outlook regarding to put weight on reversing antinociceptive pathways in persistent neuropathic pain (Ligon et al. 2016). Zhu et al. showed that curcumin can inhibit the HAT-dependent p300/cAMP response element-binding protein (CREB) activity to acetylate H3K9ac and H4K5and subsequently reduce their binding capacity to the promoter of pronociceptive genes of BDNF and COX-2 (Zhu et al. 2014). Moreover, curcumin can exhibit potent antioxidant and anti-inflammatory activities through which can explain other underlying mechanism of its antinociceptive effects (Ligon et al. 2016).

21.5.1 High-Fat Diet

High-fat diet is a well-known reason in the etiology of metabolic syndrome and related disorders. However, additionally, the expression levels of HDAC isoforms have been revealed to be upregulated in the case of high-fat diet in animal models (Funato et al. 2011). The inhibition of HDAC can associate with neuroprotective effects by acetylation of H3, elevating neurotrophic factor (BDNF) in the case of metabolic dysfunction (Sharma and Taliyan 2016). Jiang et al. showed that acetylation of *PPARγ* can potently activate the regulation downstream of genes as transcriptional factors independent on ligand induced activation. Therefore, HDAC inhibitors can enhance potently the expression levels of *PPARγ* so as to improve insulin sensitivity (Jiang et al. 2014). Diabetes mellitus is a strong risk factor for development of Alzheimer's disorder, emerging the main role of insulin resistance

as a major contributor in the etiology of Alzheimer. HDAC inhibition in diabetes have been shown to improve insulin sensitivity and consequently may be a promising therapy for treating the cognitive deficit (Sharma and Taliyan 2016). In fact, the overexpression of HDAC have been shown to be occurred when a high-fat diet implemented in animal models, whereas, imposing animals to fasting decreases acetylation of H3 and H4 in the ventrolateral part of ventromedial hypothalamus, suggesting the impact of HDAC in molecular base alterations in the medial hypothalamus under different metabolic circumstances (Funato et al. 2011).

Increasing the expression levels of HDAC in superficial dorsal horn can be a single reason to keep hypersensitivity and hold persistent pain. Inhibition of HDAC IIa in dorsal horn can stop the circle and terminate inflammatory-FCA induced thermal hyperalgesia in animal models. Sodium butyrate is a short chain fatty acid derivative has been shown to inhibit HDAC in a noncompetitive manner and selectively repress the activity of subclasses I and IIa (Kukkar et al. 2014). Moreover, studies have shown that increased TNF-alpha levels in neuronal cell culture decrease GABAergic postsynaptic activity and lead to hypersensitization, whereas sodium butyrate not only can restore the GABAergic activity through inhibiting HDAC, but also potently decrease TNF-alpha and consequently can limit the inflammatory-related disinhibition of GABA activity in neuropathic pain (Kukkar et al. 2014).

Longitude consumption of dietary fish oil which is rich in eicosapentaenoic acid (EPA, C20:5n-3) and docosahexanoic acid (DHA, C22:6n-3), and also seeds rich in alpha-linolenate (C18:3n-3) can be helpful in reducing inflammatory pain. DHA and EPA are substrates for COX-2 to produce eicosanoids with far less proinflammatory effects, when compared to arachidonate (C20:4n-6) produces highly inflammatory mediators such as PGE2 and proinflammatory LTs. EPA can enhance GABAergic activity and able to restore impaired memory in animal models (Pérez et al. 2017). However, n-3 subtype of fatty acids with promising effects on preventing and even treating inflammatory pains, have not being noticed for the possible interaction in HDAC related transcriptions thus far and warranted to be studied (Rahman et al. 2013; Yang et al. 2017).

Ketosis is a metabolic state used to define the accumulation of ketone bodies (beta-hydroxybutyrate, acetoacetate and acetone) in circulation. Ketosis is resulted from fasting circumstances as following the longitude dietary calorie restriction, metabolic defects related to insulin resistance or staying on a diet with less caloric proportion provided by carbohydrate (ketogenic diet: provides about 70% calorie from fat), all of which can reliably switch the metabolism to fatty acid oxidation and producing ketosis which is referred to ketogenesis (Pirouzpanah and Koohdani 2011; Masino and Ruskin 2013; Ruskin et al. 2013).

There is a sufficient observations to support the fact that ketone bodies, which are originated mainly from mitochondrial beta-oxidation of fat can provide a broad neuroprotective effects. Ketone bodies in ketotic state causes reduction in the ratio of aspartate relative to glutamate by inhibiting the aspartate amino transferase, and it is revealed that the subsequent reaction of glutamate decarboxylation to produce GABA is increased (Géranton and Tochiki 2015a, b). This allows inhibitory

postsynaptic GABAergic activity in anticonvulsant therapy, and ketosis similar to epilepsy possibly can influence nociception in neuropathic pain (Ruskin et al. 2013; Simeone et al. 2017). Ketogenic diet can diminish neuronal excitability through inducing mitochondrial respiration and biogenesis to increase intracellular levels of ATP (ATP to ADP ratio) and ATP efflux could close the gate of ATP-sensitive K + channels and consequently inhibit neural excitability in favor of controlling epilepsy which may be a parallel mechanism to relive nociceptors hypersensitivity (allodynia) (Ma et al. 2007; Masino and Ruskin 2013; Ruskin et al. 2013; Simeone et al. 2017). Recently, it has shown that beta-hydroxy butyrate can inhibit HDAC in experimental in vitro and in vivo studies, inducing acetylations on H3K9 and H3K14 where enhance the transcription of forkhead box O3 (FOXO3A), upregulates catalase and Mn-dependent superoxide dismutase, in favor of increasing resistance to oxidative stress (Boison 2017; Simeone et al. 2017).

Taken together, limited number of experimental observation in hand showed that HDAC activity mediated by the levels of dietary fat intake have potential role where incorporate in chromatin modelling which dynamically modulates the transcriptional activity of target genes in nociceptive pathways which of course remained highly to be resolved in refractory cancer pain.

21.5.1.1 High Carbohydrate Diet

High dietary intake of simple carbohydrate such as sugar have long been known to associates with increasing the likelihood of insulin resistance and related disorders such obesity, hyperlipidemia, metabolic syndrome, diabetes, coronary heart disease, stroke and so forth, all of which reinforce the core impact of metabolic exacerbation in association with high-sugar diet. Although the direct effect of high-carbohydrate intake on HDAC has not being studied thus far, the effect of insulin resistance on increasing the activity of HDAC could be mentioned out based on evidence provided on metabolic syndrome and supposed that high-sugar diet may interfere in HDAC activity dependent on insulin-related metabolism (Vahid et al. 2015; Sharma and Taliyan 2016).

Hypoxia is a potent reason for somatic pain. High-glucose diet and subsequent hyperglycemia can alter the stability and structure of regulatory proteins such as hypoxia-induced factor (HIF) in diabetes and maladaptive response to hypoxia and related analgesia. Given the encompassing reasons have been proposed behind this impairment are (1) methylglyoxal-induced modification of HIF-alpha and concerning regulatory factors such P300, (2) contribution of Von Hippel-Lindau (VHL), and subsequent hydroxylation of HIF-1alpha to be destabilized in high-glucose environments, (3) overexpression of glyoxalase-1 (GLO1) to enhance clearance of methylglyoxalin in the presence of abundance glucose, and (4) to prevent oxidative stress-related methylgloxal-induced modification of P300 (P300/CREB), which is a coactivator of transactivation of HIF-1alpha (Bento and Pereira 2011; Vahid et al. 2015). Bompada et al. showed that in human pancreatic islets of Langerhans, abundance of glucose in the culture motif resulted in elevated

levels of thioredoxin-interacting protein (Txinp) and P300 expression which could be reversed by P300 inhibitors over making less acetylation of H3K9 and H4 to interfere less in the promoter of *Txnip* gene, whereas HDAC inhibitors at high glucose may enhance acetylation levels of P300. Thereby, acetylation can be a promoter for glucose-induced *Txnip* overexpression and apoptosis (Bompada et al. 2016).

In addition, dietary fiber and resistant starch are major functionally bioactive component of diet can produce and support the circulation levels of short chain fatty acids such as butyrate (Tajaddini et al. 2015). Indeed, though, derivative of short chain fatty acid have well-established to be produced on fermentation of fibers and resistant starch in gut, specially colon, HDAC-related transcriptional changes remain elusive regarding to consumption of complex carbohydrate generally, and there is highly demand to be unraveled in neuroprotection by particular aspect of gene settings in nociception.

21.5.1.2 Amino Acids and Related Metabolites

Melatonin, N-acetyl-5-methoxytryptamine, is a neurohormone expressed in the pineal gland and regulate sleep and wakefulness (circadian rhythm). Melatonin is also an endogenous lipophilic antioxidant therefore easily can cross membrane and blood brain barrier (Ulugol et al. 2006). Melatonin is produced from the precursor amino acid L-tryptophan. Dysfunction of L-arginine/NO/cGMP cascade in spinal cord has been shown to take part in hyperalgesic action, whereas, it is widely accepted that this pathway involved in antinociception (Mantovani et al. 2003; Kamei et al. 2005). Melatonin exerts its anti-depressant and antinociceptive effects through which may interact in the activity of L-arginine/NO/cGMP pathway and also NMDA receptors (Mantovani et al. 2003; Ulugol et al. 2006). GABAergic pathway is also addressed to be involved actively in analgesic effects of melatonin (Mantovani et al. 2003).

L-arginine is the precursor for NO (neurotransmitter) synthesis by inducible nitric oxide synthase (iNOS), by which being regulated by calcium-calmodulin-dependent kinase (Moncada and Higgs 1993). NO mediates in the etiology of neuropathic pain. NO can bind to anion superoxide to build-up very toxic oxidant known as peroxynitrite involved in pathogenesis of inflammatory pain. Inhibitors of NOS such as N-nitro-L-argenine-methyl ester is able to inhibit cold allodynia in neuropathic pain in rats (Ulugol et al. 2006).

21.5.1.3 Bioactive Compounds

Curcumin

Curcumin (diferuloylmethane) is the active polyphenolic component isolated from rhizome of turmeric (curcuma longa) exerts dual potent inhibitions on COX-2 and

LOX-5, through which highlighted curcumin as one of the major anti-inflammatory components in the alternative medicine to be used to suppress releasing eicosanoids in inflammatory lesions and consequently capable to manage inflammatory pain (Rao 2007; Zhao et al. 2014; Zhu et al. 2014). The inhibitory effects of curcumin on PLA2 have also been shown with very low IC50 (concentration of an inhibitor exert 50% inhibition) indicating effective inhibition on PLA2.

Metabotropic GRM2 is G-protein coupled receptor expressed in the spinal cord and DRG (expressed presynaptic in the first order sensory afferents with cell bodies in the DRG), wherein responsible in dealing with glutamate (neurotransmitter)-related neuronal signaling to diminish NMDA-receptor activity and decrease risk of excitatory functions (Carlton et al. 2001). The transcription levels and activity of GRM2 can be regulated by NFkB-dependent HDAC inhibitors (SAHA) to induce acetylation of the P65 subunits at K310 to show analgesic effects. Zammataro et al. have shown that curcumin can inhibit HDAC in DRG and epigenetically can modulate GRM2 expression to improve the analgesic effects of GRM2 agonist (Zammataro et al. 2014). Meja et al. showed that curcumin can restore oxidant-impaired HDAC2 activity through downregulating the gene expression of proteins involved in the process of proteasomal degradation in order to target the anti-inflammatory effects of corticosteroids (Meja et al. 2008; Wang et al. 2016a, b, c). Curcumin may thus have potential to show antinociceptive effects on inflammatory pain in cancer patients, who suffer from sever oxidative stress particularly in association with metabolism of pathologic lesions.

Neuropathic pain associates with upregulated expression of HAT-dependent P300/CREBP in rat spinal dorsal horn. However, it is important to know that curcumin plays an important role in inhibition of HAT activity and downregulates downstream genes revealed to have nociceptive roles in neuropathic pain (Zammataro et al. 2014; Zhu et al. 2014). Zhu et al. showed that curcumin can inhibit HAT and subsequent binding of P300/CREBP and H3K9 and H4K5 to the promoter of COX-2 and neurotrophic factor, i.e., BDNF (Zhu et al. 2014). There are accumulative evidence showing that inflammatory pain is in association with upregulation of TNF-alpha and IL-1 at cerebrocentric and peripheral nerve tissue in the animal models. It is also speculated that curcumin as an agent of adjuvant antioxidant therapy can relive inflammatory pain by potently interfering in cytokine biosynthesis along with inhibiting the per-oxidant (such as nitric oxide) biosynthesis to make antinociception (Sharma et al. 2007; Zhao et al. 2014).

Sulforaphanes

Dietary sulforaphane (SFN) and its chemopreventive effects on carcinogenesis has long been studied (Daniel and Tollefsbol 2015; Jafarpour-Sadegh et al. 2016). Many studies have indicated that SFN as a major component of cruciferous vegetables (broccoli, kale, Brussels sprouts and cabbages) can markedly inhibit HDAC and its different isoforms to enhance acetylated histones H3 and H4 (Vahid et al. 2015; Jiang et al. 2016). Although studies showed that SFN can potentiate the

expression of BDNF and related TrkB signaling pathway to prevent neural dosorders such as Alzheimer's disease, SFN administration in animal diabetic models showed reduction in NGF and BDNF levels in neuronal cells of hippocampus (Wang et al. 2016a, b, c). If BDNF deficiency is a reason to explain the pathogenesis of Alzheimer's disease, SFN may induce BDNF synthesis in presynaptic neuronal terminal (first-order neurons) to prevent BDNF-dependent Alzheimer (Kim et al. 2017). However, the upregulation of BDNF in DRG and spinal cord is responsible to generate nociceptive sensitization and neuropathic pain (Obata and Noguchi 2006). Thus far, there is no study to unravel the effects of SFN on BDNF-dependent neuropathic pain in cancer.

There are sufficient amount of evidence to show that SFN can interact in cellular web of anti-oxidant defense system to detoxify oxidants generated through endogenous metabolic pathway and even exogenous resources in terms of cellular oxidative stress. The bioavailability of SFN to the brain and penetration through blood-brain barrier is a subject of research issues, specially inducing the transcription of heat shock transcription factor-1 to regulate downstream genes in CNS including synaptic transmission is an outstanding findings to explain possibly the cortical connectivity may be improved by SFN administration (Pennisi et al. 2017). Indeed, studies showed that SFN is a nutrigenomic element mainly interact in the upregulation of antioxidant transcription factor that is nuclear factor (erythroid derived-2) like-2 (NFE2L2 or NRF2) related pathway to stimulate phase II detoxification and upregulating multiple cellular antioxidant genes (such as quinine reductase, γ-glutamylcyteine synthetase and glutathione-S-transferase) (Kobayashi et al. 2009). NRF2 localized in the cytosol and usually bound to Kelch-like ECH-associated protein-1 (Keap1) as ubiquitinating agent to degrade NRF2 protein by proteasomes (Kobayashi et al. 2009; Pennisi et al. 2017). Reserving and accumulation of NRF2 can be mediated by SFN, because it is first an electrophilic compound can bind to thiols group in Keap1 cysteine residues to form thioacyl adducts, and then inactivate Keap1 to repress NRF2 from undergoing proteosomal degradation by Keap1-related polyubiquitination (Bai et al. 2013; Shawky and Segar 2017). Sustaining the integrity of NRF2 results in activating redox-sensitive signaling pathway in terms of NRF2-Keap1-antioxidant response element (ARE) to translocate NRF2 to nucleous (Hintze et al. 2003). NRF2 forms heterodimeric bind with Maf protein to promote ARE-dependent transcriptions (Shawky and Segar 2017). ARE is a short DNA sequence cis-regulatory element (5′-TGACNNNGC-3′) locates in the promoter of target antioxidant genes such as thioredoxin reductase, nicotinamide adenine dinucleotide phosphate (NADPH):quinine oxidoreductase 1 and heme oxygenase as endogenous cytoprotective enzymes against oxidative stress and rate-limiting enzymes of glutathione synthesis (Hintze et al. 2003; Kim et al. 2010; Shawky and Segar 2017). SFN exerts dual inhibitory effects on HDAC as well as DNMT, which is responsible to inhibit setting of aberrant methylation in regulatory sequence to suppress transcription of genes-related to pain (Daniel and Tollefsbol 2015).

PDGF is a mediator for neuropathic pain-like state and increased in tumoral synthesis in malignancies (Narita et al. 2005). PDGF can induce intracellular

ERK/MAPK (ras/raf/MEK/ERK signaling cascade) pathway to induce growth signal and PI3K/mTOR/Akt signaling to induce cyclin D1 expression (antiproliferative effect) and cellular oxidative stress. SFN can interact in mTOR activity independent to its antioxidant NRF2-related effects may diminish cyclin D1 expression, angiogenesis and promote P53 regulatory function to improve P21-dependent cellular arrest in G1/S (Shawky and Segar 2017).

Inflammation plays an important role in inducing chronic pain. SFN has been revealed that can: (1) inhibit the expression of proinflammatory transcriptional factors such as activity of NFkB (p65 subunit), (2) inhibit Akt-mediated phosphorylation of IκB kinase (IKK), subsequently and/or independently can regulate TNFalpha, IL1beta, IL6, and (3) upregulate IL10, all of which may be responsible in attenuating potently allodynia and hyperalgesia (Negi et al. 2011; Wang and Wang 2017). Proinflammatory eicosanoids such as PGE2 is synthesized by COX-2 and act as chemotactic agent to recruit immune system to synergize chemotactic inflammation by secretion of cytokines. SFN can substantially inhibit expression and activity of COX-2 through which can partly implicate the possible antinociceptive effects of SFN in diminishing inflammatory pain in neuropathy (Negi et al. 2011; Wang and Wang 2017). The iNOS produces nitric oxide radical and its activity can be a single reason to induce the oxidative stress. When iNOS is overexpressed in injured nerves, it can accompany with inflammation activates immune dendritic cells (such as macrophages) and neuronal microglia (Wang and Wang 2017). Thereby, based on experimental observations, it is possible to suggest that dietary SFN can influence hypersensitivity of inflammatory pains by suppressing inflammatory induced oxidative stress independent to NRF2 effects. Taken together, nutrigenomic observations suggested that SFN exerts dual inhibitory effects on both inflammatory pathways and enhancing NRF2-based antioxidant defense system studied in neuropathic pain.

Tea (Flavan-3-ols)

Drinking tea and green tea (camellia sinesis) provide abundant amounts of bioflavonoids into the diet of consumers. Flavan-3-ol and flavonols, which are considered as chief subtype of flavonoids, find in tea. Flavan-3-ol includes catechin (derivatives are gallocatechin, catechin-3-gallate, gallocatechin-3-gallate), epicatechin (derivatives are epigallocatechin, epicatechin-3-galate and epigallocatechin-3-gallate), theaflavins (theaflavin-3-gallate and theaflavin-3,3′-digallate, found in black tea) and thearubigins (found in black tea) are available by drinking tea. Tea flavonols includes mainly quercetin, myricetin and kaempferol (Nijveldt et al. 2001).

Experimental studies have shown that flavonoids-derived from tea can exert anti-inflammatory activity by inhibiting COX-2 and LOX-5 and subsequently reduce the production of various proinflammatory PGs and LTs. The potential of inhibitions can be affected by the location and number of glycosidic moiety bind to hydroxyl group of flavonoids (Hanaee et al. 2004; Pirouzpanah et al. 2009a, b). In

addition, catechins and epicatechins have been shown to have the capacity of down-regulating COX-2 expression and cytokines as well. Moreover, flavanols are also potent antioxidant and capable to inhibit synthesis of ROSs and RNSs in brain cells after stroke (Nijveldt et al. 2001; Izzi et al. 2012).

Bosch-Mola et al. indicated that EGCG can down regulate CX3CL1 protein expression in the spinal cord to show antihyperalgesic in inflammatory pain in neuropathy (Bosch-Mola et al. 2017). Li et al. showed that epigallocatechin-3-gallate can attenuate oxidative stress to overcome partly on enormously active inflammatory signaling and control cytokines such as TNFalpha (Li and Zhang 2015). Epicatechin and its derivatives suggested to have nociceptive effects in the circumstances of neuropathy (Li and Zhang 2015). Cherng et al. described analgesic effects of baicalin (7-glucoronic acid,5,6-dihydroxyflavone extracted from Haung Qin) when inhibit HDAC1 expression and increase acetyl-H3 (Cherng et al. 2014). Epigallocatechin-3-gallate (EGCG) can also potently inhibit HAT activity which might be in concerning with reduced P65 hyperacetylation and lead to suppressing the NFkB-dependent transcriptional activation of TNFalpha and related ILs to induce anti-inflammatory response in the case of Epstein bar viral infection-induced B lymphocyte transformation (Choi et al. 2009). Nutrigenomic effects of tea flavanols are promising dietary elements in inducing antinociceptive effects by interacting in the mechanistic pathways of inflammation and oxidative stress. However, as far as our knowledge is concerned, there is lack of sufficient information to support that how far epigenetic factors can be affected by tea flavonoids to regulate hypersensitivity, which partly might be speculated within the concept of oxidative-dependent inflammation that is underlined in osteolytic pain (CIBP).

Flavonols

Quercetin is a widely distributed flavon-3-ols in human diet. High quercetin content plant sources of quercetin are onion, apple and tea (Pirouzpanah et al. 2006, 2009a, b). Quercetin is one of the active component can interact in the setting of epigenetic events to regulate the transcription of important biological pathways. Quercetin effectively inhibit HAT-dependent P300/CREB activity to reduce the acetylation of histones where acetyl substituents are responsible in the activating of NFkB and COX-2 expression, thereby induce repression on NFkB-dependent expressions of downstream cytokines and suppress the transactivation of COX-2-mediated eicosanoid synthesis, all of which are crucial exacerbating mediators of carcinogenesis (Xiao et al. 2011). In addition, Lee et al. showed that H3 acetylation induced by quercetin can overexpress FasL, where quercetin also enables activation of c-jun headed transactivation of FasL. FasL binds to trimeric transmembrane receptor (Fas) to form death-inducing signaling complex (DISC) involves in extrinsic pathway of apoptosis. Lee and colleagues showed that quercetin activate HAT and in other hand by inhibiting HDAC activity can efficiently lead acetylation to be set on certain amino acid residue on targeted histones in HL-60 cell lines (Lee et al.

2011). Quercetin is also able to enhance NRF2 levels in DRG of neuropathic models (Komirishetty et al. 2017). Gao et al. have addressed that quercetin can inhibit histamine release from mast cells, repress overexpressed PKC and TRPV1 and consequently decrease neuron excitability in the case of heat hyperalgesia and mechanical allodynia in a dose-dependent manner in neuropathic pain models induced by paclitaxel in cancer (Gao et al. 2016). Calixto-Campos and colleagues have shown that introducing mammary adenocarcinoma known as Ehrlich cells to the skin of rats produce pain and potently this process of nociception can be affected by quercetin through which they explain the role of quercetin in reducing synthesis of cytokines, decreasing neutrophil recruitment, so lessen myeloperoxidase activity and subsequently can attenuate oxidative stress partly (Calixto-Campos et al. 2015). They show that quercetin can potentiate analgesic effects of administration of inhibitors of opioid receptor (Azevedo et al. 2013).

Oxaliplatin is a therapeutic agent in chemotherapy associates with neuropathy and painful sensations that limits the advantage of using this drug. Oxaliplatin has important role in increasing the hypersensitivity of thermal and mechanical nociceptors to experience neuropathic pain. Studies showed that the most integrating mechanism in the procedure of neurotoxicity is oxidative stress. They pronounced that quercetin can scavenge oxidants, inhibit the synthesis of oxidative radicals (such as anion superoxide), inhibiting iNOS and repressing the nitration induced by RNS (such as peroxynitrite and nitric oxide) in the dorsal horn of spinal cord to control pain (Azevedo et al. 2013). Given support the fact that quercetin present anti-inflammatory, antioxidant, analgesic and anti-tumor activity, all of which are mechanisms contributed in the controlling the neuropathic pain (Calixto-Campos et al. 2015). However, the nutrigenomic effect of quercetin in intervening epigenetic mechanism in regulating the nociceptive pathways remained to be elucidated.

Myricetin is a naturally occurring flavonol commonly derived from fruits, vegetables, nuts, berries and tea (Nijveldt et al. 2001; Pirouzpanah et al. 2009a, b). Myricetin exerts biological properties through its antineoplastic, antioxidant and anti-inflammatory effects. Recently, myricetin has being paid much attention in the scope of its analgesic effects. Myricetin inhibited phosphorylation of p38 (MAPK) buy PKC to interact in the activity of voltage-activate potassium channel current in sensory neurons. Myricetin can block Ca2+ influx under the circumstances of K+ induced depolarization (Hagenacker et al. 2010).

Kaempferol exists in fruits (apples, grapes, raspberries, blackberries and peaches), vegetables (tomatoes, onions, potatoes, broccoli, Brussels sprouts, squash, cucumbers, lettuce, beans and spinach) and green tea (Kim and Choi 2013). Kaempferol is an antioxidant and therefore detoxify oxidants owing to providing phenolic polyhydroxyl in the structure of polyphenol, where the number and molecular location of hydroxyl can affect the anti-oxidant potency of kaempferol (Calderón-Montaño et al. 2011). Kaempferol actively involved in a wide range of other biological activities, including anti-inflammatory, antimicrobial, anticancer, estrogenin/antiesterogenic activities (Nijveldt et al. 2001; Izzi et al. 2012). Thereby, kaempferol can participate actively in the antinociception experienced in neuropathy (Calderón-Montaño et al. 2011).

Flavonols likewise other flavonoids showed various ranges of anti-oxidant and anti-inflammatory effects through which have been widely speculated that they have analgesic effects. Although, the inhibitory effects of flavonols on HDAC and DNMT have been repetitively shown, to the best of our knowledge, no study has examined the hypothesis of whether the antinociceptive effects of flavonols might be managed through interfering in the epigenetic bases implicated in pain pathways.

Phytoestrogens

Genistein is another flavonoids (isoflavone) have estrogenic functions. Genistein can interfere in controlling outbalanced status of proinflammatory cytokines and oxidative stress which both implicated in pathogenic circuits of neurodegenerations (Valsecchi et al. 2011). Genistein binds to estrogen receptor and can potently show antagonistic effects on estrogenic signaling in premenopause women. Indeed, it could be explicated that genistein have high affinity to bind ERbeta which is found out that ERbeta could be expressed markedly in neuronal cells and immune system (Valsecchi et al. 2008, 2011). Genistein can activate ERbeta-dependent downstream signaling to bring up consequential features of antioxidant, anti-inflammatory and immunomodulatory effects. Valsecchi et al. have drawn a vision that the ERbeta antagonists are able to provide circumstances of inhibiting allodynia and hyperalgesia. Unlikely, ERalpha antagonists have been suggested to potentiate the allodynic responses, so one can assume the importance of ERbeta to be involved actively in analgesia (Valsecchi et al. 2008).

Organosulfurs

Although we observed that Allium vegetables (organosulfur containing foods) can decrease risk of breast cancer patients in Iranian women (Pourzand et al. 2016), recent controlled-clinical trials added interesting findings that onion (Allium cepa) can provide some advantages in favor of ameliorating some metabolic features and controlling cancer antigens in breast cancer patients, who underwent doxorubincine administration (Jafarpour-Sadegh et al. 2015, 2016). However, the organosulfurs as pungent component found in wasabi and mustard seed can cause inflammation and pain. Indeed, organosulfur can act as stimuli to activate nociceptive pain to be detected by TRPA1 and TRPV1 as nociceptors. Allicin and diallyl disulfide (DADS) are organosulfurs found in garlic can depolarize first-order sensory neurons (TRPA1 and TRPV1) which are also referred in terms of allyl isothiocyanate-sensitive sensory nerves (Bautista et al. 2005). Although the inhibitory effects of allicin on HDAC has been denoted in studies which resulted in given rise to histone acetylation, to the best of our knowledge, no evidence exist to support the this epigenetic pathway in influencing nociception.

21.6 Conclusion and Implications

Bone metastasis can be evoked in many types of cancer. The sensitivity of CIBP have been figured out impressively to be in association with tissue acidosis, accumulating inflammatory factors, NGF and eicosanoid, overdrive of osteolytic activity and osteoblastic lesions, extracellular lactate to make chelate with free calcium ion and focal ischemia. Drugs or nutritional manipulations can target some nociceptive pathways which are overdriving mechanisms and highlighted in studies including: (1) down regulation of mediators associated in DNMR signaling, (2) enhancing GABAergic pathways, (3) upregulating and activating glycine receptor, (4) promoting the transcription and activity of glycine transporters, (5) inhibiting purinergic receptors and (6) alleviating microglial activity, to relive pain. Many studies showed that region-specific DNA methylation can inhibit the antinociception and decrease analgesia and pain relief, whereas the hypomethylation can increase the expression levels of nociceptive genes. Increasing the acetylation at histones by inhibiting HDAC or inducing the activity of HAT are promising therapeutic approaches in cancer treatment and even the pain management.

There is consensus of findings to show overregulation of HDAC isoforms in the case of treating animal pain models with high-fat diet, thereby hyperalgesia and allodynia can be given rise to hold persistent pain. Sodium butyrate can reverse the GABAergic activity by putting inhibition on the HDAC activity, and additionally can constrict the inflammatory-mediated disinhibition of GABA activity in neuropathic pain, suggesting the type of fatty acids to play crucial regulatory role in controlling pain. Ketogenesis is a potent circumstances increase the production rate of GABA, inhibiting HDAC in favor of reactivating antinociceptive genes and inducing ATP efflux to block the gate of ATP-sensitive K+ channels to show analgesia. Melatonin exerts its antinociceptive effects by influencing the activity of L-arginine/NO/cGMP pathway, NMDA receptors and GABAergic pathway. Curcumin can inhibit HDAC, modulate GRM2 expression to improve the analgesic effects of GRM2 agonist. Curcumin may inhibit HAT to possibly downregulates downstream genes related to nociception. SFN mainly upregulate the antioxidant transcription factor that is NRF2 to activate phase II detoxification and upregulating multiple cellular antioxidant genes, in favor of ameliorating inflammatory pain in neuropathy. Flavonoids (flavanols, flavonols and phytoestrogens) have reported to exhibit notable anti-oxidant and anti-inflammatory effects through which it is strongly speculated that they have analgesic effects. They also express inhibitory effects on HDACs and DNMTs, whereas no study has examined the hypothesis of whether they have antinociceptive effects.

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Chapter 22
Metastatic Breast Cancer at a Glance: Scenarios of BC Brain- and BC Bone-Metastasis by Illustrations

Parvin Mehdipour

Abstract The machinery of metastasis is supposed to be the final effort of cancer cells' programming, but requires to be clearly defined. However, it is the success of cancer cells' journey from the primary organ to another destination(s). The occurrence of metastasis is as the result of the unpredictable and diverse cascade programming through the generations of cells and could not be generalized. So, the metastatic pattern is, more realistic, to be considered as a personalized event. The main problem with metastasis is due to the inter- and intra-diversity of different clones. Besides, there are the harmonized cascade events and cross-talk between the uncontrolled malignant scenarios including the functional behaviors of cancer cells and their territories. However, breast cancer metastasis (BCM) is, globally, a pronounced concern and causes lethality in BC patients. In this chapter, it was aimed to provide the basic requirements for metastasis, and the chain of events in BC. These are reflective of sequential cellular and molecular alteration by focusing on different signaling pathways and diverse group of genes through the road map of metastatic events. Besides, the cellular and molecular aspects in BC as a primary tumor and in two metastatic hosts including brain and bone have been explored. The impact of key genes such as growth factors (EGF, VEGF), stem cells (CD44/24 and CD133), the involved signaling pathways and their interactions are presented by illustrations. The remarkable correlations between these targets with the prognostic, prediction, and survival in BC-brain and BC-bone metastasis are highlighted. The most significant consideration in management of BCM includes the classification of the cellular/molecular characteristics, by linking and filling the gaps in genomic and somatic levels and providing most influential therapy to the metastatic patients. Finally, the personalized strategy with a multi-disciplinary approaches is essential to unveil the involved targets though the developmental processes in metastatic cancers.

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Abbreviations

6PGDH	6-phosphogluconate dehydrogenase
6PGL	6-phosphogluconolactonase
a2,6-sialyltransferase *ST6GALNAC5*	*ST6GALNAC5* ST6 *N*-acetylgalactosaminide *alpha-2,6-sialyltransferase* 5
AF1q	Protein of gene MLLT11 (myeloid/lymphoid or mixed-lineage leukemia)
AKT	Protein kinase B (PKB), also known as Akt
ALDH1	Aldehyde dehydrogenase 1
ALK2	Activin receptor-like kinase 2
ALK3	Activin receptor-like kinase 3
ANGPT2	Angiopoietin 2
BBB	Blood-brain barrier
BC	Breast cancer
BDNF	Brain-derived neurotrophic factor
BC	Breast cancer
BCM	Breast cancer metastasis
BCSCs	Breast cancer stem cells
BM	Brain metastasis
BM-MSCs	Bone marrow-derived mesenchymal stem cells
BMP	Bone morphogenetic protein
CSCs	Cancer stem cells
CD44	Cluster of differentiation 44
CD24	Cluster of differentiation 24
CD133	Cluster of differentiation 133
CD151	Cluster of differentiation 151, transmembrane proteins
c-MYC	Avian myelocytomatosis virus oncogene cellular homolog
CIN	Chromosome instability
CTCs	Circulating tumour cells
CXCR4	C-X-C chemokine receptor type 4
CRK	Crk family-adaptors-signalling complex
COX-2	Cyclooxygenase-2
CSCs	Cancer stem cells
CTCs	Circulating tumor cells
crk	for *c*hicken tumour virus no. 10 (CT10) *r*egulator of *k*inase
ECM	Extracellular matrix
EGFR	Epidermal growth factor

EMT	Epithelial–mesenchymal transition
EMT	Epithelial to mesenchymal transition
ER	Estrogen receptor
FMC	Feline mammary carcinoma
FGF	Fibroblast growth factor
G6PDH	Glucose-6-phosphate dehydrogenase
GBM	Glioblastoma multiform
HER	Human epidermal growth factor receptor
HIF	Hypoxia inducible factor
HER2	Human epidermal growth factor receptor 2
HuR (ELAVL1)	Hu antigen R
IDC	Invasive ductal carcinoma
IGF	Insulin-like growth factor
IP	Immunoproteasome
IGFBP	Insulin-like growth factor binding protein
IL-6	Interleukin 6
IL-8	Interleukin 8
JAK/STAT	Janus kinase/signal transducer and activator of transcription
Ki67	MKi67 (cellular marker for proliferation is originated from city Kiel, Germany)
MB	Medulablastoma multiform
MBSCs	MB stem cells
MMPs	Metalloproteinases
MIN	Microsatellite instability
MT1-MMP	Membrane-type. 1 matrix metalloproteinase
MYOF	Myoferlin
miR	MiRNA
MAPK	Mitogen-activated protein kinase
MMTV	Mouse mammary tumor virus
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NF-kB	Nuclear factor kappa B subunit 1
NRF2	Nuclear factor erythroid 2-related factor
NFjB	Nuclear factor kappa B
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells
OG	Oncogene
PARP	Poly-adenosine diphosphate ribose polymerase
p63	Tumor protein 63
Plexin-B1	A receptor for semaphorin 4D (Sema4D, also known as CD100)/a tumor-suppressor protein through regulation of c-Met

PrI	Proteom instability
PI3K	Phosphatidylinositol 3 kinase
PTGS2	Prostaglandin synthase 2
PDGFD	Platelet derived growth factor D
Ptprq	Protein tyrosine phosphatases receptor type Q (PTP receptor type Q)
PLLP	Posterior lateral line primordium/of wild-type and mib mutant embryos
PR	Progestron receptor
PPP	Pentose phosphate pathway
PI-3K	Phosphatidylinositol 3 kinase
P123	Pluronic *P123*
RAE1	Ribonucleic acid export 1
RBCAE1	Ribonucleic acid export 1
Rac1	Ras-related C3 botulinum toxin substrate 1
RARRES3	Acid receptor responder protein 3
RBPs	RNA-binding protein
Sspo	SCO-Spondin
SMAD	From gene *sma* for small body size
STAT3	Signal transducers and activators of transcription-3
SLN	Sentinel lymph node
SDF-1α	Stromal cell-derived factor-1
SLC8A2	Solute carrier family 8 Na-Ca
SLC7A11	Solute carrier family 7 member 11
TGF-b	Transforming growth factor-beta,
TNFSF4	Tumour necrosis factor ligand superfamily member 4 gene (also called Ox40l or Cd134 l)
Tph2	Tryptophan hydroxylase 2
TSG	Tumour suppressor gene
TkrB	Tropomyosin receptor kinas B
type II BMP receptors	Bone morphogenetic protein receptor type 2
TrkA	Tropomyosin receptor kinase A
TrkB p75NTR	Tropomyosin receptor kinas p75NTR
TKLs	Tyrosine kinase inhibitors
uPA	Plasminogen activator, urokinase
UPAR	Plasminogen activator, urokinase receptor
UPA	Plasminogen activator, urokinase
VCAM1	Vascular cell adhesion molecule 1
VEGF	Vascular endothelial growth factor
WNT	Wingless-type

22.1 Introduction

Metastasis is an antagonist mirror in which a message as 'the success of cancer cells' is hidden. This is reflective of a remarkable fact and it indicates that the programmed command from nature is, successfully, transmitted to the cancer cells.

Clinicians and Scientists have been well motivated for more than two centuries to draw a clear and Informative scheme of metastatic events with a great hope to plan an influential strategy to combat against metastasis. This event also creates a crucial problem with the complicated programmed processes. However, in spite of the serious global efforts, the survival rate seems not to be promising for more than half of a century. It is expected that the metastatic cancer cells are not common, but there is a question as 'are these cells easily detectable?' In addition, tracing the migration of metastatic cancer cells from an initial tumor to the blood stream requires different assays on the '**control migratory cells**' as well, and in fact such research is required to be performed in human cancer materials. It worth's to state that the achieved data in animal models and in vitro, even on the human cell-based cell lines, may not be translatable in cancer patients. By considering these facts, the therapeutic trials, even for the target-based strategies, may lead to a real bias. In fact metastasis is an unpredicted condition with many unexpected and diverse behaviors in different tumors of the same patients, and also in the same tumor of different patients. So, it is hard to generalize the metastatic pattern through the available pathways. It may be more realistic that the researcher individualize the metastatic patterns. So, the main problem with metastasis is due to the inter- and intra-diversity of different clones which are extremely varied. Besides, there is no normal harmonized manner, instead there are the harmonized cascade events and cross-talk between the uncontrolled malignant scenarios including the functional behaviors of cancer cells and their territories. However, breast cancer metastasis is, globally, the major cause of death in women and metastasis from breast to different organs is a pronounced concern and causes lethality in BC patients.

Kiesler and Begley (2016) has provided five explanations for being optimistic about the perspective of the global status of cancer (https://www.mskcc.org/blog/future-five-reasons-optimism) which includes "(1) Precision medicine; (2) Development of immunotherapy, (3) Cell-based therapies, and (4) Epigenetic drugs."

In spite of tremendous attempts by scientists and clinicians, the outcome still requires much more complementary efforts for being translated to human. The question is 'when would the cancer patients be able to access an effective personalized management?'

An important question regarding the spread of cancer cells to the neighboring territory of breast cancer (BC) to the sentinel lymph node (SLN) has not been well defined and answered. It was highlighted that "the cancer cells stay in the SLN acting as an incubator before launching the next phase of the metastatic journey to the systemic sites." Professor Dontscho Kerjaschki, from Vienna, has shown in a lecture that the SLN, "acts a hub for the cancer cells to enter into the blood stream for systemic metastasis."

22.2 Road Map of Metastasis: Metastatic Breast Cancer

Regarding the metastatic journey, the cancer cells have two choices including (1) Directly through the blood stream, and (2) through the lymphovascular system. However, SLN may consider as a reservoir for immigrated cancer cells for metastasis. The selected key approaches are summarized in the road map of metastasis (Valastyan and Weinberg 2011) (Fig. 22.1).

22.3 The Required Managements for the Neoplastic Disorders

By highlighting the applicability insights in cancer, a multi-strategy is required to define the unmasked puzzles by considering the routine and simple organized cultural requirements which is summarized in a schematic Figure in which the purposeful approaches are aimed to be puzzled in neoplastic disorders (Fig. 22.2).

22.4 The Cooperative Key Genes in Cell Cycle Progression

Furthermore, cell cycle progression is the target through which inhibitive strategies may lead to restrict tumor growth and proliferation. Specific signaling pathways have been previously published (Peng et al. 2009; Wang and Li 2006); besides, different pathways are involved in cell-proliferation, migration, and invasion

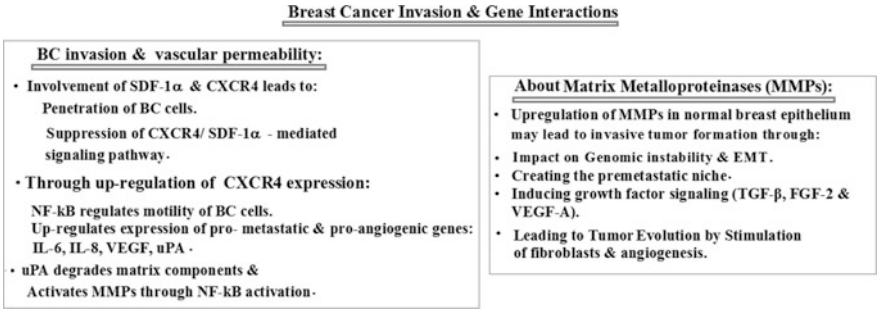

Fig. 22.1 Road map of metastasis: essential insights. *BC* breast cancer, *BCM* breast cancer metastasis

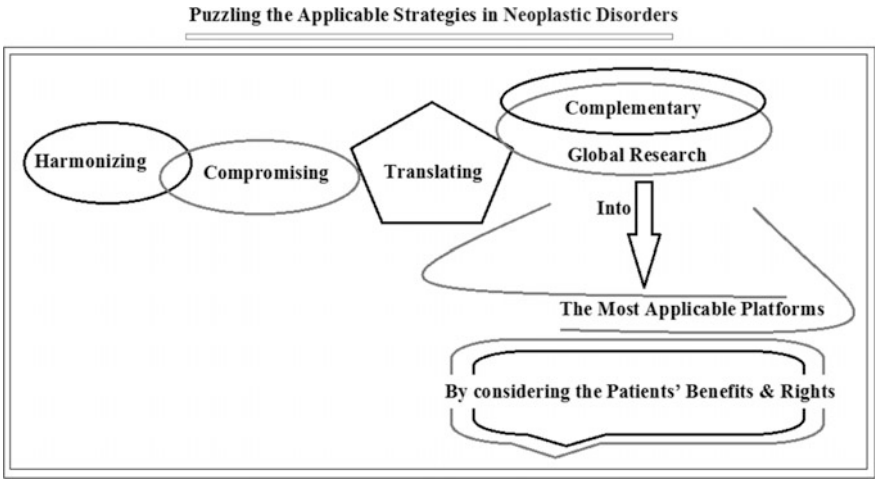

Fig. 22.2 Requirements for achieving the applicable platforms in neoplastic disorders

(Cheng and Sharp 2006; Matter et al. 2002; Weg-Remers et al. 2001). In this regard, an Informative panel are illustrated (Fig. 22.3). These events may lead to metastasis, if the individuals are predisposed to being targeted by metastatic process. So, the managements of such event are relied to an early diagnosis and an appropriate management. Besides, the key role of EGFR expression in cancer development and progression in the metastatic event has been, previously, highlighted in different cancers (Gabos et al. 2006; Rimawi et al. 2010; Torabizadeh et al. 2016). Besides, the EGF overexpression may be involved in more invasive and aggressive statue of tumor with a negative impact on prognosis prognosis (Gullick 1991; Modjtahedi and Dean 1994; Zhau et al. 1996). The attractive point is whether there is a balance between different functional targets, such as amplification and expression which has been also stated by previous publications. However, The overexpression of epidermal growth factor (EGFR) is reported in different cancers, including brain (Libermann et al. 1985), breast cancer (Sainsbury et al. 1985), bladder (Berger et al. 1987), and ovary (Gullick et al. 1986; Modjtahedi and Dean 1994; Zhau et al. 1996).

The correlations between histopathology characteristics and the key biological factors is an important prognosis for the metastatic BC patients. So, it was predicted that the BC patients characterized with high tumor grade, auxiliary lymph node (ALN) involvement, overexpression of EGFR, p63, and Ki67, are at higher brain metastasis risk (Shao et al. 2011).

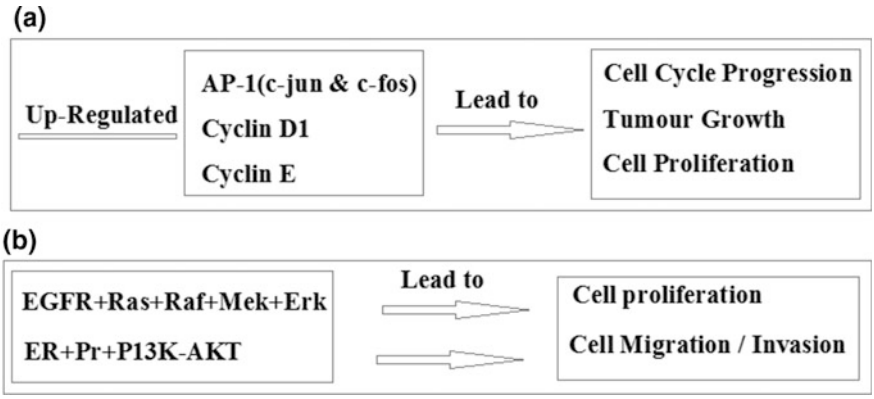

Fig. 22.3 A brief view on the involved key gens: from cell cycle progression to invasion. **a** Up-regulated genes; **b** Involved genes in pathway

22.5 From Cell Mobility to Cancer Invasion: The Role of CD44V6

It worth's to highlight the importance of Epidermal Growth Factor as a key element involved in normal and malignant tissues. The EGFR family receptors are characterized with an unique transmembrane protein and region, besides, have an extracellular ligand-binding with a cytoplasmic tyrosine–kinase domain (Modjtahedi and Dean 1994). The biological and signaling characteristic of EGF include normal development, differentiation, proliferation and invasion. Furthermore, the signaling pattern consist of EGFR and ERK. Interestingly, the signaling pathway of EGF with CD44 in different tumors is previously published on the fundamental facts in tumor cells (Lakshmi et al. 1997; Kang and Massague 2004; Cheng and Sharp 2006); in astrocytoma and brain tumor cells (Monaghan et al. 2000; Okamoto et al. 2002; Rooprai et al. 2000); in gliomas (Murai et al. 2006); and in basic paradigms (Matter et al. 2002; Weg-Remers et al. 2001; Orian-Rousseau et al. 2002) (Figs. 22.4 and 22.5). The cleavage and intracellular domain of CD44 is stimulated by the release of the soluble ectodomain region of CD44. Then the translocation to the nucleus occurs which will lead to the transcription of responsive genes (Okamoto et al. 2002). By considering the cross talk between EGF and CD44 a comprehensive illustration is provided (Fig. 22.4a). CD44 Glycoprotein as a key cell surface component is complicatedly involved with many events in different neoplasms (Fig. 22.4b).

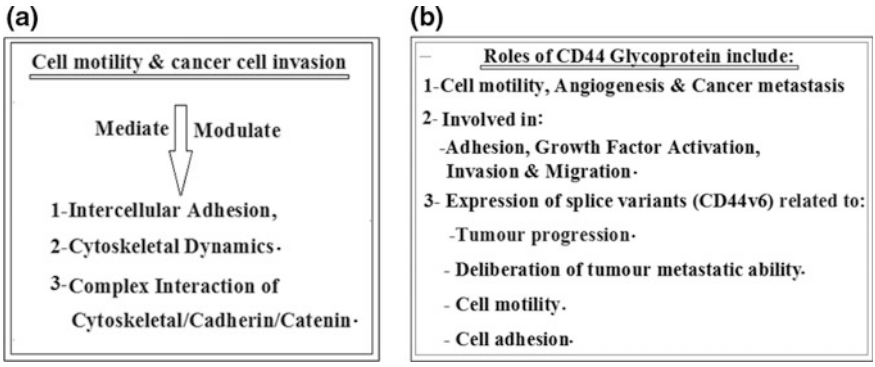

Fig. 22.4 Essential cellular behaviors and impact of CD44 in cancer metastasis **a** cell mobility and invasion; **b** roles of CD44

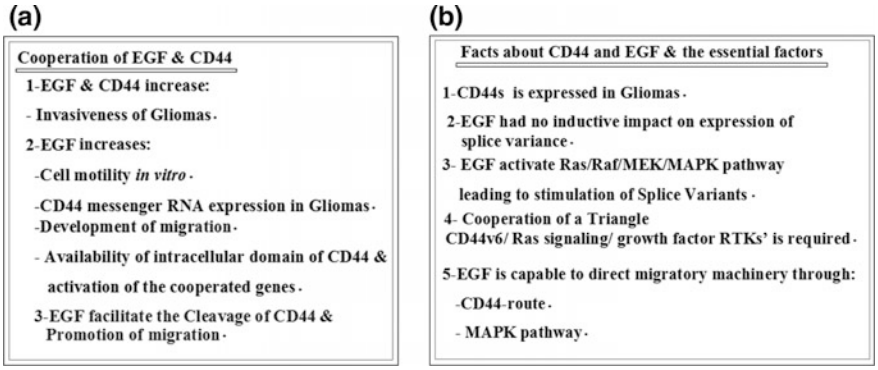

Fig. 22.5 Cooperation of EGF and CD44: a road map in gliomas. **a** Cooperation of EGF and CD44; **b** the facts about CD44 and EGF. *CD44s* standard CD44

22.6 Cooperation of CD44 with EGF

The brain, as a favorite metastatic destination for BC is required to be characterized. However, the cooperation of CD44/EGF is a challenging insight in different cancers and the performance of complementary cell based investigation is essential. However, the manner of cooperation between EGF and CD44, and the involved factors in gliomas are provided (Fig. 22.5).

22.7 Impacts of EGF and VEGF on Cancer

The key roles of breast cancer stem cells (BCSCs) as a triangle strategy include aggressiveness, metastatic potential and resistance to therapy. In previous publication the microRNA-760/NANOG, its' relevant genes and metastasis was explored in two different breast cell lines (Han et al. 2016). They have reported that the overexpression of miR-760-760/NANOG led to suppression of CD44(+)/CD24 (−) cell population as well as inhibition of cell proliferation and migration of BT-549. As conclusion, they have highlighted that miR-760-760/NANOG may provide a new therapeutic insight in suppressing BCSCs and prevention of metastatic event.

To highlight the key roles of growth factors, EGF and vascular endothelial growth factor (VEGF) are briefly addressed:

EGF and VEGF are located at chromosome 4q25 and 6p21.3. Respectively. The crucial roles of EGF includes differentiation, cell proliferation, tumorigenesis and apoptosis. The mode of expression of EGF is diverse either in different tumors or in normal tissues. However, the overexpression of EGF—Receptor is reported in the majority of epithelial-based tumors (Sainsbury et al. 1985; Libermann et al. 1985; Gullick et al. 1986; Berger et al. 1987) (Fig. 22.6a). In this Figure the mode of expression of EGF reveals to be directive through cancer development to metastasis (Sanson and Cornu 2000; Gullick 1991).

The VEGF family is involved in vascularization with a crucial role in angiogenesis (Holmes and Zachary 2005). Angiogenic vessels are originated from

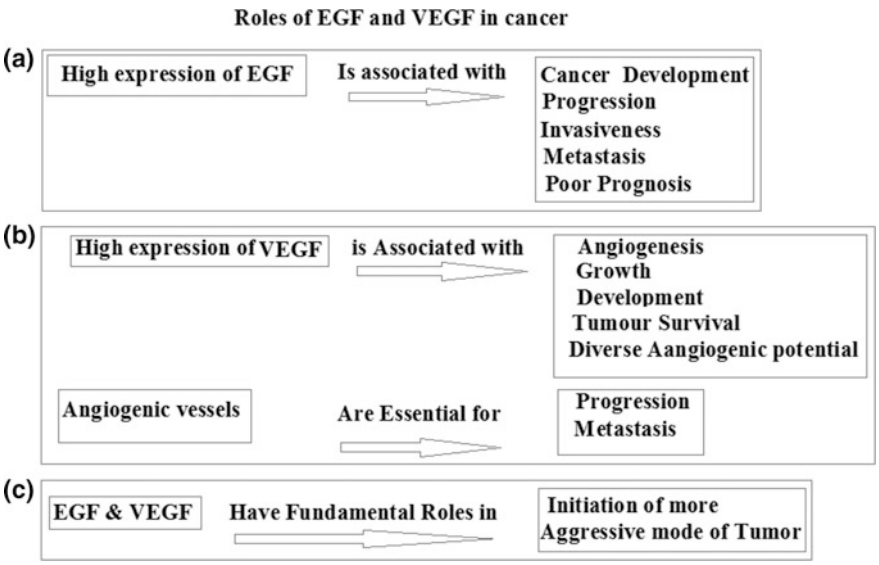

Fig. 22.6 Roles of epidermal growth factor and vascular endothelial growth factor in cancer. *EGF* Epidermal growth factor, *VEGF* vascular endothelial growth factor

previous vessels and is found to be essential for progression and metastasis of tumor (Hanahan and Weinberg 2000) (Fig. 22.6b). Besides, VEGF has the key role in development and progression of non-cancerous diseases (Luo et al. 2011). As Fig. 22.6 illustrates, both EGF and VEGF have negative impact on the tumor, but their cooperation leads to more severe outcome for tumor. So, manner of function for growth factors may lead to plan an appropriate management for cancer patients.

Besides, proliferation and cell motility are the key concerns and are involved in two different pathways.

22.8 Cooperation of CD24, CD44 and EGF: Importance of Heterogeneity in Breast Cancer

The highlighted points a functional role of CD24, Cd44 bout metastasis included the regulation of immune machinery, cytokines, chemokines and the relevant receptors, the epithelial-mesenchymal transition (EMT), and the crucial role of cells in microenvironment. Besides, the final focal concerns revealed to be invasiveness, metastasis and poor prognosis (Sleeman 2016). For further informative evidences, the protein expression images of the cellular targets including CD24, CD44 and EGF are presented (Figs. 22.7a–h and 22.8a–h). As Fig. 22.7 shows, very few stem cells are present. Furthermore, in spite of low frequency of cells with high expression of EGF, the co-expression of CD24/CD/44/EGF is remarkable. In order to highlight the heterogenic pattern of protein expression of these gens, another image of CD24, CD44 and EGF of the same patient is also provided (Fig. 22.8). This Figure is reflective of a limited number of stem cells (Fig. 22.8d). The co-expression either of low expression of CD44 and high expression of FGF or the same manner with CD24/FGF was remarkable (Fig. 22.8e, f respectively).

22.9 Impact of Stem Cells on Tumorigenesis and Metastasis of Breast Cancer

As the matter of fact, the heterogeneous architecture of stem cells in the tumor of gliomas has been previously published and the self-renewal capability has been highlighted (Sugimori et al. 2015; Soeda et al. 2015). Besides, the involved mechanisms in cancer metastasis have not been totally unraveled yet. So, cancer stem cells (CSCs), and the metastatic event as two ends of a line play key roles in cancer initiation and the progressive processes. As an example, it was, previously, reported that breast cancer stem cells (BCSCs) play as a tumorigenic initiator of breast and is involved in metastasis as well (Dragu et al. 2015; Ritter et al. 2015). In addition, the altered profiles of microRNAs in metastases and self-renewal of BCSCs have been also reported (Liu et al. 2015; Sun et al. 2015; Duru et al. 2015).

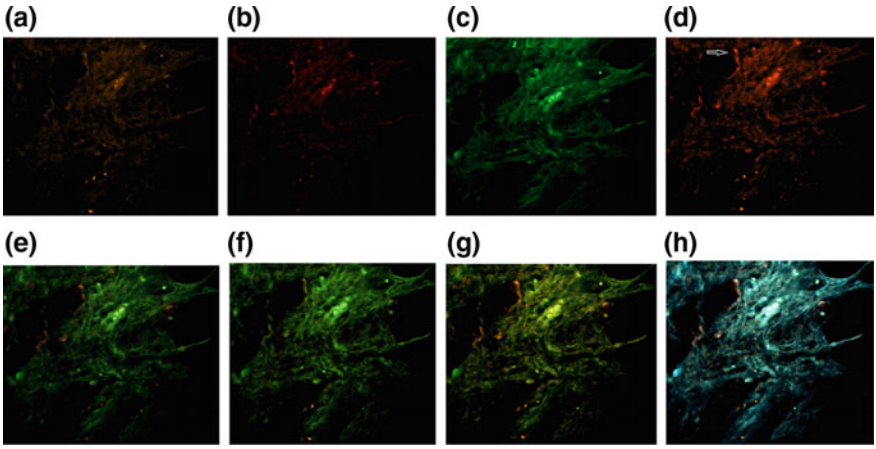

Fig. 22.7 Protein expression of CD24, CD44 and EGF in the tumor of a patient affected with primary breast cancer. **a** Cells conjugated with Rpe indicative of CD24, **b** the same cells conjugated with pe-cy5 indicative of CD44, **c** the same cells conjugated with FITC reflecting the expression mode of EGF, **d** co-expression of CD44 and CD24, the *arrows* indicate the presence of stem cells characterized with CD44+/CD24-expression status, **e** co-expression of CD44/EGF, **f** co-expression of CD24/EGF, **g** co-expression of CD24/CD44/EGF, and **h** co-expression of dapi/CD24/CD44/EGF. Magnification: ×100 (Adapted from Mehdipour's archive)

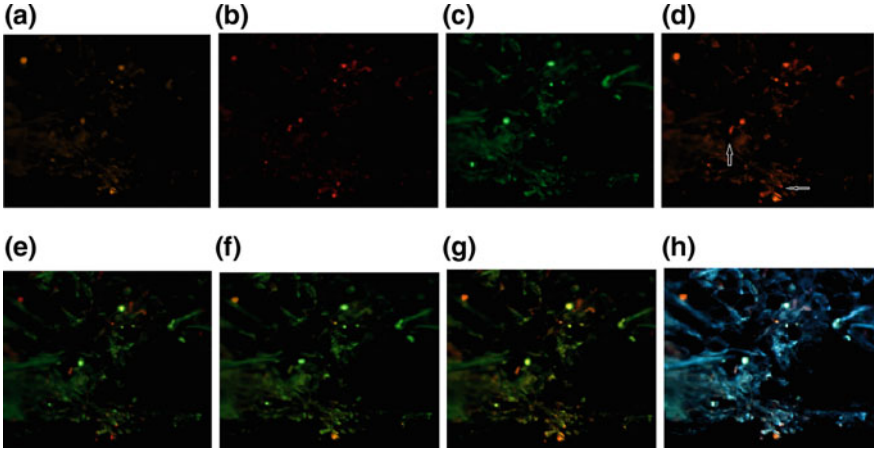

Fig. 22.8 Protein expression of CD24, CD44 and EGF in the tumor of a patient affected with primary breast cancer. **a** Cells conjugated with Rpe indicative of CD24, **b** the same cells conjugated with pe-cy5 indicative of CD44. **c** The same cells conjugated with FITC reflecting the expression mode of EGF, **d** expression status, **e** co-expression of CD44/EGF, **f** co-expression of CD24/EGF, **g** co-expression of CD24/CD44/EGF, and **h** co-expression of dapi/CD24/CD44/EGF. Magnification: ×200 (Adapted from Mehdipour's archive)

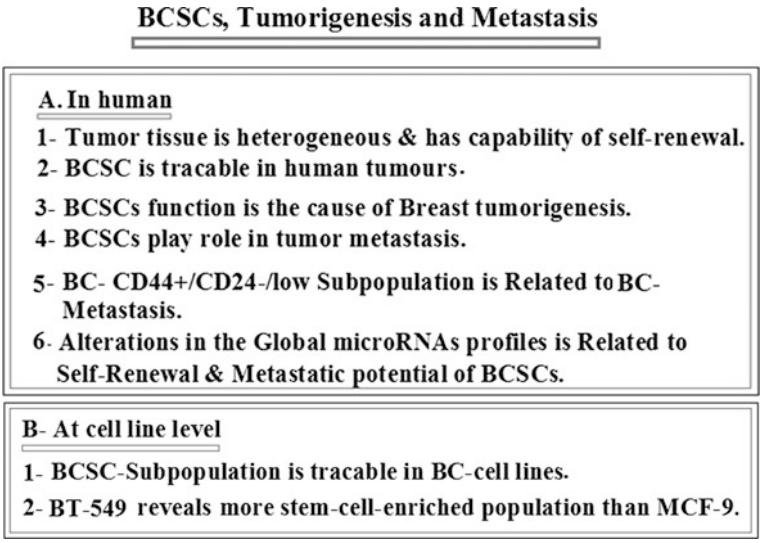

Fig. 22.9 Role of breast cancer stem cells in tumorigenesis and metastasis. *BCSCs* Breast cancer stem cells

Furthermore, comparison between different cell line-based investigations is rather challenging (Han et al. 2016). They have reported more stem-cell-enriched population in BT-549 than MCF-9 cell lines. Upon these data, the key role of CSCs in breast tumorigenesis, metastasis and its interaction with other molecular and functional territories in human and in vitro have been provided (Fig. 22.9).

An experimental model has been, previously, created in which the human BC cells could be traced in "immunocompromised mice (Al-Hajj et al. 2003). They could recognize the tumorigenic cells from non-tumorigenic cells by CD44+/CD24− cell surface marker. Interestingly, they could also found that about 100 cells were capable to initiate tumor in mice. These results, is suggested as a supportive experiments to the therapeutic innovation against these cell population.

22.10 Genomic Instability and Cancer Development

Genomic instability play the key role in cancer development. The involved genetic alterations include a single nucleotide, complex molecular-based changes, chromosomal structural and/or numerical aberrations in which the gain or loss of chromosomal materials, and the fused genetic materials are the key event in cancer initiation. Furthermore, the harmonized manner of the cell cycle events and the occurrence of gene mutation are the critical channel as the outcome of the vital link between genomic instability and cell transformation. However, cluster of the global events include genetic, epigenetic, genomic instability (microsatellite and

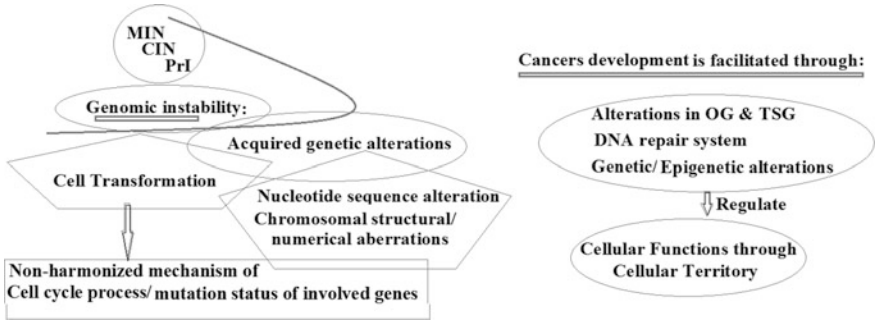

Fig. 22.10 Cancer genomic instability and cancer development. *MIN* Microsatellite instability; *CIN* chromosome instability, *PrI* proteom instability; *OG* oncogene; *TSG* tumor suppressor gene

chromosome variability). In addition, the cell functional based such as protein expression, proteome, and broad abnormalities in the cellular territory (telomere length and status of sub-telomere) are involved in cancer development (Fig. 22.10).

22.11 Highlights in Brain Territory

22.11.1 From Characterization of Glioblastoma to Metastatic Cascade Events

Tracing of the circulating tumor cells (CTCs) are the key strategy for early detection in different cancers. But it was not successful in brain tumors due to the limited cases with extracranial metastasis (Fonkem et al. 2011; Kalokhe et al. 2012). It has been also proposed that the glioma cells have an exceptional territory in which the migration into the blood stream is restricted (Piccirilli et al. 2008). Later on, the circulating brain tumor cells were defined as "more invasive property" (Sullivan et al. 2014). This statement was accompanied by further finding in which the brain CTCs were characterized with "enriched for mesenchymal gene expression". They have also reported that the primary mesenchymal origin of metastatic glioblastoma multiform (GBM) is rare. Furthermore, the brain CTCs could be traced in the peripheral blood of patients affected with GBM and they have also stated that CTC are characterized as "intrinsic property" within the biological territory of GBM (Müller et al. 2014).

Now, why do the brain cells circulate into the blood territory, do not migrate to the distant organ? The answer was stated by Gao and the colleagues due to (1) The inappropriate condition of the host "soil"; (2) the CTCs as "seed" anchorages the specific alterations by inhibiting the extracranial metastatic of the GBM (Gao et al. 2016). Furthermore, it is vital for the glioma cells to express PTEN in peripheral organs (Zhang et al. 2015). This tumor suppressor gene could be function as a

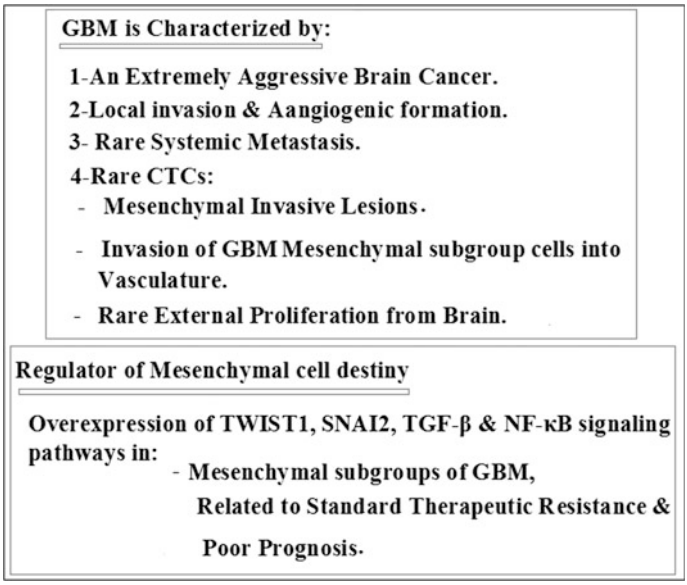

Fig. 22.11 Characterization of glioblastoma multiform and the regulatory mechanisms

partial guarantee element for the survival of these cells in the host territory. More characteristics of mesenchymal GBM cells, and the CTCs strategy are previously highlighted (Bhat et al. 2013; Phillips et al. 2006; Tso et al. 2006; Verhaak et al. 2010). Characterization of some facts in gliomas by considering signaling pathway are presented as a cascade manner (Figs. 22.11 and 22.12).

22.11.2 Brain Metastases, and Circulating Cancer Cells

Brain metastasis (BM) is an event with late diagnosis in clinic that lead to the complicated management and disappointing outcome. BM is the major cause of deaths in patients affected with BC (Weigelt et al. 2005). Breast cancer and especially breast cancer brain metastasis have a negative impact on the BC patient's life style including the survival and the psychological status (Pelletier et al. 2008; Miller et al. 2003). The most frequent metastatic destination of BC includes brain as the most common, followed by bone, lung and liver (Shao et al. 2011). As a brief illustration, brain metastasis and selected complications are provided (Fig. 22.13).

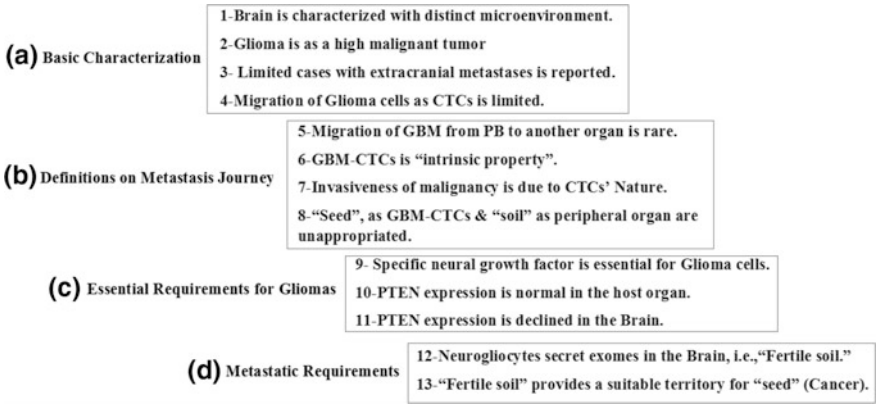

Fig. 22.12 GBM: Glioblastoma multiform; CTCs: Circulating tumor cells

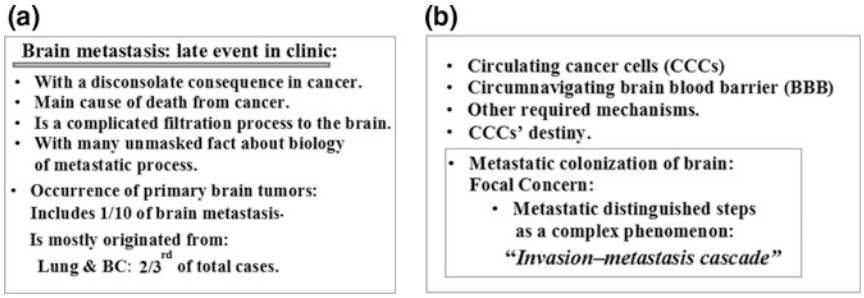

Fig. 22.13 Brain metastasis and complications. **a** Brain metastasis. **b** Circulating cancer cells

22.11.3 Breast Cancer Brain Metastasis and Impact of Relevant Factors on Brain Destination

Machinery of brain metastasis is rather complicated in which different factors including the molecular and cellular insights play the key roles (Shao et al. 2011; Nishizuka et al. 2002; Okamoto et al. 2002; Valastyan and Weinberg 2011; Irollo and Pirozzi 2013). The interactive factors are presented in an illustration (Fig. 22.14).

22.11.4 Machinery of Metastases

The important characteristic of CSCs is to facilitate the processes of metastatic cascade through epithelial–mesenchymal transition, the programmed expression

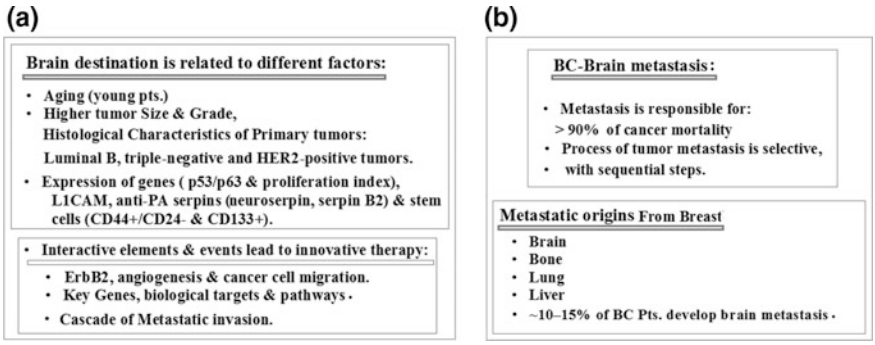

Fig. 22.14 Machinery of brain metastasis. **a** *Up* Influential factors on brain destination; **a** *Down* required element for an innovative therapy. **b** Breast cancer brain metastases

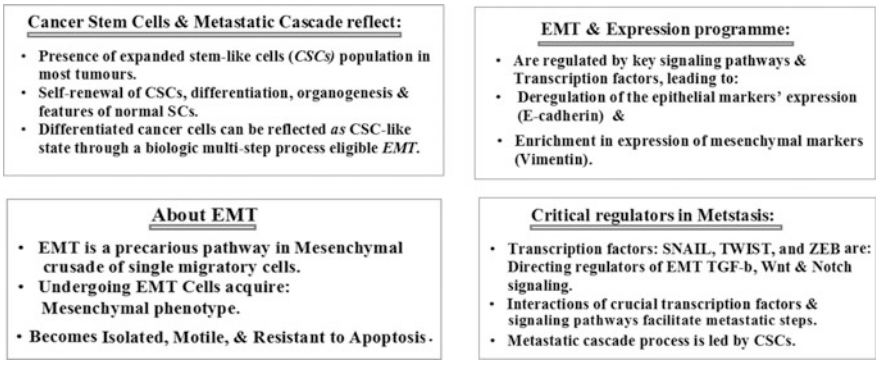

Fig. 22.15 Cancer stem cells and functional role of involved genes in metastasis. *EMT* Epithelial–mesenchymal transition. *SCs* Stem cells

and acting as the regulators (Valastyan and Weinberg 2011; Bahrami and Mehdipour 2015) (Fig. 22.15).

22.11.5 Brain Metastatic Cascade and Regulatory Factors

Metastatic machinery is characterized with the multi-cascade and multifunctional processes which is unpredictable with late diagnosis. Survival is also a serious concern for the clinicians (Valiente et al. 2014). The key facts in such complex machinery is presented (Fig. 22.16).

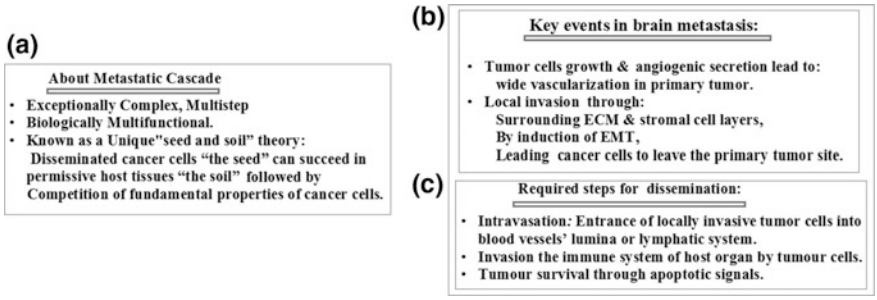

Fig. 22.16 Metastatic machinery and dissemination metastatic cascade. **a** Metastatic events steps of dissemination. **b** *ECM* extracellular matrix; **c** *EMT* epithelial–mesenchymal transition

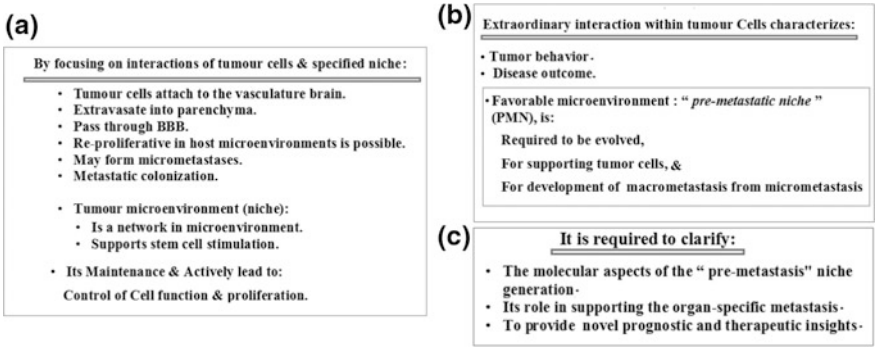

Fig. 22.17 Interactions, tumor cells, specialized niche, and requirements in micro- and macro-metastases. **a**, **b** Interactions; **c** requirements

22.11.6 Interactive Events and Metastatic Requirements

Niche, as a key item in metastasis, consist of nutrients, soluble factors, vascular networks, stromal cells, and ECM architecture which has been previously reviewed (Bahrami and Mehdipour 2015). By considering interactions between tumor cells with specialized niche, the programmed events are summarized (Fig. 22.17).

22.11.7 "Seed and Soil Theory": Managements and Predictive Facts of Breast Brain Metastasis

The "seed and soil theory" is supported by the biologically progressive and successive processes. The management of such manners rely on different signaling pathways to guarantee the metastatic proliferation. The scenario of the metastatic

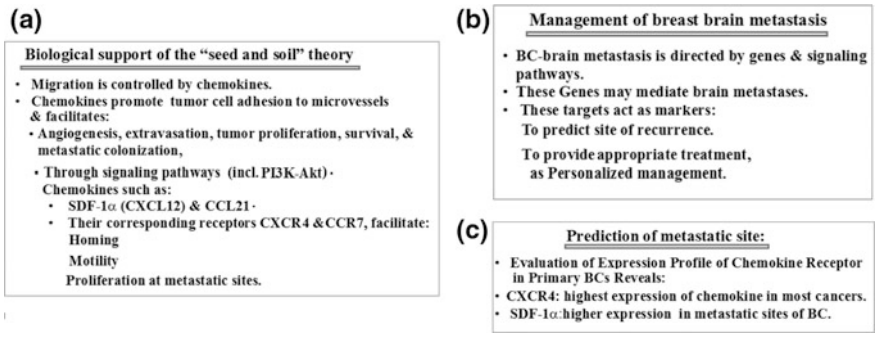

Fig. 22.18 The key biological facts and management in breast cancer brain metastasis. *CXCR4* C-X-C chemokine receptor type 4; *SDF-1α* Stromal cell-derived factor-1. **a** Biological events in "seed and Soil theory", **b** managements in BCBM; **c** prediction of metastatic site; *BC* breast cancer

requirements has been previously reviewed by us and the requirement of breast cancer brain metastasis (BCBM) is provided as an illustration (Fig. 22.18). The SDF-1α chemokine (Stromal cell-derived factor-1) is a bioactive chemoattractant capable to play a key role in hematopoietic stem cell establishment and cancer progression. The related SDF-1α/CXCR4 receptor signaling is reflective of the tumor aggressiveness, leading to distant organ metastasis (Liu et al. 2017). They have engineered "a biomimetic tumoral niche of a soft polyelectrolyte film" which provided an atmosphere through which the BC cells could, appropriately, response to chemokines. They have stated that this tool would offer an inventive therapy in metastatic status through targeting of chemokine receptor type 4 (CXCR4) and CD44 receptors or the related signaling elements including CXCR4 and Rac1 (Fig. 22.18).

22.11.8 The Complementary Mechanisms in Breast Cancer Brain Metastasis

Additionally, according to the complementary mechanisms in Fig. 22.19, and in spite of an order-based machinery in BC brain metastasis, it is difficult to judge for a universal application of the available sequential events in breast cancer bone metastasis. However, a personalized approach is an essential endeavor in the metastatic patients.

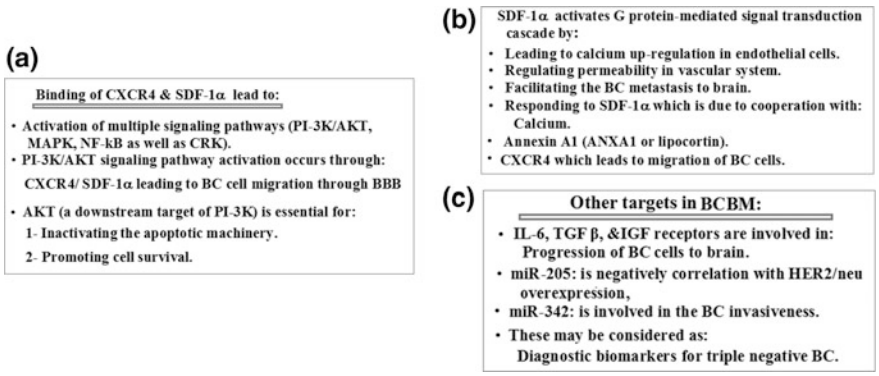

Fig. 22.19 Further mechanisms of breast brain metastasis. **a**, **b** Cooperation and machinery of CXCR4 with SDF-1α; **c** other targets in BCBM. *ANXA1* Annexin A1. *BC* Breast cancer. *BCBM* Breast cancer brain metastasis. *CXCR4* C-X-C chemokine receptor type 4; *SDF-1α* Stromal cell-derived factor-1; miR miRNA; *MAPK* mitogen-activated protein kinase; *NF-kB* nuclear factor kappa-light-chain-enhancer of activated B cells; *CRK* an adapter protein family; *PI-3K* phosphatidylinositol 3 kinase; *AKT* Protein kinase B (PKB), also known as Akt. *Rac1* Ras-related C3 botulinum toxin substrate 1

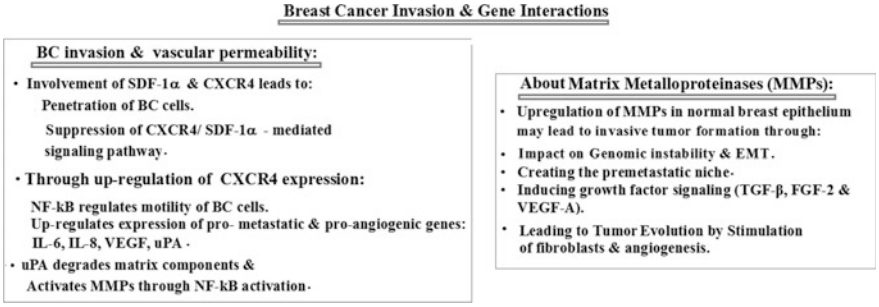

Fig. 22.20 Inhibition of breast cancer invasion and gene interactions. *BC* Breast cancer. *NF-kB* Nuclear factor kappa-light-chain-enhancer of activated B cells; *IL-6* Interleukin 6; *IL-8* Interleukin 8; *VEGF* Vascular endothelial growth factor; *uPA* Plasminogen activator, urokinase; *MMPs* Metalloproteinases. These genes are included in the 17 involved genes in BC/brain metastasis

22.11.9 Breast Cancer Invasion and the Roles of Involved Genes in Metastasis to Brain

Inhibition of BC invasion and vascular permeability is a promising strategy in which the involved factors many play influential roles (Fatunmbi et al. 2012). Amongst these, Matrix Metalloproteinases (MMPs) has multi-potentials role in both normal breast tissue and neoplastic tissue. The tactical road map of tumor evolution by facilitating many events is provided in two schematic Figures (Figs 22.20 and 22.21). These two figures illustrate how different gens are capable to harmonize the metastatic process from breast cancer to brain.

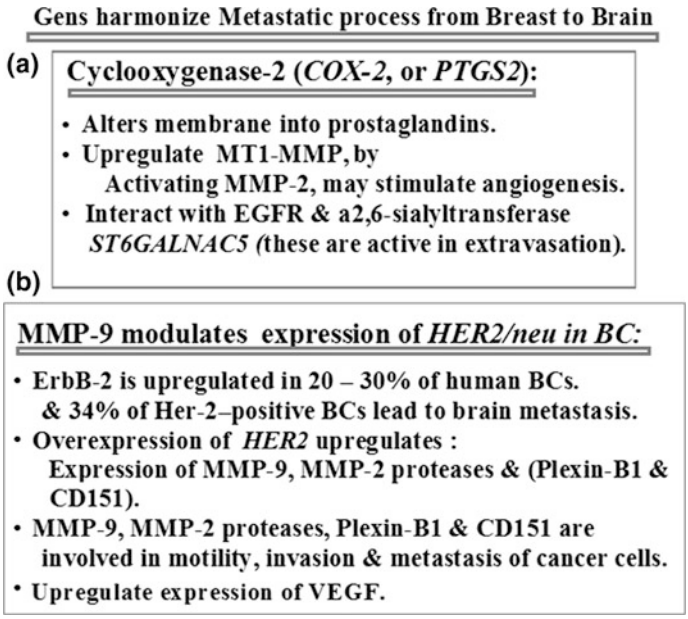

Fig. 22.21 Gens harmonize the metastatic process from breast cancer to brain. **a** *MMPs* Matrix metalloproteinases; *EMT* epithelial–mesenchymal transition; *TGF-β* Transforming growth factor *beta* 1; *FGF-2* fibroblast growth factor 2; *VEGF-A* Vascular endothelial growth factor-A. **b** *COX-2* Cyclooxygenase-2; *PTGS2* prostaglandin synthase 2; *MT1-MMP* membrane-type. 1 matrix metalloproteinase; *EGFR* epidermal growth factor; a2,6-sialyltransferase *ST6GALNAC5* *ST6GALNAC5* ST6N-acetylgalactosaminide *alpha-2,6-sialyltransferase* 5; Plexin-B1 & CD151: transmembrane proteins

22.12 Breast Cancer Bone Metastasis at a Glance

By focusing on breast cancer bone metastasis, and based on the "van't Veer's, Smid's, and Kang's signatures", Fig. 22.22 illustrates the genes with specific functions which are classified as: angiogenesis (red), invasion (brown), migration (yellow), proliferation (blue), survival (green), and the genes with common functions are presented in the circles in between of the primary BC and the bone metastatic destination. The genes as red highlighted revealed to function against the involved gene (as circle).

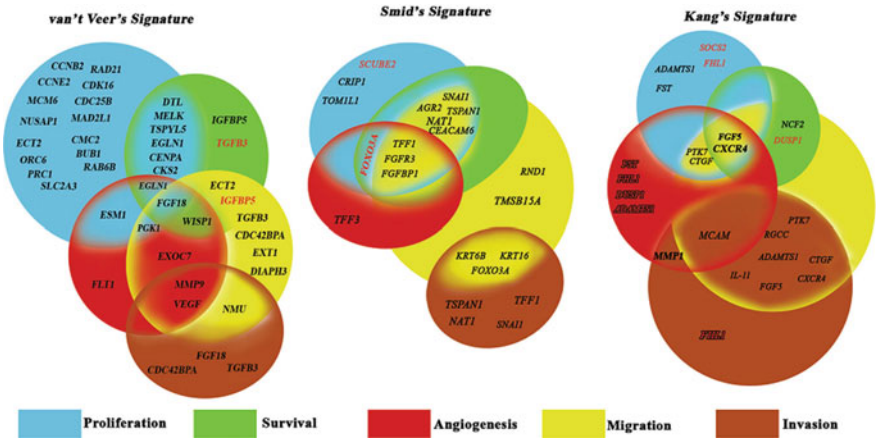

Fig. 22.22 Schematic view of breast cancer bone metastasis based on van't Veer's, Smid's, and Kang's signatures. Breast cancer bone metastasis is directed by the coordinated gens through the molecular and cellar pathways. The genes involved in van't Veer's, Smid's, and Kang's signatures direct a inclusive signaling network in breast tumors including primary tumors (*PT*) and secondary tumors (*ST*). TGF-b, FGF, JAK-STAT, NFjB, WNT, and PI3 K pathways in primary tumor, and TGF-b, FGF, NFjB, and PI3K pathways in ST are regulated by group of genes in three provided signatures. Six signaling pathways are included in the PT creating a signaling network that lead to tumor growth, proliferation, survival, angiogenesis, migration and invasion. Genes from van't Veer's, Smid's signatures and their signaling molecules are presented in primary tumor (*upleft*). HIF have remarkable influential impacts on PT primary tumor development and dissemination. Induction of EMT and CSC capability rely on different genes and signaling pathways. The capable CTCs are aimed to disseminate, home, and extravasate into the secondary organ (bone). Most of the differentiated CTCs (*yellow*) could not survive the "soil"; but in circulation, and in a minor clone of CSCs (*red*) CTCs survive at distant organ and form metastasis. In metastatic tumor (*down-right*), genes in Kang's signature participate in activating five pathways, leading to invasion, migration, EMT, and CSC formation. The level of differentiation in PT and ST could be same or different (In triple negative BCs are mesenchymal). *TGF-b* Transforming growth factor-beta, *FGF* fibroblast growth factor, *JAK-STAT* Janus kinase/signal transducers and activators of transcription, *NFjB* nuclear factor kappa B, *WNT* Wingless-type, and *PI3K* phosphatidylinositol 3 kinase, *HIF* hypoxia inducible factor, *EMT* epithelial to mesenchymal transition, *CSCs* cancer stem cells; *CTCs* Circulating tumor cells. This Figure is adopted from Fazilaty and Mehdipour (2014)

22.12.1 *Impacts of Three Signatures on the Metastatic Machinery of Breast Cancer Bone Metastasis*

The architecture of metastasis is rather complicated which is directed by many gens involved in multi-pathways. The scenario of breast cancer bone metastasis is illustrated in Fig. 22.23 (adopted from Fazilaty and Mehdipour 2014).

Three signatures are reflective of three territorial interaction of specific genes Each signature is diversely characterized, but all are reflective of the key events including proliferation, survival, angiogenesis, migration and invasion, but through

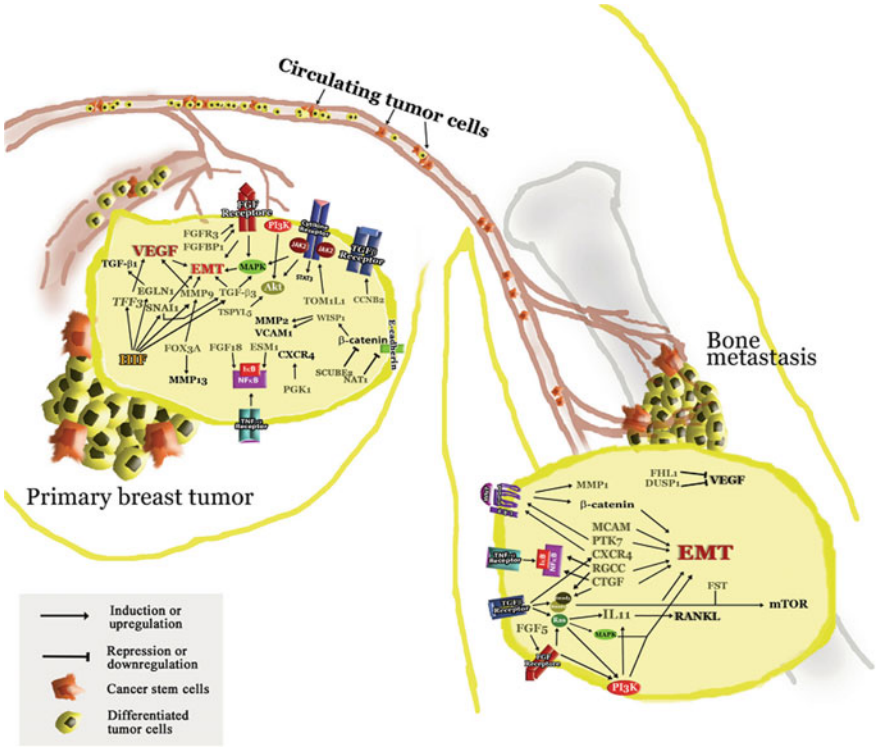

Fig. 22.23 Breast cancer bone metastasis through three different signatures. The genes included in van't Veer's, Smid's, and Kang's signatures are specialized for the programmed functions. These genes are marked as: proliferation (*blue*), angiogenesis (*red*), survival (*green*), migration (*yellow*), and invasion (*brown*). The genes having same function in common are located at interconnected zones of circles. Size of circles are reflective of the deviation in direction of the relevant function in individual signature. This Figure is adopted from Fazilaty and Mehdipour (2014)

three diverse powers. The question is which signature has more influential impact on the metastatic process?

In spite of the provided highlighted metastatic events in our review (Fazilaty and Mehdipour 2014), some major questions could be addressed including (1) could all differentiated CTCs and/or CSCs survive and succeed to overcome the unreceptive program by host destination? And (2) regarding the manner of molecular and biological characteristics, do the primary tumors, behave as same as metastatic-tumor?

However, in addition to molecular alterations in the primary breast- and host bone-tumor, the cell biological processes play key role either in initiation or progression or in survival of metastatic events. This paradigm is required to be investigated through a systematic follow up study on the human samples.

22.13 Targeted Based Therapy in Cancer with Emphasize on BC

The well-defined cell-based and molecular-based targets and their influential interactions are the fundamental items for application of targeted therapy in cancer including BC (Liu et al. 2013; Rodrigues-Ferreira et al. 2012; Soffietti et al. 2012; Fokas et al. 2013). So, different target-based therapies are provided at a glance (Fig. 22.24) in which the required mechanisms and the manner of their inhibition are also presented.

22.13.1 Cellular and Molecular Targets Direct the Metastatic Process

Figure 22.25 is reflective of involved molecular and functional mechanism in cell-migration and metastasis of the breast cancer brain tumor metastasis (BCBM) by focusing on the specific target-based factors for further therapeutic innovation (Soffietti et al. 2012; Bahrami and Mehdipour 2016).

22.13.2 The Required Elements in BCBM

In addition, the conclusive considerations from the recent commentary on breast cancer brain metastasis are highlighted (Fig. 22.26) (Mehdipour 2017). Furthermore, the requirement for a successful extravasation include the function of growth factors such as VEGF, EGF and the essential microenvironment signals of tumor. In BCBM, the key components are presented in an illustration (Fig. 22.27).

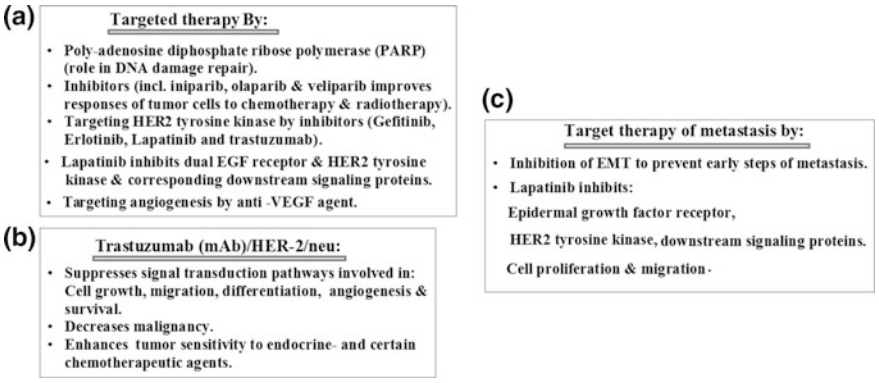

Fig. 22.24 Strategies of targeted therapy in breast cancer. *BC* Breast cancer. **a** *PARP* Poly-adenosine diphosphate ribose polymerase. *HER2* Human epidermal growth factor receptor 2; *EGF* epidermal growth factor; *VEGF* vascular endothelial growth factor

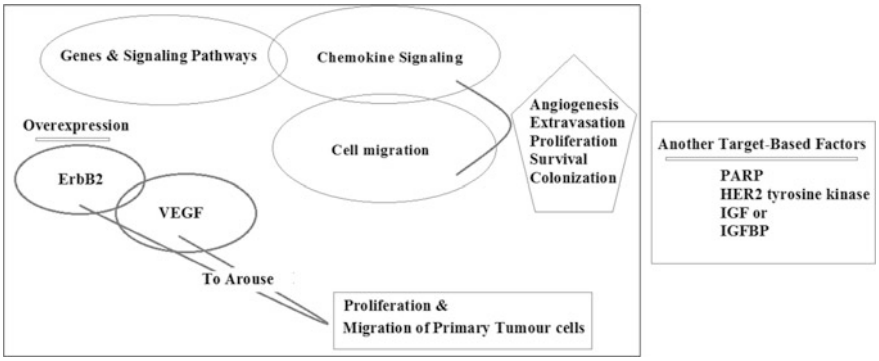

Fig. 22.25 The key facts about breast cancer brain metastasis and target-based factors. *PARP* Poly-adenosine diphosphate ribose polymerase; *IGF* insulin-like growth factor; *IGFBP* insulin-like growth factor binding protein

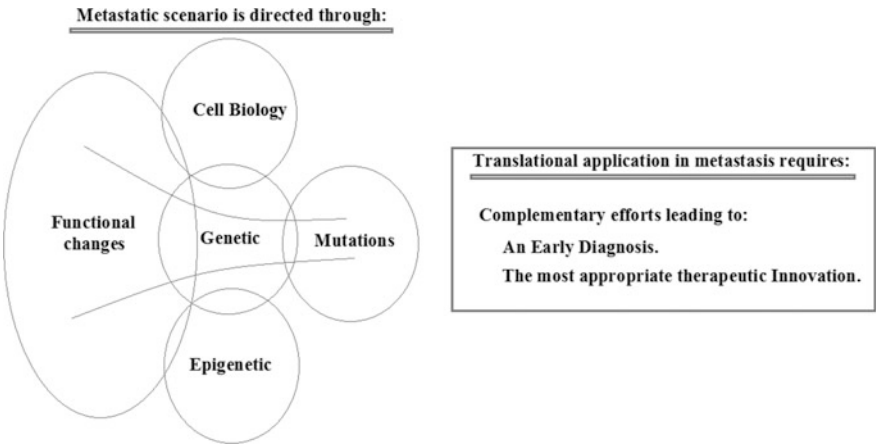

Fig. 22.26 Metastatic scenario

22.13.3 The Essential Strategy in Management of Neoplasms

A road map as a complementary design, either for genetic- and tumor biology-based paradigms, or for the clinical approaches, is provided (Fig. 22.28). This strategy may be applied in all types of neoplasms including malignant, non-malignant, metastatic and non-metastatic cancers.

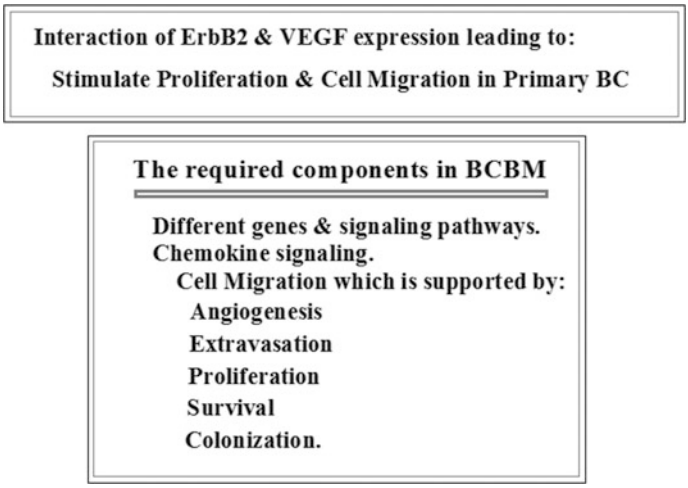

Fig. 22.27 Involved elements in breast cancer brain metastasis

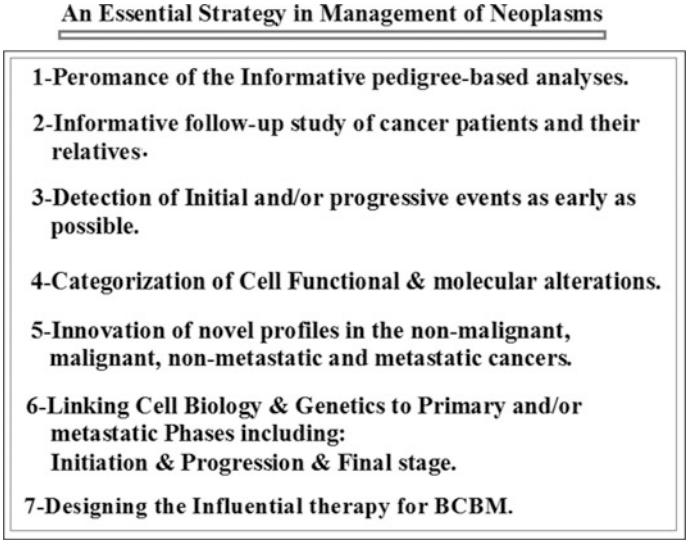

Fig. 22.28 Suggestive managements as road map in neoplasms

22.14 Complementary and Supportive Insight in Metastatic Breast Cancer: Emphasizes on BCBM

In breast brain metastasis, involvement of two channels were highlighted in our previous review including Channel 1 Characteristics of pathology/ Immuno-Histochemistry (Luminal B, high tumor grade and size, triple negative and Her2-neu positive and overexpression of p53, p63, ki67), and Channel 2 (ALN involvement, presence of lung metastasis and Multi-distant metastases) (Bahrami and Mehdipour 2015). However, such profiles are, apparently, inadequate to unmask convincible machinery of the metastatic events. So the complementary profiles are required for promotion of a successful metastatic event of those some key cell cycles' genes and growth factors are provided in this chapter: (1) in breast tumors (Figs. 22.29, 22.30, 22.31) and (2) in brain tumors (Figs. 22.32 and 22.33).

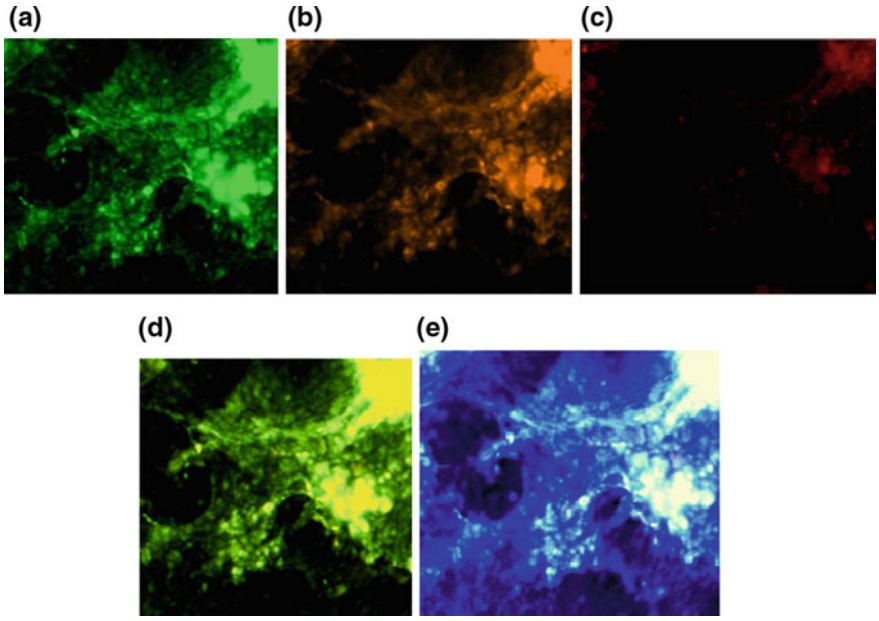

Fig. 22.29 Protein expression of Ki-67, VEGF and EGF in the tumor cells of patient affected with breast cancer. **a** Tumor cells of a BC patient with FITC (*green*) reflecting of expression of Ki-67; **b** Same cells conjugated with Rpe expressing the protein of VEGF; **c** Same cells conjugated with Pe-cy5 reflective of the EGF expression; **d** co-expression of Ki-67/VEGF/EGF is indicative of the harmonic interaction between these three proteins; and **e** merged images of dapi/Ki-67/ VEGF/EGF. Magnification of cells: ×100. This figure is adopted from Parvin Mehdipour's archive

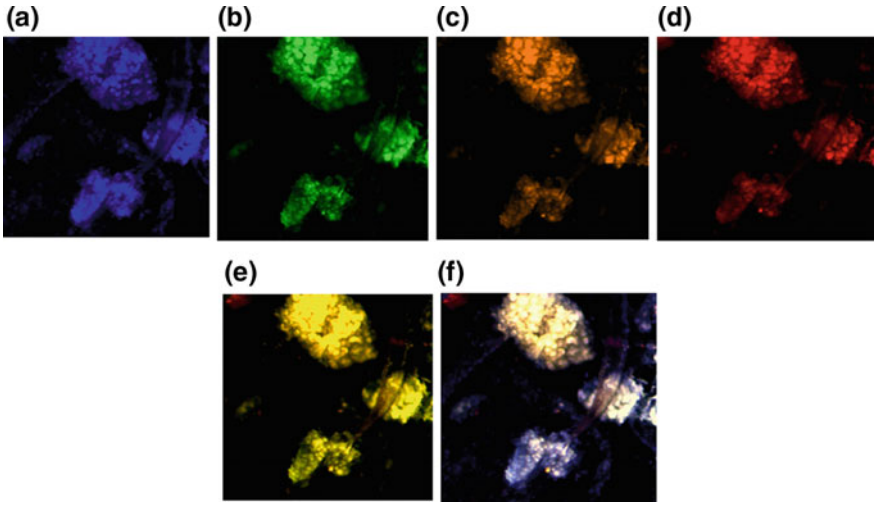

Fig. 22.30 Protein expression of Ki-67, ATM and P63 in the tumor of patient affected with breast cancer. **a** Tumor cells of a patient with BC with dapi: **b** tumor cells with FITC (*green*) reflective of the heterogenic expression of Ki-67; **c** Same cells conjugated with Rpe with the heterogenic expression of ATM; **d** same cells conjugated with Pe-cy5 illustrating hetrogenic expression of P63; **e** co-expression of Ki-67 ATM/P63; and **f** merged images of dapi/ Ki-67/ ATM/P63. Magnification of cells: ×100. This figure is adopted from P. Mehdipour's archive

22.14.1 Protein Expression in Breast Cancer

As Fig. 22.29 shows, a harmonic co-expression between Ki-67 and VEGF is indicative of a successful cooperation which could lead to a remarkable-proliferation, and angiogenesis. Through such event, the partial progressive manner of tumor by involvement of the facilitator metastatic factors could occur.

Regarding Fig. 22.30, initiation and/or promotion of proliferation could be directed through the clone of cells with high expression of Ki-67. In contrast, in spite of the heterogenic expression of P63 and ATM, the cells with high expression are the supportive clones through which a successful tumor progression could be restricted and could be considered as a counter function against tumor progression. Furthermore, the harmonic co-expression of ATM and P63 could be as the supportive machinery against Ki-67 function (Fig. 22.30a–f). In addition, the key cell cycle proteins including cyclin E, cyclin D1 and CDC25A are considered in this chapter (Fig. 22.31a–e). In this figure, all three involved proteins are reflective of the heterogenic expression pattern including low, high and lack of expression, but the remarkable point is a harmonic co-expression which facilitates the cascade of transmission from G1 to S and G2.As the matter of fact CDC25A has a crucial rule all through different cell cycle phases.

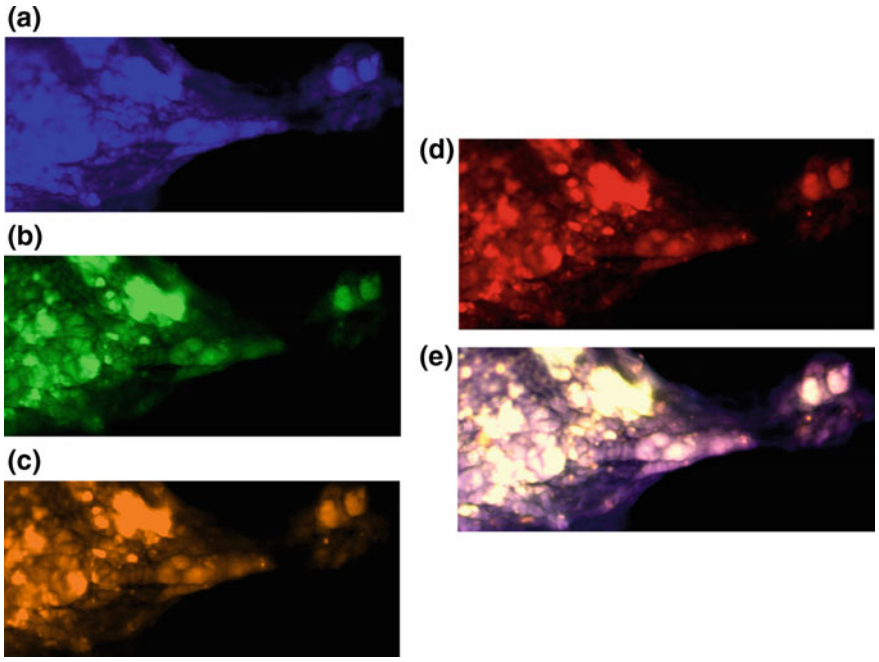

Fig. 22.31 Protein expression of cyclin E, cyclin D1, and CDC25A in the tumor of patient with breast cancer. **a** Tumor cells of a patient with BC with dapi filter; **b** tumor cells with FITC (*green*) reflective of the heterogenic expression of cyclin E; **c** same cells conjugated with Rpe with the heterogenic expression of CDC25A; **d** same cells conjugated with Pe-cy5 of cyclin D1; and **e** co-expression of dapi/ cyclin E/cyclin D1/CDC25A. Magnification of cells: ×200. This figure is adopted from P. Mehdipour's archive

22.14.2 Protein Expression in Brain Tumors

Figure 22.32 shows the protein expression status of cyclin D2 and cyclin E responsible for transition from G1 to S-phase in the tumor cells of brain tumor, classified as an anaplastic glioma; and fos as a transcription factor. Lack of expression in cyclin E and fos is remarkable, but in contrast, cyclin D2 is characterized with high expression (Fig. 22.32). Whether cyclin D2, as a sole, with high expression is capable to facilitate tumor progression by passing from G1 to S-phase is a challenging item. However, co-expression between fos and cyclin D2, even between few cells, may be considered as a poor prediction for this cancer patient which highlights the crucial impact of personalized insight in cancer management.

Another profile which could unmask some influential impact on the personalized classification in brain tumor is also provided (Fig. 22.33). This image is

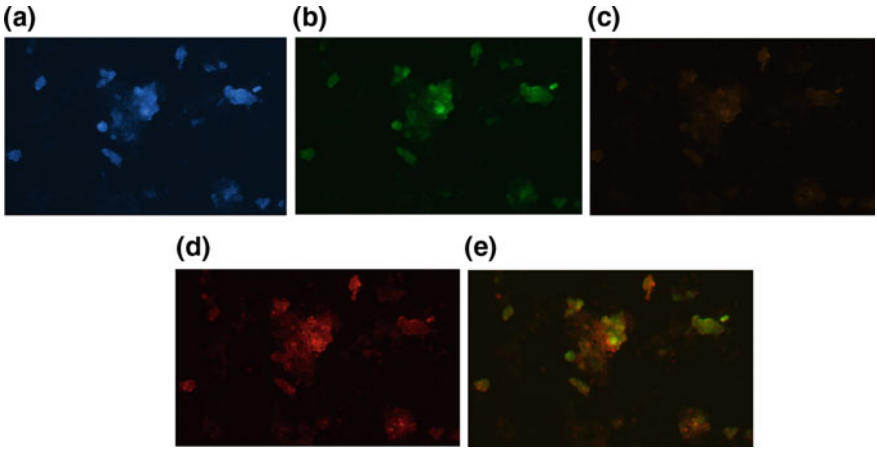

Fig. 22.32 Protein expression of cyclin D2, cyclin E and fos in tumor cells of a patient with anaplastic glioma. **a** Tumor cells of a patient with anaplastic glioma with dapi filter; **b** tumor cells with FITC (*green*) reflective of the lack of expression, except in very few cells of fos; **c** same cells conjugated with Rpe with an absolute lack of expression of cyclin E; **d** same cells conjugated with Pe-cy5 and reflecting heterogenic pattern of expression including a clone of cells with high protein expression of cyclin D2; and **e** co-expression of fos/cyclin E/cyclin D2 illustrating a prominent expression of cyclin D2. Magnification of cells: ×100. This figure is adopted from Parvin Mehdipour's archive

presentative of an interaction between Ki-67, P14, and P63. Furthermore, in spite of slight diversity in the degree of expression between these three targets, but a harmonic co-expression is remarkable.

22.15 An Update on Basic Insights in Cancer and Metastasis

22.15.1 miR-34a and Diagnostic Application in Breast Cancer

Wang and the colleagues have recently reported that miR-34a with 5 fundamental characteristics may be applied as a diagnostic factor in BC (Wang et al. 2017) which includes the following points: (1) Declined expression of miR-34a was in reverse manner against the increased ErbB2 levels in BC, (2) High expression of miR-34a leads to the reduction of ErbB2 expression and followed by suppression of invasion in BC cells and growth status in vitro, (3) declined expression of ErbB2 facilitates the inhibition of BC cell proliferation, (4) "re-expression of ErbB2" has an opposite function against miR-34a, and (5) An reverse correlation was found between miR-34a levels with BC malignancy.

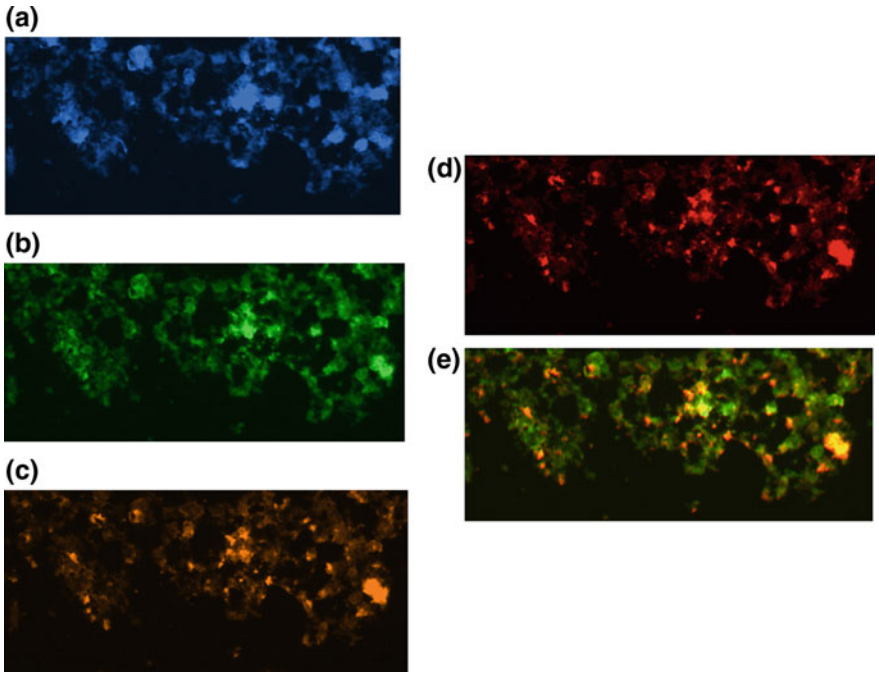

Fig. 22.33 Protein expression of Ki-67, P14 and P63 in tumor cells of a patient with glioblastoma multiform. **a** Tumor cells of GBM with dapi filter; **b** tumor cells with FITC (*green*) reflective of the heterogenic pattern of expression; **c** same cells conjugated with Rpe which reflects the heterogenic pattern of P14 expression; **d** same cells conjugated with Pe-cy5 which also shows the heterogenic pattern of P63; and **e** co-expression of Ki-67/P14/P63 illustrating a harmonic co-expression expression between these proteins. Magnification of cells: ×100. This figure is adopted from P. Mehdipour's archive

22.15.2 Diagnostic and Prognostic Roles of Ribonucleic Acid Export 1 in Normal and Neoplastic Breast Tissue

Oh and the colleagues, by considering the key roles of ribonucleic acid export 1 (RBCAE1), have highlighted the roles of RBCAE1 as, (1) Involvement in mRNA export; (2) A regulator of mitotic checkpoint; and (3) "RAE1 haplo-insufficiency leads to chromosome missegregation and early aging-associated phenotypes." (Oh et al. 2017). They have stated that the abnormal copy numbers of this gene is correlated with gene amplification in human BC cells. Besides, as a challenging points, they have emphasized on, (1) Upregulation of RAE1 in BC tissues was found to be comparative to normal breast cells by in silico analysis; and (2) Functional assays at cell lines level revealed an induction of invasion and migration through EMT signals. They have also found correlation between

clinical/histopathological characteristics and RAE1 expression, and also between expression of this gene with high tumor grad, by performance of tissue microarray and immunohistochemistry respectively. Finally, they have stated that RAE1 might be considered as a target for therapeutic innovation for the patients with BC.

22.15.3 Fundamental Multi-roles of AF1q in Cancer

It was recently stated that Wnt signaling pathway play pivotal role in controlling different stem cells. Besides, it may play a role as niche for facilitating the self-renewing status of stem cells (Tse et al. 2017). They have also emphasized on an association between A1fq, as an oncogene, with poor prognostic outcome in many cancers including BC, and its over-expression in stem cells. However, it is suggested that AF1q plays a role as "an enhancer" in generating the stem-like cells which is found to be relied on activating status of Wnt signaling pathway.

22.16 Experimental Models and Strategies in Cancer Metastases

22.16.1 Basic Model to Identify Signature in Breast Cancer

As it was stated, feline mammary carcinoma (FMC) is defined as human BC with late age of onset, histopathologic-, biological characteristics and metastatic pattern which is suggested as a reliable model in aggressive human BC (Hassan et al. 2017). Beside the cell-based experiments, they have developed a "nude mouse model of FMC" to assay angiogenic functions and tumor progression and found 15 genes of those 10 were over-expressed including PDGFA, PDGFB, PDGFC, FGF2, EGFR, ERBB2, ERBB3, VEGFD, VEGFR3, and MYOF. Furthermore, they have confirmed that the PDGFD, ANGPT2, and VEGFC genes were originated from stroma. These finding would be useful to identify the molecular signature of FMC involved in progression and metastasis.

22.16.2 The Xenograph Model for Metastatic Suppression in Breast Cancer

Involvement of more than 600 genes in the processes of breast cancer metastasis to brain, lung or bone, and the lack of adequate informative data on the machinery of these gens in immune system was a convincing sign to unmask the new fact in BC metastasis (Anderson et al. 2017). They have found a novel role of the retinoic acid

receptor responder protein 3 (RARRES3) to inhibit the immunoproteasome (IP) expression through "a gene co-expression network approach which deals with bone marrow-derived mesenchymal stem cells (BM-MSCs) and CSCs". Besides, the Xenograph was considered as a model to assay the mode of expression at differential stage of metastasis. In conclusion, their goal was to improve the available data to unmask pathways which may lead to design for "breast cancer driven immune modulation."

22.16.3 Mouse Model: Creation of the Triangle Genes' Profiles in Brain Metastasis

Metastasis plays a manipulator role to letdown the therapeutic targets including those affected with breast cancer. In recent publication, the highlighted items include, (1) Frequent migration of tumor cell to the brain in the cancer patients affected with three major cancers including breast, non-small cell lung cancer, or melanoma; (2) The unmasked biological and molecular facts on the migration of primary tumor cells to the brain; (3) Interaction and cooperation between metastatic cancer cells with the brain's microenvironment (Sato et al. 2017). So, they have created the human BC- or melanoma-based mouse models, and have performed RNA sequencing and expression assay of two genes' profiles including "the mouse genes *Tph2*, *Sspo*, *Ptprq;* and human genes *CXCR4*, *PLLP*, *TNFSF4*, *VCAM1*, *SLC8A2*, and *SLC7A11* which were upregulated in brain tissue harboring metastases." They aimed to create a basic panel of these genes for further task to improve the cancer management including the prognostic and therapeutic strategies for the patients affected with cancer.

22.16.4 Basic and Methodology in BC Brain Metastasis

Survival has been always a serious concern for the clinical oncologists, cancer patients, their family, and friends. So, a reliable and complementary prediction tools is an essential requirements for the relative appropriate management for cancer patients. The "Graesslin's nomogram external validation" has been recently, applied for the patients with BC who has later developed brain metastasis (BM) who are characterized with HER2 amplification/overexpression, Er-/PR-/Her2-,and some with cerebral metastatic BC. In addition, the risk factor for an extra tumor spread to cerebral was also estimated by "Fine and Gray's competing risk analysis" (Genre et al. 2017). They have concluded that these estimating tools are capable to be exported and reproduced to create the "competing risk model analysis" through which the early managements including prognosis, prevention and designing the more influential therapies for the patients either with bone metastasis, or with "extra-cerebral metastatic BC" would be possible.

22.16.5 Basic Methodological Aspects in Metastatic Brain Tumors

The vascularization is a supportive channel to facilitate permeability of tumor cells in the brain territory to oppose the blood-brain barrier (BBB). Mittapalli and the colleagues have updated the standard "mathematical modeling by considering by quantitative fluorescence microscopy in two tumoral models (Mittapalli et al. 2017). They have proposed a hypothetical insight on therapeutic efficacy of trastuzumab which apparently was not successful in the metastatic brian tumor due to the inadequate penetration through central nervous system. In stead when they tried the same size antibody as bevacizumab revealed to achieve a fruitful result in similar tumor type. The reason for such diverse result was due to triggering VEGF in the vascular territory, which decreases edema and invasion.

22.16.6 Basic Insight on the Role of Cluster of Differentiation 133, Epithelial–Mesenchymal Transition (EMT) Programme and Prometastatic Event

The cooperative manner of cancer cells creates a wonder land in the tumor territory. Although the EMT plays a fundamental process in development of embryo and morphogenesis, but cancer cells take an advantage of such normal cellular behavior and pave the way towards the formation of tumor and metastasis (Latorre et al. 2016). They have highlighted the role of CD133 (PROM1), as a marker of cancer stem cells which enable EMT in different type of cancers. However, as, the manner of CD133 function is diverse in various tumors, the precise definition of the monitoring network on CD133 expression is not available. Latorre and the colleagues reported how fascinating a ribonucleoprotein complex, that is the "long noncoding RNA MALAT1 and the RNA-binding protein HuR (ELAVL1) (RBPs)" facilitate the CD133 to regulate its expression. They have, conclusively, stated that any miss-directional action of the suppressive complex could lead to the upregulation of CD133 and "an EMT-like program" which possibly direct the process toward prometastatic phase.

22.16.7 An In vivo Humanized Bone Model for Bone Metastases

The available in vivo models, based on the injecting human cancer cells to target mouse, may not provide a successful translation for experimenting the human

primary bone tumors. However, an engineering approach of humanized bone model has been recently created (Martine et al. 2017). In this model the "immune-deficient hosts" is considered which is capable to facilitate an interactive media between human cancer cell population and "a humanized bone microenvironment in vivo" within few months. The categorization and validation of "the humanized organ bone model" support a tracing strategy to unmask the involved mechanisms in bone metastasis originated from prostate and BC.

22.17 Therapeutic Aspects and Targets in Cancer

22.17.1 Pentose Phosphate Pathway as a Therapeutic Target in Breast Cancer Brain Metastasis

In the recent paper, expression of "the pentose phosphate pathway (PPP) as a metabolic pathway parallel to glycolysis" originated from tissue of metastatic BC was assayed by microarray (Cha et al. 2017). The related proteins of PPP including glucose-6-phosphate dehydrogenase (G6PDH), 6-phosphogluconolactonase (6PGL), 6-phosphogluconate dehydrogenase (6PGDH), and nuclear factor erythroid 2-related factor (NRF2) were correlated with diverse prognoses and metastatic destinations. Based on these results, the remarkable overexpression in brain metastasis was highlighted as a "potential therapeutic target". The highlighted marks are illustrated (Fig. 22.34).

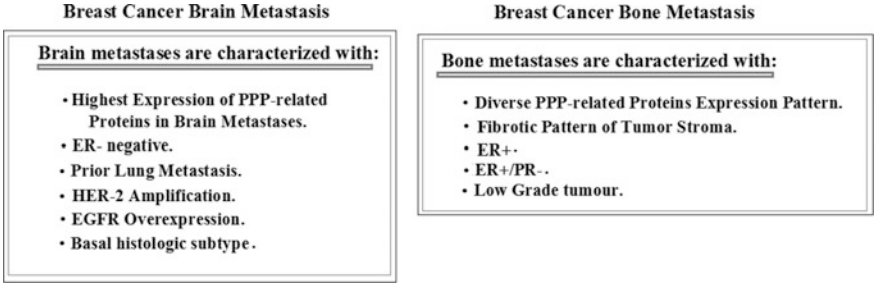

Fig. 22.34 Diverse breast cancer metastases signature pattern based on different destination metastatic site. *PPP* Pentose phosphate pathway; *G6PDH* glucose-6-phosphate dehydrogenase; *6PGL* 6-phosphogluconolactonase; *6PGDH* 6-phosphogluconate dehydrogenase; *NRF* nuclear factor erythroid 2-related factor; *ER* estrogen receptor; *PR* progestron receptor; *HER* human epidermal growth factor receptor; *EGFR* epidermal growth factor receptor

22.17.2 Therapeutic and Preventive Strategies by Targeting the Cancer Stem Cells

Cancer stem cells are the key elements for occurrence of relapse and metastasis, and clinically lead to the poor prognoses. Due to the resistance of CSCs to the standard therapies, available strategies are not, clinically, convincing. Recent strategy is based on "novel peptide agonist of bone morphogenetic protein (BMP) signaling, P123" through inhibition of activin receptor-like kinase 2 (ALK2) and ALK3, and type II BMP receptors. This innovation may control the cell cycle pathway by activating "SMAD 1/5/8" signaling (Bosukonda and Carlson 2017). Furthermore, P123 could block the induction of transforming growth factor-β, as is essential for EMT, which could lead to the progressive stage and metastatic process. They have also reported that the co-therapy with paclitaxel and P123 will lead to rise the cancer cell apoptosis. They have, conclusively, stated that P123; (1) is an influential therapeutic candidate for suppression of CSCs and cancer cells; (2) is capable to prevent and eradicate the primary tumor cells; (3) has preventive capacity against metastasis and cancer recurrence; and (4) Plays a supportive role in therapy of BC. Furthermore, CSCs are an interesting model which reflects the functional heterogeneity of tumor cells. Besides, CSCs may be resistant to the current anti-cancer drugs which is a challenging item and indicative of therapeutic limitations as well (Zekri et al. 2016).

22.17.3 CD133 as Therapeutic Target and Applicable Prognostic Marker in Breast Cancer

The meta-analyses was performed to estimate the impact of CD133 expression on the clinical prognosis of breast cancer patients (Li et al. 2017). The mortality risk of BC patients with a positive CD133 and cancer progression revealed to be higher than the negative CD133. They have also suggested that CD133 may be considered as a predictive-, prognostic marker and therapeutic target for the BC patient.

22.17.4 A Triangle Based-Therapy Through ALDH1/CD133/VEGF in Breast Cancer

Angiogenic factor plays a fundamental role in tumor growth and metastasis, but its relation to CSCs is not fully understood. In recent paper, the expression of aldehyde dehydrogenase 1 (ALDH1) and "cluster of differentiation 133 (CD133)" were assayed in invasive ductal BC (IDC), and according to result, a triangle correlation was found between ALDH1+/CD133+ and VEGF expression (Lv et al. 2016). Based on these results, they have suggested a new therapeutic target for exploring the growth and metastasis of tumors.

22.17.5 Therapeutic and Predictive Impact of TKLs and Amplified Her2 Clones, in Response to Radio-Therapy

Response to treatments including radiotherapy, and survival are the challenging dilemma which is, mainly, due to lack of comprehensive information of the cellular behavior in cancer. In this regard, the recent publication is reflective of data on molecular subtype-based in BCBM within two categories including with or without "targeted therapies" (Miller et al. 2017). As a conclusion, the molecular subtypes, such as TKLs and the amplified Her2 clones, were considered as a reliable prognostic factor either as a predictive element for the radio-therapeutic response, or for survival duration.

22.17.6 The BDNF/TkrB Pathways as a Targeted Therapy

The importance of brain-derived neurotrophic factor (BDNF) as an influential neurotrophic element has been highlighted (Tajbakhsh et al. 2017). In this review article, the machinery of BDNF is reported to be upon to stimulation of the growth and metastasis of BC cells through TrkA, TrkB and the $p75^{NTR}$ receptors. Furthermore, different signaling pathways such as "Akt/PI3 K, Jak/STAT, NF-kB, UPAR/UPA, Wnt/β-catenin, and VEGF" and estrogen receptors reported to be manipulated by miss-motivation of BDN/TrkB pathway. Eventually, this pathway could be intonated by different miRNAs accompanied by overexpression of the involved genes which lead to an undesirable clinical outcome and reduction of survival duration. They have also emphasized on the key role of BDNF/TkrB pathways either as the pathogenic protagonist or as a targeted therapy.

22.17.7 In vivo Therapeutic Target: Correlation Between CD133 Elevation and Metastatic Event

Medulloblastoma (MB) with the therapeutically challenging pediatric tumor requires the multi-management including "surgery, craniospinal radiotherapy and chemotherapy" (Garg et al. 2017). Regarding the MB stem cells (MBSCs), they have assayed the CD133 expression in MB patients and found a correlation of CD133 upgrading with higher metastatic rate, followed by a negative impact on the clinical status. They have also reported the activation of "signal transducers and activators of transcription-3 (STAT3) pathway in overexpressed $CD133^{+}$ MBSCs". Furthermore, promotion of tumorigenesis was conducted by c-MYC. By considering the tumor size, they have also stated that inhibiting the STAT3 signaling in medulloblastoma stem cells may be considered as a therapeutic target in vivo.

22.18 Conclusions

This chapter highlights the different aspects of metastasis by focusing on BCM and departure from breast to brain- and bone-destination. However, the following conclusive points could be also applicable for any primary metastatic organ and the target organ.

As a brief conclusion, the most applicable strategy would be the personalized cancer management by considering the translational research, and multi-punitive insight to uncover the involved molecular- and functional-alterations though the evolutionary courses in cancer metastases. In addition, cancer control by early detection is the most appropriate management for metastatic cancers. Such systematic plan requires a global harmonization of the research and clinical schedules in cancer world. Besides, the rapid and personalized base updates are required for managing the BCM including; (1) Multidisciplinary programme for the metastatic cancer management, (2) Performing pedigree-based cancer genetic counselling, (3) Classification of the cellular/molecular characteristics, (4) Bridging and unmasking the gaps at genomic and somatic levels, (5) Providing tumour-based guidelines by considering genetics and cell biological disciplines, (6) Applying Personalized cancer management, and (7) Innovating the most influential personalized therapy. As a final statement, it seems that there is an evolutionary timing process. However, the prompt/surprising/global attempt is required for arriving to the nearest island of cancer territory.

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Chapter 23
New Three-Dimensional NLS-bio-feedback Approaches in Site Specific Diagnosis of Cancer

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Abstract Despite the complexities of human organism, human being acts in accordance with the same biological and biophysical universal rule as other biological systems. Human organism is a thermodynamically open non-equilibrium system that at every level manifests itself as an interaction of thermo-dynamical processes. In terms of thermodynamics every organism, considered as non-isolated open non-equilibrium system, which exist only in stationary state when its internal parameters are harmonized on the level of good health status. Stationary state stability can be preserved only as a result of continually exchange between biological system and its environment by energy, matter, entropy and information. cancer is a complex disease that develops as a sequence of gene-environment interactions in a progressive process that occur in field of dysfunction in multiple systems, including genetics apparatus, immune functions and deviation of bio-signal. Recent evidence supports a concept that deviation of hemostasis due to abnormalities in information itself is the primary cause of malignancy. New non-invasive approaches to cancer diagnosis and therapy are suggested via three-dimensional NLS bio-feedback system. Human cells are composed of molecules and atoms. In this paper we discuss Information, Biological noise, Biophysical noise and Entropy in the context of biological systems. Particular attention is paid to the information and noise in accordance with the Quantum-entropic logic theory. The noise/information ratio and how the degree of destruction rate in the given object can be evaluated as the level of noise/information around biological object changes will also be discussed. Non-linear-system and entropy method is very fruitful on the study of body and its interaction with (intra-extra) environment.

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Keywords Bioresonance · Biological noise · Biophysical noise · Cancer · Entropy · Information · NLS-bio-feedback system · Noise/information index · Spectral-entropy analysis · Torsion field communication

Abbreviations

BHPG	Benign hyperplasia of prostate gland
CPG	Cancer of prostate gland
DRI	Digital rectal investigation
Gy	Gray
MeV	Mega electron volt
NLS	Non-linear system
SEA	Spectral-entropy analysis

Section I. Bio-resonance Approaches in Diagnosis of Prostate Cancer

23.1 Introduction

According to recent World Health Organization (WHO 2007) projections, cancer will have replaced ischemic heart disease as the overall leading cause of death worldwide in 2010. Cancer is a group of abnormalities characterized by uncontrolled cells growth and progressive accumulation of clonally derived cells from un-differentiated abnormal cells. If the spread of cancer cells in organism is not controlled, it can be resulted in death. Cancer is caused by interaction of both environmental and genetics factors (Juran and Lazaridis 2007; Vidal et al. 2011).

The development of most cancers occurs in long period of time and requires multiple steps (Tritter 2002). Then, early detection of cancer in initiation period is vitally important. Early symptoms of cancer, as in almost any other chronic disease, are usually nonspecific. Because of complexity of cancer, we need the holistic and comprehensive approaches for its early detection.

23.1.1 Human as a Thermodynamically Open Non-equilibrium Systems

Human nature is highly complex (Buss 1984). The introducing of the concept of living organisms as thermodynamically open non-equilibrium systems can be traced to *Gurwitsch* (Beloussov 1997), *Ervin Bauer* (Bauer's principle, 1920) (Elek and Müller 2013), *Vernadsky* (Zhukovsky 2000), and *Ludwig von Bertalanffy* (Bertalanffy 1950). One of the major achievements of Soviet theoretical biology is the discovery of the *Gurwitsch* phenomenon mitogenetic radiation. The organic

connection with this discovery and its relation to the processes of embryogenesis he created the theory of the biological field (Vanwijk 2001).

According to *Gurwitsch's* Morphogenetic Field theory, behaviour of both individual cells and organ rudiments is controlled by a field of forces common to all the elements of an embryo. This field regulates behaviour of individual cells in a developing embryo, directs their movements, controls their divisions and differentiation, and evolves itself with embryo growth.

Numerous experiments confirmed the existence of "mitogenetic" radiation, a term given by *Gurwitsch*. Such phenomena of weak bioluminescence were later on termed Ultra-weak Photon Emission (UPE) or Biophotons in modern bioelectromagnetic field theories (Brizhik 2008).

Human system is not close system, but an open system and continuously exchanges matter, energy and information with the environment. The biological field theory of *Gurwitsch* reflects a further conceptual shift from the linear view of particle-field dichotomy in classical physic to a non-linear view of particle-field interactions, in modern self-organization theory (Nicolis and Prigogine 1979).

The concept of living organisms as non-equilibrium and open systems suggested that living order exists in a state of non-equilibricity due to the action of biological fields (Gurwitsch and Gurwitsch 1942; Vanwijk 2001).

Bio-chemical and bio-physical interaction within and between cells is well proved. Bio-chemical and bio-physical interaction follows the non-linear pattern, which are fundamental elements in modern biological field theories. Nonlinearity implies evolution and novelty as inherent properties of a living system which its whole parts are in constant dynamical interaction, thus not determined by initial conditions, and its description cannot be deduced from the properties of its elements and molecules alone. The importance of network interactions has gained considerable attention in self-organization of the organism through feedback control mechanisms among the whole parts of body (Gilbert and Sarkar 2000; Longo and Montévil 2011), taking into account the need for integration of epigenetic processes and non-genetic informational pathways as higher levels of cellular control (Strohman 1997; Nicholson Daniel 2014; Jablonka and Lamb 2006; Atkinson 1965; Schneider 2010).

Positive feedback loops in combination with negative feedback are a common feature of oscillating biological systems.

It is also showed that malignant and normal tissues have different electric properties and that these modifications are efficiently revealed through non-linear resonance interaction (Barbault et al. 2009). In the study of electromagnetic characteristic of cancer, *Barbault* and his colleagues concluded that, cancer-related frequencies appear to be tumor-specific and treatment with tumor-specific frequencies is feasible. Another study showed that, cancer cells have cell membrane potentials that are lower than the cell membrane potential of healthy adult cells (Szent-Gyorgyi 1968). *VanWijk* study presented that characteristic of bio-photon emission in normal cells and tumor cells have the opposite pattern. Whereas normal cells show decreasing emission with an increasing number of cells, the photon emission of tumor cells increases in a nonlinear way to further values, displaying thus a qualitative, not only a quantitative, difference (Vanwijk 2001).

It is supposed that human as a superorganism, normal and cancer cells as Interactome Networks, cancer as a highly complex disease and dysregulation of the critical signaling molecules and carcinogenic process in genomic and supragenomic level follow the NLS model and system biology approach (Proal et al. 2009; Dinicola et al. 2011; Huang and Ingber 2006; Marmarelis 1997; Higginsa 2002; Lango and Weedon 2007; Vidal et al. 2011; Hameroff 2004, Nesterov 2012; Brabek et al. 2010; Kuznetsov et al. 1994).

23.1.2 Wave Regulation of Organism Homeostasis at the Open Non-equilibrium Systems

Homeostasis means keeping things constantly and comes from two Greek words: 'homeo,' meaning 'similar,' and 'stasis,' meaning 'stable.' Homeostasis is "the tendency of a living organism or cell to maintain sustainable equilibrium and balance with the environment". Human body systems maintain homeostasis by using input (sensor) and output (effector) mechanisms (Boggs 1966; Cohen et al. 2012).

There are several mechanisms of organism's homeostasis control. The first mechanism is humoral (humoral regulatory chain) control of homeostasis and consists of biologically active substances. This is a slow process which may take hours or days. Quick physiological processes cannot be regulated just by this mechanism.

The next mechanism is nerve regulation (neural regulation chain). This is a relatively quick method of regulation, but there are certain cells in an organism (red blood cells, white blood cells) which cannot be innervated and at the same time respond to influence immediately. The third mechanism is the regulatory molecules of immune system (immunological regulation chain). Of course, there are, growing evidence supports the notion that these are three parts of a unique system, the neuro-immune-endocrine system (Boggs 1966; Velázquez-Moctezuma et al. 2014; Poletaev 2013; Gordon 2008; Maier et al. 1994; Pert et al. 1985; Kiecolt-Glaser et al. 2002; Ironson et al. 2002).

Besides these, there should be the forth principal mechanism of homeostasis control. This mechanism was called *wave method of homeostasis regulation* (Stanley et al. 2015; Bonnemay et al. 2015).

Biological systems are capable of changing, not only in response to and interaction with the environment, but also self-organize the internal microenvironment, so that it must closely corresponds to the optimal conditions for their survival and life.

A nonlinear system, in contrast to a linear system, is a system that the output is not directly proportional to the input. Typically, the behavior of a nonlinear system is described by a nonlinear system of equations.

On the other hand, the concept of linear relationship suggests that two quantities are proportional to each other. Nonlinear relationships, in general, are any relationship which is not linear.

All cells and biological systems need to biological signal and information for cooperative and integrative physiological function. Biological Information and signaling can lead to the reconstruction and modification of physiological-state of organism. Information is need for maintain of body organization, and coordination of its functions.

There is a growing recognition that biological information for normal function of body may not be located solely in genomic database. In Homo sapiens, information has different level and various types. The source of Bio-information may be biochemical (that is: DNA-information, immunological—information or immunculus and neural-information) and bio-physical-information (quantum-entropic interactions) (Jablonka and Lamb 2006; Nicholson 2014; Nesterov 2012).

23.1.3 Quantum-Entropic Logic Theory and Bio-information

Non-linear (NLS) diagnostic approaches are based on a new physics of quantum-entropic interactions or Quantum-entropic logic theory. The concept of information (negentropy) cannot be separated from that of thermodynamic free energy. In 1956, *Leon Brillouin* coined the term "negentropy" for the negative entropy. He then connected it to information in what he called the "negentropy principle of information."

The concept of negentropy (the same as the negative entropy or syntropy) is also applicable to living systems, it means entropy that a living system is exporting to reduce their own entropy (Shannon 1948; Brillouin 1962; Adami 2004; Mcnamara and Dall 2010).

In terms of thermodynamics every organism should be defined as nonisolated open non-equilibrium system, which exists only in stationary state when its internal parameters are stabilized on the level of survival. Stability of stationary state can be preserved only as a result of intensive exchange between living system and its environment by energy, entropy, matter and information (Zhukovsky 2000; Levin 2011; Reinagel 2000).

The postulates of Quantum-entropic logic theory are the following:

1. Any physical object of biological or non-biological nature increases its structural level, when it absorbs information (negentropy) from environment, and at the same time it becomes more complex and stable (Brillouin 1964; Vanwijk 2001).
2. Any physical object of biological or non-biological nature decreases its structural level, when it loses information, thus it becomes less stable and more disorganized. For biological object loss of structural organization (information)

means worsening of adaptive behavior, development of diseases and, finally, death of an organism.
3. There is always information noise around any destructing object which that loses information (Nesterov 2011; Stern 1999).

Biological noise (signal processing) contains information and information of noise embodied in small fluctuation of the spectral components (Johnson and Mildred 1972; Steinberg 1987; Nesterov 2011). In this context the term of noise is different from the molecular noise in gene expression. Cell to cell variation in gene expression also called noise. Cell-to-cell variation or "noise" in gene expression is also very important regulatory tools in cell physiology and follow also the non-linear model (Pilpel 2011; Chen and Wang 2006).

Since information transfer in biological system is carried out by the frequency principle, body signals are considered as noise, which is a source of important information (Reinagel 2000).

The more intense destruction of biological system, the higher level of noise/information around that system and the more acute pathology process can be registered. Therefore, if we measure level of noise/information around biological object we will be able to evaluate about degree of destruction velocity in this object. If we measure frequency properties of noise background, we will recognize which tissues in an organism were destructed, because every tissue in a living organism has its own specific frequency spectrum that differs from the others.

Noise in cellular physiology also plays an important role in many fundamental cellular processes, including transcription, translation, stem cell differentiation and response to medication (Johnston 2012; Schulte and Andino 2014).

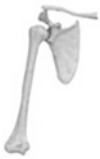
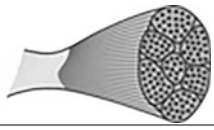
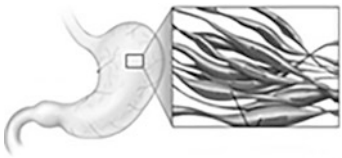
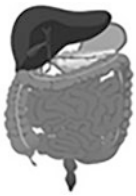
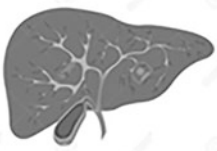
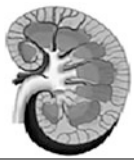
Each cell receives and transmits wave-signal. Signal of biological regulation used in information exchange in biological systems has complex structure. Biological systems have hundreds of frequency levels. Each high-frequency component is modulated by a component with lower frequency; this principle goes down to very low frequencies and just low-frequency components are carrier of information. That is why we should study information noise signal in extremely low frequency range (from 1.8 to 8.2 Hz in 9 standard values) to get more information. All biological tissue is represented within whole range of these frequencies, and each tissue has its certain point of signal amplitude (Nesterov 2011) (Table 23.1).

This frequency is called self-frequency of a tissue. The law is the following: the higher structural organization of tissue, the higher its self-frequency. For example, bone tissue has self-frequency of 1.8 Hz, brain cortex—8.2 Hz.

Prof Vladimir Nestrov, head of Institute of Practical Psychophysics in cooperation with International institute of theoretical and applied physics of RANS and Clinic Tech Inc. (USA) has completed a series of scientific studies which proven that information interactions in biological organism are carried out by means of certain physical fields, named torsion fields afterwards.

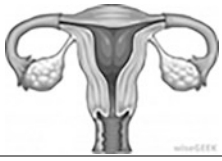
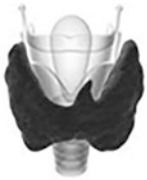
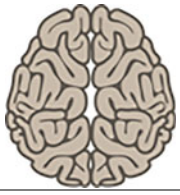
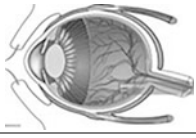
As the electromagnetic field is produced by electric charge and the gravitational field by mass, rotation or spin of a mass generates torsion field. All these fields have effects on long distances. The term torsion may be defined as a variable which

Table 23.1 Table of organ frequencies

Organ	Frequency (Hz)	Example
Osseous tissue	1.8	
Rough connective tissue, joints, and heart valves	2.8	
Loose connective tissue, striated musculature, cardiac muscle	2.6–3.4	
Smooth muscles	3.4	
Simple columnar epithelium of the digestive tract	4.2	
Stratifies squamous and columnar epithelium. Parenchymatous liver tissue and tissue of the biliary tract	4.9	
Kidney tissue epithelium and reproductive organs	4.9–5.8	

(continued)

Table 23.1 (continued)

Organ	Frequency (Hz)	Example
Lymphoid ring of the pharynx, upper section of the respiratory tract, lymphatic system, spleen, ovaries, prostate	5.8	
Peripheral nervous system, bronchus epithelium, adrenals, thyroid	6.6	
Central sections of sensory compartments except the optic one, subcortical structures of the brain, pons; cerebellum, limbic system and lungs parenchyma	7.4	
Retina, optic nerve, cerebral cortex	8.2	

describes the rotation. According to torsion field theory the interaction of spin–spin can be transmitted by electromagnetic waves, except, that this does not possess energy and mass but only information (Laura and Criveanu 2014).

On the other hand, a number of particles have charge. Charge generates electric field. Besides all particles have certain energy. There are particles which have energy, but do not have mass (for example: photon—quantum of light). These particles have interactions energy too. But all elementary particles, regardless of having mass or energy only, have one common quantum-mechanical parameter—spin. Spin of a particle may be left-hand or right-hand. Information interaction both in animate and inanimate nature is the interaction of elementary particles spins; any elementary particle has spin, so information or torsion field is always a component of any physical field (Nesterov 2011; Akimov and Tarasenko 1992).

Recently existences of particles have neither mass nor charge and energy which is close to zero was foretold. These particles have only one parameter—spin. It is hard to conceive them as physical particles, easier to visualize them as vacuum vortexes. These virtual particles may carry information along with other elementary particles (electron, bozon, photon, gluon and so on). Information emission has a great penetration power.

The vast majority of our DNA "text" is not used in the coding of proteins and enzymes, it is non-coding DNA and scientists generally do not have any idea what its purpose is. Thus, they initially dubbed it "junk" DNA.

During 1984–85, *Gariaev* made a startling discovery. He found that an in vitro DNA sample in a test tube had the ability to attract and attach coherent laser light, causing it to spiral along the DNA helix. This alone was an unexpected discovery, but it was not all. After the DNA sample and apparatus were removed, the photons continued to spiral as if the DNA were still there. This was dubbed the "DNA phantom effect," and it signified that some "new" scalar/torsion field structure had been excited from the vacuum/aether and was entraining the light even in the absence of the DNA.

This effect has been observed to last for up to a month, showing that the new field structure possesses remarkable persistence and stability. Even after blowing the phantom away with gaseous nitrogen, it returns within eight minutes. Torsion fields are known to be stable and persistent.

Significantly, *Gariaev* also remarked that DNA not only absorbs and emits light, but radio frequencies and phonons as well.

Animal and human's DNA harnesses both sound and light in its every moment operations (Gariaev et al.1992; Rein and McCraty 1994).

As it was said earlier elementary particles have 2 values of spin. Therefore torsion fields are divided into 2 types: right-hand polarized torsion (information) field and left-hand polarized torsion (entropic) field. Influence of right-hand polarized torsion (information) field to any physical object, including biological ones, improves level of its structural organization because of information saturation. Effect of left-hand polarized torsion (entropic) field is related to worsening of structural organization of any material object due to loss of information. Therefore right-hand polarized torsion field is the universal protector of all physical objects and vice versa left hand polarized torsion field is the universal destructor of all material objects.

We cannot register torsion fields directly due to their great penetration power. We can evaluate about effect of torsion fields to a biological system by indirect signs. It was mentioned that torsion field is a component of magnetic field. Torsion field has 2 types of polarization—left-hand and right-hand; magnetic field also has 2 poles—north and south. In accordance with laws of physics left-hand polarized torsion field will be generated around north magnetic pole. At the same time right-hand polarized torsion field will be generated around south magnetic pole.

Permanent magnet always has two poles—north and south: where north magnetic pole is universal destructor—when it influences a system it will lose information; and south magnetic pole, is the universal protector and accompany with accumulating information in a system. Therefore all information processes influenced by permanent magnetic field in biological systems will have only one direction—from N pole to S pole.

Actually, scientist cannot to comprehensive explain how variation at the cellular and tissue level is coordinated into variation at the whole-organism level, especially

as priority of cellular and tissue functions change over an individual's lifetime and are influenced by environmental variation.

Any biological system may be regarded as cybernetic device or black box. Cybernetics is a science that studies systems of any nature that are capable of perceiving, storing, and processing information, as well as of using it for control and regulation (Nesterov 2012; Martin et al. 2011; Mcnamara and Dall 2010).

Consistent with cybernetics laws, each system will function if two signals are present: input and output. At the same time we can be unaware about character of processes inside the system. In order to evaluate condition of the system we should evaluate input and output signals of the system. According to quantum-entropic logic input signal of a system (related to receiving of information) may be correlated with effect of south magnetic pole; output signal of a system (related to loss of information)—with effect of north magnetic pole (Li and Hopfield 1989; Rubner and Schulten 1990).

Thus S-magnetic signal (input signal) will characterize effect of higher regulating mechanisms in relation to biological system, N-magnetic signal will characterize response of a system to regulating influence. If a system is integral, level of noise in the system is close to zero, so in this case input and output signal will be relatively similar. If a system is in the stage of destruction, there will be a gap between input and output signals, it is called dissociation of a signal. The higher dissociation, the higher level of noise/information background exists around destroyed system and the higher speed and wider extent of system destruction.

Therefore one can judge about speed and extent of destruction by value of noise/information background, which is manifested by dissociation of input and output signals. Frequency analysis of dissociation in graph spectrums allows us to understand what tissues are being destructed faster and extensively (Woods Arthur and Wilson Keaton 2013; Nesterov 2011).

In the recent decade the use of informative-wave technologies have been widely implemented into practical medicine. The Nonlinear Diagnostics Systems (NLS) have been extensively used lately and are gaining ever growing popularity (Nesterov 2011, 2012).

23.1.4 3D NLS-Diagnostic System in Monitoring of Prostate Gland Diseases

Diseases of prostate gland (PG) represent major part of pelvis organs diseases in men. The most widespread among them are chronic prostatitis, benign hyperplasia of prostate gland (BHPG) and cancer of prostate gland (CPG), which may be quite often combined. Prostate cancer is the most common nondermatologic malignancy and the second leading cause of cancer mortality in men (Landis et al. 2000; McNeal 1968; Jemal et al. 2009). Prostate cancer is a disease of mainly older men,

then; much effort is being placed on detecting prostate cancer in an early and curable stage to decrease the rate of mortality from this disease.

Prostate cancer, as other kind of cancer, generally is asymptomatic until it becomes locally advanced or metastatic disease. Prostate cancer demonstrates the unusual biological heterogeneity and demands distinctive classification (Bostwick 1989; Chikezie and Yi 2010).

Problems of early diagnostic and monitoring of these diseases treatment are still the most urgent ones in onco-urology.

Nowadays NLS-method becomes more and more important in diagnostics of disease (Nesterova et al. 2012). It became possible by introducing of a new system "Metatron"-4025 with "Metapathia GR Clinical" software, which allow carrying out of three dimensional (3D) visualization of an organ, acquire accurate data about

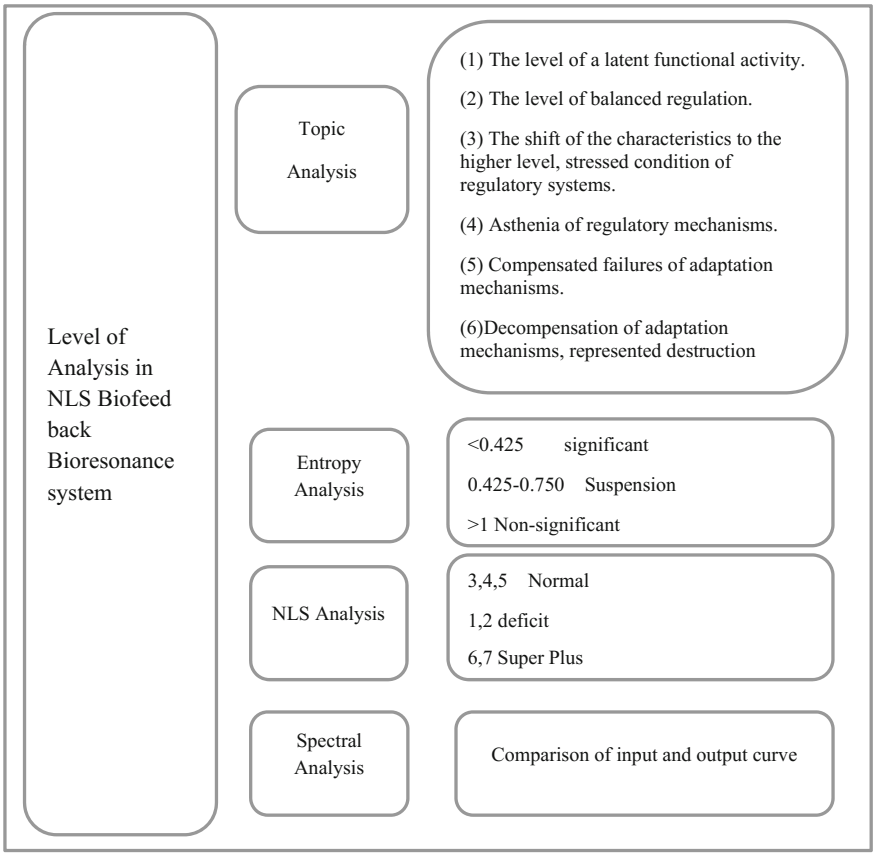


Fig. 23.1 After the examination, the results can be seen immediately on the computer screen of the NLS-biofeedback device of metatron. In order to assess a person's status one needs to test several organs and analyses the result in all the four different ways (Topic, Entropy, NLS and Spectral analysis)

dimensions and volume of affection tissues, condition of gland capsule and its internal structure owing to application of ultramicroscopic scans. NLS system has characteristic such as: easy application, possibility of multiple uses, high information value of the method and non-invasiveness (Fig. 23.1)

Appearance of 3D NLS-scanning with spectral-entropy analysis (SEA), give powerful tool for diagnostic of prostate gland diseases. By means of new NLS-technologies of prostate gland tissues and acquiring its vascular structure pictures, it became possible to fulfill highly accurate diagnostic of the earliest forms of diseases.

23.2 Materials and Methods

To examine possibilities of new NLS methods in diagnostics and monitoring of prostate gland diseases, 323 patients with different form of pathologies of prostate gland from January, 2007, till December, 2008 were researched. 181 patients of them (56%) suffering from benign hyperplasia of prostate gland (BHPG), 100 patients (31%) suffer from prostatitis and 42 patients (13%) suffer from cancer of prostate gland (CPG). Also examination of 50 control with no clinical and laboratory data on prostate gland diseases was carried out.

In this study the exclusion criteria were: (1) history of epilepsy and mental Disorders, (2) hyperthermia (more than 38 grade), (3) after treatment of myocardial infarction or stroke, (4) implanted heart pacemaker and (5) presence of foreign objects such as endoprostheses etc.

And inclusion criteria were: diagnosis of disease according to the clinical and paraclinical findings.

Spectral-entropy analysis (SEA) results were proven by analysis of cytological material taken with puncture biopsy, histomorphological study after transurethral prostatic resection or adenomectomy and by dynamic monitoring during whole period of the study.

The NLS-diagnostic is carried out using the hardware-software complex metatron device registration no FSNO 022a2005/222105 (IPP-Russia).

The device belongs to diagnostic systems with a biological NLS feedback between the patient and the central processor-telemetering module and can be used for determining the functional state of the target organs at the patients.

Investigation with Metatron carried out in special room (60–70% humidity and 20–22 °C) with minimal electromagnetics wave contaminant and all patients know that after abundant meal or extensive physical exercise can not to undertake this procedure. Examination involved in the measuring of intensity of functional changes on the targeted organ, the results assessed on Fleindler's six-point polychrome scale (Fig. 23.2). The examination which carried out with using the Metatron device, allowing obtain frequency spectrum from the researched structures, which are compared with the available spectral standard. The obtained

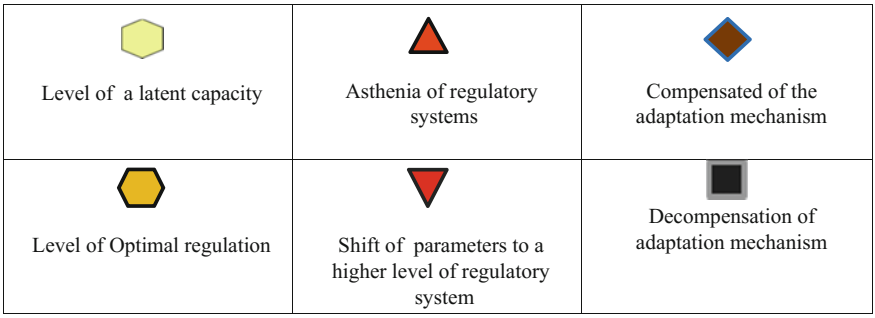


Fig. 23.2 Fleindler's six-point polychrome scale in NLS biofeed back bioresonance analysis

coefficient of the spectral differences allowed evaluating the probability of the preliminary diagnosis. All patients signed the consent form.

23.3 Results and Discussion

The results are evaluated according to Fleindler's six-point polychrome scale with analysis of Spectral-entropy (SEA) data.

In majority of cases (63%) tumor area at 3D NLS was moderately hyperchromogenic (4–5 points at Fleindler's scale) and was localized in peripheral zone in 70% of those cases. Retrospective analysis of all hyperchromogenic areas showed that hyperchromogeneity was not strictly specific for malignant pathology.

It is impossible to evaluate whether studied area was benign or malignant with using two-dimensional NLS approaches. So it was revealed that up to 45% of moderately chromogenic areas detected by usual two-dimensional NLS study to be benign after histological research. These areas were detected at acute prostatitis, benign hyperplasia, infarction and muscular hyperplasia. Besides due to absence of chromogeneity difference between tumor and normal tissue, up to 35% of CPG cases were not detected by two-dimensional NLS study. As a rule it was isochromogenic tumors and tumors characterized by infiltrating growth. Up to 40% of isochromogenic prostate tumors were detected only after gland surgical operation (transurethral prostatic resection or adenomectomy). In screening large diffuse tumors of prostate gland were rarely detected by common two-dimensional study. It was explained by the fact that after CPG spreading to central part gland, tumor chromogeneity was changed and border between tumor and healthy tissue disappeared. Informative value of NLS studies in two-dimensional method showed its low specificity (50%) and low positive prediction value (55%) in diagnostic of CPG. Great number of false-positive conclusions is related to impossibility of hyperchromogenic areas and to difficulties with visualization of isochromogenic areas.

New three-dimensional NLS methods with application of SEA allowed the differential diagnostic of hyperchromogenic areas, detection of isochromogenic tumors and more precise evaluating of malignant tissue in prostate gland.

Using of ultramicroscopic scanning, it became possible visualization of prostate gland's ultrafine structures more precise. Also ultra-microscanning approach allowed us to detect areas with overall size of less than 0.3 cm, to detect subcapsular invasion more precisely in 27% of cases.

Three-dimensional NLS-angiography method permitted to evaluate vessels condition of hyperchromogenic areas. It is widely known that tumorous angiogenesis differs from normal one (Papetti and Herman 2002; Nishida et al. 2006). Tumorous vessels have incorrect structure and increased permeability of vascular walls due to defective endothelial lining and irregularly distributed layer of smooth muscle fibers. Study of angiogenesis state is very important for CPG diagnostic; it increases positive prediction value of NLS method in detection of infiltrating isochromogenic tumors. In recent years three-dimensional NLS-angiography became the most promising method of prostate gland bloodstream evaluation. Three-dimensional scanning of vessels reconstruction showed that this method has high quality and allows to evaluate condition of vessel wall and to detect pathology zones at various parts of bloodstream (Nesterova et al. 2012; Nesterov 2012).

Using of NLS-angiography greatly decreased number of false-positive conclusions and increased number of correct diagnoses of prostate gland cancer. Study of affected vessels pattern and bloodstream condition helped us to detect up to 15% of CPG cases additionally, at the same time two-dimensional NLS method in 25% of cases did not detect any changes and CPG was diagnosed only when we used three-dimensional NLS-angiography. This indicated that evaluation of bloodstream must become an integral part of NLS researches.

Evaluation of tumor spreading beyond capsule is very important for more precise defining of cancer stages and, therefore, for determination of CPG treatment tactics. Common two-dimensional NLS research not always can precisely evaluate size of tumor area. That is why in more than 50% of cases values of tumor size, detected by two-dimensional NLS research, are incorrect. Potentials of modern 3D NLS researches in defining of disease stage increased with introducing of spectral-entropy analysis on ultrafine scans for modern devices. In our studies analysis of vessel wall condition and bloodstream affection character by means of NLS-angiography helped us to evaluate borders of tumor in 30% of patients. Study of vascular pathology at infiltrating growth of tumor helped to evaluate tumor invasion state.

Therefore application of NLS-angiography methods together with SEA only increased sensitivity of NLS researches in diagnostic of CPG from 73 to 89%, specificity from 38 to 82%, positive prediction test value from 41 to 79%, negative prediction test value from 70 to 88%.

23.3.1 Using NLS System in Monitoring of Prostate Cancer Treatment

Application of new NLS methods became especially important for monitoring of prostate gland cancer treatment. To our knowledge, monitoring of CPG treatment by means of 3D NLS-angiography was not studied properly until now. It is obvious that application of radiation therapy results in slow tumor regress and gradual fibrosis forming at the place of tumor (Iczkowski 2009). Digital rectal investigation (DRI) cannot be regarded as reliable method of treatment efficiency monitoring (Chikezie and Yi 2010). By introducing of new 3D NLS methods, efficiency monitoring of CPG radiation therapy reached more optimal level. Potential of 3D NLS system in evaluation of CPG treatment outcome was significantly extended because of development of non-invasive NLS-angiography methods. It is clear that circulation system responds first to radiation and hormonal therapy that is why information about vessel wall changing is so important in evaluation of early therapeutic effect.

Individualities of vessel wall changes in tumor tissues under influence of combined radiation and hormonal therapy allowed us to evaluate therapy efficiency in large group of patients.

We carried out research of 150 patients suffering from CPG of various stages, who were treated with combined radiation and hormonal therapy, and studied possibility of 3D NLS methods with angiography at CPG treatment monitoring. By means of SEA we traced hyperchromogenic and vascular changes in tumor tissue and parenchyma of prostate gland that occur under influence of therapy. We developed SEA parameters of CPG treatment efficiency and terms of their evaluation. Also we carried out correlation of SEA parameters dynamically changes and prostate-specific antigen (PSA) decreasing in researched group of patients.

All patients were subjected to external-beam radiotherapy on prostate gland and seminal vesicles area by deceleration emission beam of 6 and 18 meV in classic dose fractionation mode of 2 Gy 5 times in a week, with “Clinac-1800” and “Clinac-600” devices. Total basic dose to prostate gland was from 68 to 76 Gy in two stages. In 80 patients radiation was combined with antiandrogenic therapy (fluocinolone acetonide, dose of 750 mg), 35 patients were subjected to maximum androgen blockade (MAB) (Flucine, 750 mg + Zoladex, 3.6 mg once in 28 days or Flucine, 750 mg + bilateral orchectomy), in 2 patients MAB was combined with chemotherapy (5-Fluorouracil).

Average duration of monitoring period was 1 year (6 months–2 years) from external-beam radiotherapy. Digital rectal investigation, PSA level evaluation, NLS-monitoring of all patients was carried out together with oncologist and radiation therapist with 3–6 months interval. In 6–12 months after DRI radionuclide skeleton imaging was carried out in order to exclude metastases.

Changes of tumor chromogeneity also were important criteria of efficiency evaluation.

In our research we registered gradual decreasing of tumor chromogeneity (from 5–6 to 3–4 points) after exposure to radiation therapy. In period of 6 months

chromogeneity of tumor and normal tissue almost matched and it was confirmed by SEA.

In 67 patients in central parts of residual tumor hypochromogenic area (2–3 points according to Fleindler's scale) was formed; this area corresponded to "Fibrosis" formation ($D < 0.425$), its size increased during dynamic monitoring. When we used SEA analysis it was much easier to detect fibrosis zone and true size of residual tumor.

Therefore results of our work verify that combined approach in 3D NLS researches allows us to evaluate tumor response to therapy completely. Consideration of all abovementioned criteria is necessary during monitoring of CPG treatment and recommended for practical application.

23.3.2 3D-NLS System in Diagnostic and Monitoring of Chronic Prostatitis Treatment

Clinical presentation of chronic prostatitis in the half of researched patients was characterized by extraordinary variety of symptomatology, more than 30% patients was asymptomatic and 20% showed mild symptoms. Thereby it was clinically impossible to diagnose chronic prostatitis at the first stage. After 3D NLS research in 75% of patients in the first group we detected changes in parenchyma, which was specific for chronic prostatitis and were confirmed by SEA. In 75% patients who suffered more than 5 years from chronic prostatitis, we detected disproportion of affected area pattern. In patients of this group we detected zones of increased chromogeneity of various intensity and sizes with unclear pattern in periurethral area, calcification of roundish and oval form sized from 0.2 cm and more.

In 34% of these patients we identified marked outlines of urethra and ejaculatory ducts. In 16% we detected visualized cyst of ejaculatory ducts in form of local achromogenic roundish dilatations of various sizes, linked with duct by narrow achromogenic linear stripe. In 85% of patients suffering from chronic prostatitis we detected affection of prostatic plexuses veins, which evidenced stable hemodynamic disorders and phlebostasis.

Using of NLS-research methods at exacerbation of chronic prostatitis or at acute prostatitis allowed us to carry out accurate evaluation of severity of inflammatory process.

23.3.3 Diagnostics and Monitoring of Acute Prostatitis Treatment

We detected in congestive prostatitis specific NLS sign; it was so-called "small-honeycomb" parenchyma in ultramicroscanning manner. We encountered

this sign in 68% of cases both with acute and chronic prostatitis. Intensity of this sign was in direct proportion to congestive degree of gland tissue. In NLS-angiography this picture was accompanied by affected gland vascular wall. Acquiring of this NLS-graphic picture in many aspects defined further tactics of such patient's management.

23.3.4 Diagnostic of Benign Hyperplasia of Prostate Gland

Benign hyperplasia of prostate gland (BHPG) or adenoma is considered to be the most widely spread disease in men above 50 years, frequency of which increases with age (Briganti et al. 2009). In men above 40 years old adenoma is detected in 25%, above 50—in 32 and 84% of all men above 60 suffer from adenoma. Such prevalence of this disease provokes increased interest in early diagnostics and monitoring of this pathology treatment. New methods of NLS diagnostic, such as three-dimensional reconstruction, ultramicroscanning, three-dimensional NLS-angiography and SEA contribute greatly to detection and treatment monitoring of benign hyperplasia of prostate gland.

As a rule, adenomatous changes of prostate tissue are not combined by clinical symptoms of infravesical obstruction that is why early detection of this disease is quite often complicated. But when adenoma grows in transition areas, clinical symptoms appear rather late, when irreversible changes of gland in forms of fibrosis and sclerosis are already happened. NLS research with application of ultramicroscanning and three-dimensional modes allow us to quite accurately predict development of the disease and primary form of adenoma even at the early stage. By analysis of bloodstream in three-dimensional mode we found out that urethral arteries change in this group of patients is minimal. However intensity of vascularization of central part of the gland, where adenomatous tissue was formed, was higher than in screening group.

At the benign stage of hyperplasia we often face with hyper- and isochromogenic plexuses in transition area. It is known that 20% of malignant tumors may develop in transition area and that is why differential diagnostics of this area is so important. Variability of NLS-graphic picture at adenomatous tissue does not let us to limit examination only by macroscanning mode and requires involvement of additional features—ultramicroscanning and SEA.

In our studies in 65% of examined patients we detected plexuses of various chromogeneity located in central part of the gland. At the same time, spectral-entropy analysis showed only in 3% of patients malignancy, which confirmed by histological research. Evaluation of vascular pattern helped us to carry out differential diagnostics of various focal neoplasms located in central part of the gland.

Results of our studies make believe that new methods of 3D NLS-research have more informative value in comparison with standard two-dimensional

NLS-researches. We think that combined application of all abovementioned techniques must become a part of NLS approaches.

In conclusion, three dimensional NLS-graphy methods, is non-invasive, quickness, safe, high informative and efficient methods in detection of tumoral changes. It also helps to identify disease stage and qualitative evaluation of treatment. Of course, it must mention that, NLS method should be supplemented by other paraclinical procedures. The final diagnosis only should be made on the basis of the clinic lab data and the results of digital rectal examination in combination with biopsy.

Section II. Noise/Information Index as a New Systemic Diagnostic Approach in Modern Medicine

23.4 Introduction to Noise and Information

For a long time, classical biology science and medicine has been relay on reductionism approaches. The reductionist approach undoubted enfold a significant influence on biology science and especially after the progress of molecular biology, biological reactions began to be interpreted as a molecular process regulated by genetic information. Reductionism supposed as an influential analytical tool in investigation of biological phenomena in level of basic molecular and cellular processes. However as time passed and scientific data are collected, the limits of the reductionist approaches in biology have become increasingly evident. Today, with various evidences and data obtained by the reductionism methods, our knowledge about cancer compared to last decades is not comparable, but the incidence of cancer dramatically rising worldwide. Diabetic patients still need to be treated for all period of life and the rate of death because of its complications is rising. Life science cannot be enlightened only on a molecular level, without the consideration the other aspect of life. Biological systems should instead be understood as complex, stochastic and open systems, in which dynamic process of many different components at very different levels interact, from biochemical and bio-physical aspect as highly organized entity. Today more than ever medicine need for alternative and holistic methods.

Living organism made from huge number different cells, with various biological components, which participate in different biochemical reactions. These cells need to exchanges information for vital process, and this information regulates all life processes. Transfer of information plays a fundamental role in all living organisms.

Recent experiments on isogenic populations of microbes or single-cell have demonstrated that genetically identical cells in the same environment are not identical. It is partly because, that for any gene in same group of cells, the quantity of protein it produces can vary among cells: this is called biological noise. In addition to this kind of noise, from point of bio-physical sense, it is supposed the flow information in living organism almost always come along with the emergence

of noise. With the evaluation of noise/information level in the target tissues or system, one can assay the health status of that organ.

23.5 Biological Information Flow in Living Organism

Until recently, the central core of the conceptual structure of molecular biology can be summarized in the following precepts: All hereditary information resides in the nucleotides sequences of the DNA molecule. This information is transferred from DNA to RNA through the process of transcription, and from RNA to protein through translation. It also supposed that this information flow is never transferred from protein to nucleic acid sequences or from protein to protein. The last precept is usually called the "Central Dogma" of molecular biology (Piras et al. 2012).

Subsequently the advent of systemic, holistic and high throughput approaches over the last decades; introduce several intermediary steps, such as the DNA proofreading/repair mechanisms and alternative splicing of pre-mRNA. Scientists in genetics believe that the excessive potentialities of the genome are due to the occurrence of "alternative splicing", utilized in order to generate many different proteins from the same gene (Roy et al. 2013; Brett et al. 2002).

In addition, epigenetics, or modified chromatin structures, DNA methylation and histone modifications also seem to go in contradiction with the simple one-way of the central dogma (Shapiro 2009; Luco et al. 2011). Protein splicing and the ability of a protein (inteins) to alter its own sequence, discovered recently (Volkmann and Mootz 2012) and prions, which modify other protein sequences (Prusiner 1998; Nicastri Michael et al. 2013). These additional finding interfere with the key steps of the dogma and expected alter the conception of information flow in living organism. Central dogma is incomplete in terms of what's really going on in the interactive communication between DNA and others micro/macro molecules inside and between cells.

This assumption about the one-way transfer of information did not arise from physical considerations. Even the last finding along with the central dogma does not consider, in physical terms, the collective action of information exchange at the quantum level. Cellular communication and information flow in living organism have absolutely physical elements.

Information processing is essential in all fields of cellular activity. Historically, the term "Information" entered molecular biology to describe partially the biological specificity, when became clear that molecular and biochemical interactions in living organisms are highly specific. Biological interactions between molecules are stereospecific, in the sense that, in living organism, special enzyme act on the specific substrate, antibodies interact with its antigen, hormones just attached to their receptors, etc. In genetics the specificity came from one gene-one enzyme conception. Stereochemistry is the arrangement of the molecule's constituent atoms in three-dimensional space of bio-molecules. Biological specificity is studied at the cellular and molecular levels of organization, where the construction of individual

molecules allows them to selectively recognize and bind to one another (Bray 1995; Dittrich and Speroni 2007; Davies David and Gerson 1996; Schneider et al. 1986).

Therefore, by information it was mean the specification of the nucleotides sequences in DNA molecule or amino acid sequence in protein. Then, information is stored in sequences in DNA and protein. According to the *Zuckerkandl*, information in biological molecule contain in Semantophoretic molecules (primary semantide as genes, secondary semantid as Messenger-RNA molecule and tertiary semantide as Polypeptides), Episemantic molecule and at last Asemantic molecule (Zuckerkandl and Pauling 1965).

Information have critical role in organism. *Smith* and *Szathmary* suggest that major transitions in evolution depend on expansions in the amount and accuracy of information is transmitted across the generations. And it is supposed that one can understand better the evolutionary role of genes by recognizing an informational "domain" that exists alongside the sphere of matter and energy (Szathmary and Smith 1995). Information may be defined as the capacity to reduce statistical uncertainty in the communication of messages between a sender and a receiver (Paquette 2011; Adami 2004).Biological information is conveyed by particular sequences of signals and messages that originate within and between the cells of body (Gatlin 1972).

According to the *Lila Gatlin*, Life may be defined operationally as an information processing system—a structural hierarchy of functioning units—that has acquired through evolution the ability to store and process the *information* necessary for its own accurate reproduction. Cells are dynamic systems and information has message that aids cells in decision making and constructing its behavior to adapted organisms with its environment (Adami 2012; Balázsi1 et al. 2011). Therefore, the role of biological informational networks is to reliably transmit specific message about the extra/intra cellular environment to receiver, allowing the cell to modify its physiological state to changing conditions.

23.6 Entropy Production in the Cell

Along with the information flow in body, identifying the entropy production within a cell has been part of debates and studies in the last decades. Several models approach offered to identify the entropy production within a cell earth. Living system, including human organism, and cell apparatus considered as open and nonequilibrium thermodynamics system, wherein flow, growth and change are not static, not in equilibrium and are chaotic. Non-equilibrium biological or physical systems are driven by differences in intensive thermodynamic variables, which result in flows of matter, energy and information through the system (Brent 1978; Epstein et al. 2006; Himeoka and Kaneko 2014).

Entropy is often defined as a measure of the randomness of a system. According to the information theory, Entropy is a measure of the loss of information in a transmitted signal or message. In other word, Entropy is a measure of 'randomness'

or the 'disorderliness'. Second law of thermodynamics denotes that "The amount of energy available for useful work in a given system is decreasing and the entropy is always increasing", as entropy increases; the information within a biological system becomes more complex (Demirel 2010, 2014).

Living organisms are complex and systematized structures and therefore have low entropy. The entropy of an isolated system increases until it reaches a maximum, at which point, the system has reached a state of thermodynamic equilibrium. The low entropy of living systems means that they are very far from this equilibrium (Schneider and Kay 1994).

23.7 Biological Noise

Essentially, there are two strategies to control living cells, genetic modifying and changing the environment in which cells reside. But it does not absolutely mean that cells with identical genomes exposed to the same environmental factors will necessarily have identical trait. Scientist also has found that even genetically identical individuals can be very different. Such phenomen among genetically identical individuals are usually may be owing to some small chance differences in conditions and deviation that occur as individuals develop (Org et al. 2016; McGue and Bouchard 1998; Joseph 2001).

Most of the cells in human body are genetically identical, but the different kinds of cells in various tissues are not similar in appearance, behaviour and biochemical profile. In some degree, it is may be due to the variability and random fluctuations in the gene expression. That is, although, cells contain the same genetic information, but only a subset of genes are expressed in any given cell, and the ones that are expressed, determine what the cell does. However, even in cells of the same type cell-to-cell differences still occur, triggered by random differences in features like cellular size, available energy levels and micro-environments (Johnston et al. 2012; Elowitz et al. 2002; Fedoroff and Fontana 2002).

The term of *noise* or *stochasticity* in gene expression is commonly used to refer to the measured level of variation in gene expression among genetically identical cells, grown homogeneously in a common environment. In genetics term, for any gene, the quantity of protein it produces can vary among cells: this is called noise, measured as the coefficient of variation of the quantity of protein (Stewart-Ornstein et al. 2012). In biochemical sense, Stochasticity inherent to biochemical reactions is named as intrinsic noise and variability in cellular states as extrinsic noise (Cinquemani et al. 2008; Reinker et al. 2006). This inherent stochasticity is ubiquitous in physiological processes, development and disease (Kaern et al. 2005). Random differences within a cell refer as intrinsic noise (to a certain extent because of transcriptional and translation effects) and cell-to-cell differences as extrinsic noise (Stewart-Ornstein et al. 2012). Although, gene expression noise increases with cellular stress and contributes to the emergence of cellular diversity (Neildez-Nguyen et al. 2008), but it can provide the flexibility needed by cells to

adapt to changing environments or respond to cellular stresses, and a mechanism by which population heterogeneity can be established during cellular differentiation and development (Kaern et al. 2005). Gene expression is susceptible to micro environmental fluctuation, owing to the low copy number of genes and their transcripts (Ozbudak et al. 2002; Rao et al. 2002; Zhu et al. 2012).

In recent time, almost all scientific clarification about the source of biological noise is concentrated on the biochemical noise, which is gene regulation, transcription and translation (explained intrinsic noise) and cell growth and development (explained extrinsic noise) (Xue Lei et al. 2015), but in this field less attention has been paid to the physical aspects of noise (biophysical noise) in living organism (Nesterov 2011, 2012).

23.8 Physical Carriers of Information and Its Interactions in Biological Structures

From view of biophysic, several studies have been done, focusing on noise sources and their physical representations. Cells are self-replicating independent units of life. All cells are composed of Molecules. Atoms bonds together to form the molecules that makes up living organisms. Specific combinations of atoms yield an amazing diversity of molecules within the cell, each with unique functional characteristics. All atoms possess energy. According to an estimate made by engineers at Washington University, there are around 10^{14} (100,000,000,000,000) atoms in a typical human cell. Interestingly, the number of cells in the human body is estimated to be about the same as the number of atoms in a human cell.

Any substance in the universe that has mass and occupies space is defined as matter. All matter is composed of atoms. Mass generates gravitational field. Particles have charge. Charge generates electric field. Besides all particles have certain energy. There are particles which have energy, but do not have rest mass, like photons. These particles carry energy interactions. But all elementary particles, regardless of having mass or energy only, have one common quantum—mechanical parameter—spin. Spin of a particle may be left-hand or right-hand. Influence of right-hand polarized torsion (information) field to any physical object, including biological objects, improves level of its structural organization because of absorption of information. Effect of left-hand polarized torsion (entropic) field is related to worsening of structural organization of any material object due to loss of information. Therefore right-hand polarized torsion field is the universal protector of all physical objects and vice versa left-hand polarized torsion field is the universal destructor of all material objects. Information exchange, expressed in amount of information transferred inside or between cells and systems, may be relatively efficient only with relatively equal ratio of left-hand and right-hand polarized virtual particles. It appears from this that the more right-hand polarized particles in a system are, the more information it can contain. Thus information capacity of any

system is directly related to increasing gradient of right-hand polarized torsion field in relation to left-hand polarized field.

In this sense, and according to the Quantum-entropic logic theory, information is a material category, just like energy and mass of a system. Assumptions of Quantum-entropic logic theory are the following:

1. Any material object (biological or non-biological nature) increases its level of structural organization when it absorbs information from environment and becomes more complex and stable.
2. Any material object decreases its level of structural organization when it loses information, and becomes less stable and more disorganized. For biological object loss of structural organization (information) lead to the worsening of adaptive behavior, development of diseases and, finally, death of an organism.
3. There is always information noise around any destructing object which that loses information.

Each pathologic process in the given tissue associated with increased cell death (Apoptosis). The more intense destruction of biological object is, the more acute course of disease is happened and the higher level of noise/information around that object is registered. Therefore if we measure level of noise/information around biological object we will be able to judge about degree of destruction speed in this object; and if we measure frequency properties of noise background we will learn what tissues in an organism were destructed and changed more than others, because every tissue in a living organism has its specific radiated spectrum different from the others. It is showed that the higher structural organization of tissue is, the Experiments carried out in a number of higher its self-frequency (bone tissue has self-frequency of 1.8 Hz, brain cortex—8.2 Hz).

Last experiments showed that spinal fields are not of electromagnetic nature, but the nature of it not yet studied well enough by modern science. The Institute of Practical Psychophysics in cooperation with International institute of theoretical and applied physics of RANS and Clinic Tech Inc. (USA) has completed a series of scientific studies which proven that information interactions in biological objects are carried out by means of certain physical fields, named torsion fields afterwards. Therefore information (entropic) and torsion fields are identical concepts in many respects.

The registration of torsion fields directly due to their great penetration power practically is hard. We can judge about effect of torsion fields to a biological system by indirect signs. With this purpose, the NLS biofeed back system (metatron) is designed.

It is said that torsion field is a component of magnetic field. Torsion field has 2 types of polarization—left-hand and right-hand; magnetic field also has 2 poles—north and south. In accordance with laws of physics left-hand polarized torsion field will be generated around north magnetic pole and right-hand polarized torsion field will be generated around south magnetic pole. Permanent magnet always has two poles—north and south: where north magnetic pole is universal destructor—when it

influences a system it will lose information; and south magnetic pole, which in its turn will be the universal protector—accumulating information in a system.

Therefore all information processes influenced by permanent magnetic field in biological systems will have only one direction from N pole to S pole.

23.9 Conclusion

Any biological system and living organism may be regarded as cybernetic device (Trosko 1998; Sit and Miikkulainen 2006). In accordance with cybernetics laws system will function if two signals are present: input and output. At the same time we can be unaware about character of processes inside the system. In order to evaluate condition of the system we should evaluate input and output signals of the system. In accordance with quantum-entropic logic, input signal of a system (absorption of information) may be correlated with effect of south magnetic pole; output signal of a system (loss of information)—with effect of north magnetic pole.

If a system is integral, level of noise in the system is close to zero, so in this case input and output signal will be relatively similar. If a system is in the stage of destruction, we will see a gap between input and output signals, it is called dissociation of a signal. The higher dissociation is the higher level of noise/information background around destroyed system and the higher speed and wider extent of system destruction.

Therefore one can judge about speed and extent of destruction by value of noise/information background noise, which is manifested by dissociation of input and output signals. By help of frequency analysis of dissociation in graph spectrums allows us to understand what tissues are being destructed faster and extensively. Medical device with NLS-bio feed back mechanism are able to assay the level of noise/information in different system of body. This new approaches is non-invasive, holistic and safe.

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Chapter 24
Human Cancer Cell Lines: Potential to Evaluate the Therapeutic Efficacy of Anticancer Agents

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Abstract Efforts to discover new anti-cancer drugs are limited by the fact that human and animal models for testing the effectiveness of drugs are not completely faithful for recapitulation of this complex disease. These models cancer have mainly consisted of cell lines derived from human tumors or Xenografts in mice and more recently, genetically engineered mouse models of human tumor. Cell lines offer an almost infinite supply of similar cells to the genotypes and phenotypes of original tumors. Application of cell lines help scientist to cope with the ethical issues and also resolve the challenge of discrepancy associated with animal studies and human clinical trials.

Keywords Cancer genetics · Cell lines models · Tumor cells · Anti-cancer agent · Aurora kinases inhibitor

Abbreviations

3D culture	Three-dimensional culture
ABL	Abelson murine leukemia viral oncogene homolog 1
ALK	Anaplastic lymphoma kinase
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
APAF1	Apoptotic protease activating factor 1
APL	Acute promyelocytic leukemia
AURKB	Aurora kinase B
CDH1	Cadherin-1
CENPA	Centromere protein A
CML	Chronic myelogenous leukemia
CMT	Center for molecular therapeutics
DTP	Developmental therapeutics program

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EDTA	Ethylene diamine tetra acetic acid
EGFR	Epidermal growth factor receptor
FLT-3	Fms like tyrosine kinase 3
HER2	Human epidermal growth factor receptor 2
INCENP	Inner centromere protein
JAK2	Janus kinase 2
JFCR	Japanese foundation for cancer research
MCAK	Mitotic centromere-associated kinesin
MCTS	Multicellular tumor spheroids
NSCLC	Non-small cell lung cancer
PDGFR	Platelet-derived growth factor receptor
PP1	Protein phosphatase 1
PVDF	Polyvinylidene fluoride
SNP	Single nucleotide polymorphism

24.1 Introduction

Cancer cells are altered cells—a series of changes that allow them to form tumors, which behave differently from normal cells. These transformed cells depending on the genetic alterations acquired features. There were six identifiable "Hallmarks" of cancer cells, which listed as follows (Hanahan and Weinberg 2011):

1. Self-sufficiency in growth signals
2. Insensitive to Antigrowth signals
3. Escape to the programmed death of cells (apoptosis)
4. Unlimited replicative capacity
5. Continuous angiogenesis
6. Invasion to tissue.

Cancer cell lines used in many biomedical research labs. A misconception about of these cells which have unlimited potential to growth in patients, is easiness to culture and the boundless growing potential of these in the laboratory (Table 24.1) (Masters 2000). For many kinds of cancer, is much easier to grow human normal cells more than the cancer cells, even for cancers that are comparatively easy to reproduce, such as melanomas. Only cells of metastatic cancers in most cases can be established as a continuous cell lines (Masters and Palsson 1999).

Table 24.1 Cell line models (society for in vitro biology)

Cell line	A primary culture becomes a cell line when it is transferred to the next container culture. For adherent cultures, cells detached house with a protease such as trypsin, and/or one chelating agent such as EDTA and subdivided, this process is known as passage. For cells that grow in suspension Culture is divided into new containers of culture
Immortal cell line	Normal human cells have a restricted lifespan in culture medium and almost never naturally make immortal. Most cancers of human express telomerase enzyme, but either cannot be cultivated or have undergone aging. Retardation of aging and extension of lifespan can be occurred by transfection with viral genes. Viral gene products repossess proteins p53 and RB, permitting cells to continue dividing for more times
Conditionally immortalized cell lines	The benefits of immortal cell lines (a continuous supply of almost same cells) can be reached without the drawback of cell conversion to the same of cancer cells using conditional Immortalization with a temperature-sensitive mutant of viral gene. For example, a mutant of SV40 T-Antigen is operating at 33 °C, but inactive at 39 °C conformationally (Jat and Sharp 1989)
Continuous cancer cell lines	Mostly, only highly aggressive cancers (that have accumulated the genetic changes essential for infinite growing in vitro) impulsively develop continuous cell lines

24.1.1 Evidence of Similarity Between Tumor Cells and Cell Lines

When a biopsy is taken from cancer and their cells are cultured in medium culture, these derivative cancer cell lines represent the origins of cancer, from which they are derived (Broeks et al. 2000). Supporting evidence to this claim comprises the following:

Histopathology:
When human cancer cell lines are transplanted subcutaneously into immunodeficient mice such as the nude mice can create tumors. Of 127 human cancer cell lines that produced tumors in nude mice after subcutaneous injection, the histopathology correlated with the tumor of origin in every case (Fogh et al. 1977).

Genotype and phenotype correlation:
A series of two unexpected study consist of breast cancer cell lines and lung cancer cell lines from which they were resulted, showed phenotype property (i.e. the expression of the estrogen receptor) and genotype property of tumors compared to primary cancer cells, maintained over a period of time in many passage. Cyclin-dependent kinase genes mutations and similarly, p53 mutation are almost always have seen in derived cell lines and lymphomas or leukaemias from which they were derived (Barretina et al. 2012; Drexler 1998; Drexler et al. 2000; Wistuba et al. 1998, 1999).

Expression of Genes:
Reliable similarities between cell lines from the identical tissue of source, and consistent differences between cell lines of different origins has shown by means of cDNA microarray studies of over 8000 genes in 60 human cancer cell lines (Barretina et al. 2012; Ross et al. 2000).

Tumors that produce a continuous cell line have a tendency to be the fast-growing, high stage and tumors with poor differentiation, which have accumulated mutations requisite for unlimited growth in vitro. There are several cell lines derived from basic well differentiated tumors, which are mostly certain types of cancer, including tumors of bladder (Masters and Palsson 1999).The genetic modifications required to immortalize cells are mostly late events in the progression of the cancer, so it is not astounding that most primary cancers are not immortal (Masters 2000).

Cell lines of human cancers characterize a pillar of tumor biology and drug discovery through comprehensive and detailed mechanistic studies, simplistic trial manipulation, and various high-throughput applications. Many studies have used panels of cell-line noted with both genetic and pharmacological data, either within the lineage of the tumor, or various types of cancer (Dry et al. 2010; Garraway et al. 2005; Greshock et al. 2010; Lin et al. 2008; McDermott et al. 2007; Neve et al. 2006; Solit et al. 2006; Sos et al. 2009; Staunton et al. 2001; Weinstein et al. 1997).

24.2 History of Cancer Cell Lines

HeLa cell that is the most commonly used model cell line for studying the different aspects of human cellular and molecular biology is the oldest and the first human cell successfully cloned. This cell line was derived from cervical cancer cells taken on February 1951 (Capes-Davis et al. 2010). Over the time and by the progress of cell culture techniques, as well as the increase of researchers' knowledge of cancer, they inaugurated different studies based on the use of cell lines to characterize tumor cells' features (Table 24.2).

24.2.1 Cancer Cell Line Panels

NCI60
National Cancer Institute 60 (NCI60) platform that was the first high output screening program of cancer cell line launched in 1990 (Table 24.2). This platform involves 60 human tumor cell lines, demonstrating nine cancer types; namely, leukaemia (represented by 6 cell lines), melanoma (8 lines), lung cancers (9 non-small-cell lung cancer lines), colon (7 lines), brain (6 lines), ovary (7 lines), breast (6 lines), prostate (2 lines) and kidney (8 lines) (Monks et al. 1997).

Table 24.2 History of evaluating anticancer agent by cell lines

Developmental history of evaluating anticancer agent by cell lines	
1950	Animal cell culture becomes routine
1951	HeLa: first human cell line, that is developed from a cancer patient
1955	Cell-free cell culture media developed and DTP established.
1963	Approaches for cryopreservation of mammalian cells advanced
1977	Culturing of human tumor cells as MCTS
1986	(1986–1990) NCI60 model development
1989	COMPARE algorithm reported
1990	(1990–2000) NCI60 as a drug discovery screen
1992	Clustered heat maps reported
1995	Culturing human tumor cells in PVDF hollow fibres
1997	DTP external review
1998	JFCR39 launched
2000	(2000–present) NCI60 operates as service screen for drug profiling
2006	CMT1000 launched. Human tumor cell lines preserve genomic features of the primary tumor from which they were derived

National Cancer Institute (*NCI*); Center for Molecular Therapeutics (*CMT*)
DTP Developmental Therapeutics Program; *JFCR* Japanese Foundation for Cancer Research; *MCTS* multicellular tumour spheroids; *PVDF* polyvinylidene fluoride

However, the numbers of unique cell lines in the NCI60 have revised by new advanced genetic techniques panel to 57.

One of the first revelations that arisen from the screening program of NCI60 was drugs with similar sensitivity profiles of cells lines tend to pass through a common mechanism that led to the development of the COMPARE algorithm. This algorithm allows a quick comparison of compounds screened recently with compounds previously screened to determine whether the compounds exhibited new or previously described actions. In the year 1990s supplementation information from gene expression data by screening data leads to the development of cluster heat maps (Paull et al. 1989; Scherf et al. 2000; van Osdol et al. 1994; Weinstein et al. 1992, 1997).

JFCR 39

On the basis of experience of NCI60, researchers in the cancer chemotherapy Center of Japanese Foundation for cancer research (JFCR), founded JFCR-39 in 1999 (Table 24.2). It is composed of 39 cell lines derived from human tumors containing a subset of the NCI60 cell lines, and due to the prevalence of gastric cancer in Japanese population, additional cell lines from gastric cancer. By means of the COMPARE algorithm and advanced data mining technique for the identification of several new agents successfully against cancer as well as cancer biomarkers (Dan et al. 2002; Naasani et al. 1999; Nakatsu et al. 2005; Shiwa et al. 2003; Yaguchi et al. 2006; Yamori 2003; Yamori et al. 1999).

The CMT1000 platform

One platform contains human tumor cell lines that most recently created and currently contain 1200 cell lines (Table 24.2). This panel is referred to Center for molecular Therapeutics 1000 (CMT1000), as an empirical determination of their characteristics proposes that only ∼80% of such lines are amenable to profiling for drug sensitivity, largely reflecting limitations of technics such as insufficient doubling times or atypical culture necessities. This platform is now being used to analysis the genetic basis for sensitivity to approve and investigational anticancer agents.

Concisely, this profiling platform involves a 72 h of cells over a plastic under standard culture conditions to detect changes in the number of cells after treatment with different experimental agents. Sensitivity profiling with this platform started in May 2006 and, as of May 2009, 127 candidate and established anticancer agents have been interrogated for cytostatic and/or cytotoxic activity against approximately 700 human tumor derived adherent cell lines, cumulatively corresponding to around 70,000 drug–cell line pairings (Sharma et al. 2010).

24.2.2 Supplementary Applications of Panels of Cancer Cell Line

Other studies have either used smaller cancer cell line panels comprising of short-term tumor-derived cultures or established cell lines to recapitulate the genomic diversity of cancer As Tissue-specific cell line panels. Such studies via 51 breast cancer cell lines (Neve et al. 2006) 101 melanoma-derived cell lines (Lin et al. 2008) and 84 NSCLC cell lines (Sos et al. 2009) reached the important conclusion that the genetic landscape of tumor-derived cell lines is considerably similar to that of the primary tumors from which they originated, which means that cell lines derived from cultured tumors are valid genetic surrogates of tumors in vivo.

24.3 Applications of Cancer Cell Line

An individual cancerous cell line provides a snapshot of the tumor at the time of the biopsy be taken. One of the main challenges arising from biomarkers related to drug sensitivity is the combination of data from several platforms ‘systems’ that income genomic, Transcriptomic, proteomic and epigenomic information to create “molecular signatures” that can be useful in the clinic. Such signatures may be useful to predict the drug sensitivity in patients who received and clarify the mechanisms of action of the drug. However, despite all these efforts to detect a new drug, the emergence of drug resistance in many cancers is undeniable. Therefore this issue is being studied from two points of view: Detection of anticancer agent and Evaluation of drug resistance in cancer.

24.3.1 Detection of Anticancer Agent

The systems biology approaches have been applied to panels of cell lines derived from a specific tumor type to determine significant molecular associates of sensitivity to drugs (Sos et al. 2009). Genomic heterogeneity and its effects on cancer therapeutic agents are very important, So that genotype differences potentially establish a necessary basis for sensitivity of drug differences between established cancer cell lines and primary tumor cells.

Genomic heterogeneity in tumors of different patients, it becomes increasingly evident that the diversity of cell types in tumor cell populations also likely plays a key role in drug susceptibility. i.e. subsets of tumor cell with distinct States of differentiation or with properties of "stem cells" may have distinctive sensitivity to drugs (Rosen and Jordan 2009; Trumpp and Wiestler 2008). In vitro studies have shown that this cellular heterogeneity can influence the response to treatment with anti-cancer agents.

Distinctive short-term treatment assays are probable to produce readouts that are not affected by the potential existence of a small subpopulation of cells with distinct drug sensitivity, but such heterogeneity could certainly contribute to the nature of the responses to the clinical treatment of patients.

The analysis of cell lines cultured in vitro is certainly associated with alerts to potentially effects attributed to a long-term transition in culture medium and no physiological environment. However recent discoveries showing that the cell lines derived from tumors maintain genomic primary tumor characteristics largely still strengthen the validity of this approach to the discovery of clinically significant correlations between the genetics of tumor cells and the sensitivity to drug (Neve et al. 2006; Sos et al. 2009).

This observation that specific genotypes are closely related to the drug susceptibility has been studied further in the context of NSCLC and by the CMT1000 platform, sensitivity to inhibitor KLA is well correlated with ALK-activation of chromosome translocations that occur at low frequency (3–7%) in these tumors (McDermott et al. 2008; Rodig et al. 2009; Shaw et al. 2009). In addition, by means of the platform of CMT1000, NSCLC cell line with sensitivity to PDGFR kinase inhibitor has shown co- amplification of genes coding for receptor (PDGFRA) and unique ligand of its (PDGFC). PDGFRA activation has seen in about 13% of NSCLCs (McDermott et al. 2009; Rikova et al. 2007).

As a result, you can divide the NSCLCs in genetically distinct subsets based on activating mutations that they host, and each of these subsets may correspond to cohorts of patients probably to benefit from treatment with specific inhibitors that target products of these specific genetic lesions.

One of the first surprises that resulted from the CMT1000 platform were the remarkable degree to which derivative cell lines from human tumor recapitulate clinical results, qualitatively and quantitatively about their response to inhibitors of the target. Initial studies shown that the responses of cell lines to agents that target protein kinases were very limited, and cell lines with exquisite sensitivity to HER2,

EGFR, platelet-derived growth factor receptor (PDGFR), MET, anaplastic lymphoma kinase (ALK) or BRAF kinase inhibitors were characteristically marked by activating mutations or gene amplification for the encoding the drug target. In most cases, these changes are related to the types of specific tumors (e.g., EGFR mutations in about 10% of NSCLC); although, rare cell lines of other tumor types (for example, gastric cancers) were also found the identical genetic damage and demonstrate sensitivity to that specific inhibitor (McDermott et al. 2007).

Thus, cancer subsets that genetically defined, regardless of the tissue of origin, appear to be associated with response to definite inhibitors of kinases, emphasizing the potential profits of patients classification based on genotype, rather than the tissue of origin, as is at present the practice in medical oncology.

Cancer Cell line Project as one part of the Cancer Genome project is a major effort to re-sequencing, which focuses on the most common genes related to cancer in cell lines derived from human tumors. So far, this ongoing effort resulted in the re-sequencing genes of 51 'cancer genes' in 785 cell lines derived from human tumors, which gives a rich database of common oncogenes mutations in human cancer cell lines (genes and cell lines that are part of this database details on the cancer cell line project website). Most human tumor cell lines in CMT1000 are represented in the Cancer Cell line project and efforts are made to contain all cell lines in the CMT1000 in the Sanger Institute's Cancer Cell line project. As a result, you can now begin to integrate mutational information associated with these cell lines with drug sensitivity data in order to better apprehend the genomic determinants of clinical response to different cancer therapeutics (Sharma et al. 2010).

Moreover, the Cancer Cell Line Encyclopedia (a compilation of gene expression, chromosomal copy number and massively parallel sequencing data from 947 human cancer cell lines) can be as a forecaster modeling of sensitivity of anticancer drug (Barretina et al. 2012).

The ability to show a correlation between data resulted from sensitivity of drugs by a large number of human cancer cell lines and different forms of genomic information (mutation data from re-sequencing studies, microarray gene expression information and gene copy number from single nucleotide polymorphism (SNP) arrays) is a powerful and feasible approach. This approach aims to facilitate the identification of signatures or biomarkers of sensitivity to drug that can potentially be used to identify patients who may to derive benefit from a particular targeted therapeutic (Sharma et al. 2010).

24.3.2 Evaluation of Drug Resistance in Cancer

A main limitation and problem for the clinical profit derived from drug treatments of cancer is quickly acquired cancer's drug resistance. As a result, the majority of cancer patients who exhibit a response to drug therapy will ultimately relapse with drug-resistant disease. This process occurs often quickly. Therefore, it is important to improve a better comprehension of the molecular mechanisms underlying the earnings of drug resistance.

An experimental strategy that has successfully used to solve this problem involves using drug-sensitive cancer cell lines as a model for the establishment of mechanisms of acquired resistance to drug. Using constantly exposing such drug-sensitive cells to treatment in vitro over a period of time, it is often possible to eliminate most of the cells during the selection for the expansion of relatively rare drug-resistant clones. By comparing the different properties of the selected drug-resistant cells and parental drug-sensitive, it is conceivable to identify the particular molecular mechanisms of drug resistance. This approach has been used successfully. E.g. several mechanism have established for acquired drug resistance are overexpression of CRAF in melanomas as a potential mechanism of acquired resistance to BRAF inhibitor therapy and amplification of *MET* in non-small-cell lung cancer as a mechanism of acquired resistance to EGFR kinase inhibitor therapy (Engelman et al. 2007; Montagut et al. 2008).

In both of these cases, such mechanisms of acquired drug resistance might also be the cause of some cases of de novo drug resistance, raising the possibility that similar efforts to model acquired drug resistance in larger panels of cell lines with established drug sensitivity could also be beneficial for identifying extra mechanisms of de novo drug resistance.

Since all screens based on cell lines involving monocultures, a limitation of this analysis is focuses on the unique cell autonomous sensitivities to tested compounds can be scored. Therefore, agents that potentially function to affect the interaction of tumor cells with their environment (for example, inhibitors and angiogenesis or tumor–stromal interaction inhibitors) cannot be evaluated with these platforms.

24.3.2.1 Three-Dimensional Culture

In recent years, better awareness of the importance of microenvironment of tumor and solid tumors three-dimensional (3D) aspects in the process of tumorgensis and the response to treatment has provoked efforts to model these features of tumor cell growth in vitro more exactly. Tumor cells growing in 3D cultures are generally supposed more closely imitate their counterparts in vivo.

As a result, 3D cultures have inaugurated to discover aspects of tumor biology and metastasis of the tumor (i.e. Potential of invasiveness, polarity changes, matrix independent survival and sensitivity to drugs and radiation). Models of 3D culture systems take in matrix-embedded 3D cultures, multilayer cell systems, hollow fibre-based assay approaches, ex vivo tumor cultures and multicellular tumor spheroids (MTCS) (Abbott 2003; Griffith and Swartz 2006; Jacks and Weinberg 2002; Wang et al. 1998; Weaver et al. 1997).

Hollow Fibre Assay

The protocol includes short-term in vitro culture (24–48 h) of 12 human tumor cell lines as a panel in biocompatible hollow fibres and then kept on by the implantation

of these structures intraperitoneally or subcutaneously in nude mice. Thus, this system examines the capability of the administered drug to access two pharmacological compartments and can be used to evaluate responses of tumor to drug treatment in these two compartments. In general, the mice are treated with the candidate anticancer agent for 4 days, after which the fibres are removed and the viability of the tumor cells is analyzed by standard cell viability assays.

Beneficial features of this short-term assay method are significantly decrease the time and amount of drug required for standard in vivo efficacy testing. Also it simplifies the in vivo analysis of effects of drug on human tumor cell lines that can't generate tumors in animals, as well as good correlation was observed between the sensitivity of drugs in the NCI60 and hollow fibre assays. Moreover, drugs that show evidence of efficiency in hollow fibre assays usually indicate good anti-tumor activity in human xenografts, and so this system is usually used as a pre-screen before more expensive and time-consuming human xenograft testing is assumed (Decker et al. 2004; Hall et al. 1999).

Multicellular Tumor Spheroids (MTCS)

The MTCS system is a most well described 3D culture systems and supposed to precisely simulate in vivo growth of tumor cells in terms of their both pathophysiology and response to therapy. The MTCS system has also been used to examine various aspects of cancer therapeutics such as metabolic and chemical gradients, tumor hypoxia, cell–cell and cell–matrix interactions, chemoresistance and radioresistance (Friedrich et al. 2007a; Gudjonsson et al. 2003; Khaitan et al. 2006; Lee et al. 2007).

Limitation of 3D Culture

So far, fewer than 100 human tumor cell lines exhibited that they have the ability to grow in spheroid cultures (Friedrich et al. 2007a). Around half the cell lines in the NCI60 panel do not form MTCS (Friedrich et al. 2009), and the hollow fibre assay as it is presently applied uses only 12 human cancer cell lines (Decker et al. 2004). The test with a small number of cell lines restricts the potential to capture the cancer genomic heterogeneity, and the necessity for implantation in nude mice with the hollow fibre assay system prominently increases the cost and time of the assay, which excludes its use as a high-throughput platform. In addition, the different 3D cell culture systems are not relevant for blood cancers that account for about one third of all human cancers.

Many studies have documented differences in cancer drug sensitivity between monolayer cells cultured and those grown in 3D cultures (David et al. 2008; dit Faute et al. 2002; Serebriiskii et al. 2008). For example such previous studies have shown that some drugs are more effective in 3D cell culture systems compared with 2D systems (Barbone et al. 2008; Howes et al. 2007), While other drugs demonstrate better activity in the 2D cell culture systems. This issue Potentially reflect difference in gene expression between the two conditions (Dardousis et al. 2007; Friedrich et al. 2007b).

Most of the screens based on cells for detecting cytostatic or cytotoxic activities are geared to rapidly dividing cell lines of the tumor (those that have a doubling time less than the duration of the analysis) and therefore some slow—growing tumor cell lines will not be screenable on this platform. The composition of the culture media and the existence of fetal bovine serum in standard cultures almost definitely do not accurately characterize the features growth of tumor cells in vivo. Also the non-physiological oxygen levels, commonly used in cell culture studies, can have a big impact on the response to agents that target hypoxia-dependent pathways or DNA damage. Further warnings associated with the use of cell line panel as a model of human diseases encompass the fact that certain types of tumors (e.g., the prostate) are difficult to proliferate in vitro and therefore may be under-represented.

24.4 Animal Models and Xenografts of Human Cancer Cell Lines to Assess Drug Efficacy

Although Xenografts of human tumor cell lines were advantageous to establish the pharmacological properties of new agents, have been less reliable to display for the effectiveness of the drug. Unsatisfactory performance of many candidates of clinical cancer agents who showed great promise in xenograft models is underlined this subject. One potential problem associated with xenotransplantation models drug efficacy relevant to an inappropriate dosing. Consequently, recent studies demonstrated that at clinically relevant doses, tumors growing as xenografts in mice responded to the original tumors in humans in a similar way. With developed animal models of human cancers such as metastatic models, orthotopic models and autochthonous models, also genetically engineered models of cancer, predictability of determining clinically useful agents are likely to improve in the future and animal models will continue to play an important role in the development of anticancer drugs (Frese and Tuveson 2007; Gopinathan and Tuveson 2008; Kerbel 2003; Peterson and Houghton 2004; Suggitt and Bibby 2005) (Table 24.3).

Table 24.3 Summary of policies and their features

Policy	Classification	Drug detection	Drug development	Output
Cancer cell line panels	NCI60	Yes	Yes	High throughput for drugs low throughput for cell lines
	JFCR36	Yes	Yes	High throughput for drugs low throughput for cell lines
	CMT1000	No	Yes	High throughput for cell lines low throughput for drugs
	Tissue-specific cancer cell line panels	No	Yes	Low throughput for both (drugs and cell lines)
3D culture	Hollow fibre assays	No	Yes	Low throughput for both (drugs and cell lines)
	MTCS	No	Yes	Low throughput for both (drugs and cell lines)
Animal models	Xenografts	No	Yes	Low throughput for both (drugs and cell lines)
	Genetically engineered mouse models	No	Yes	Low throughput for both (drugs and cell lines)

24.5 Aurora Kinases Family and Their Cell Line Studies

Aurora kinases are involved in some control pathways of the control point, including spindle assembly control point, alignment of metaphase chromosomes and chromosome biorientation. The aberrant expression of Aurora kinases may alter control functions, especially in mitosis and this can lead to genetic instability and trigger the development of tumors. Aurora kinases have gained much attention since they were identified as oncogenes in good faith (Kollareddy et al. 2008).

24.5.1 Definition of Aurora Kinase

The Aurora family of serine/threonine kinases (consisting of Aurora-A, -B and -C) are an important group of enzymes that controls various aspects of cell division in mammalian cells (Vader and Lens 2008).

Aurora homologues have been identified in different species (Table 24.4). Saccharomyces cerevisiae has a single Aurora kinase: IPL1. In Caenorhabditis elegans, Drosophila, and Xenopus there are two types of Aurora kinases: Aurora-A and Aurora-B. Mammals possess at least three auroras kinases: Aurora A, Aurora-B and Aurora-C (Fu et al. 2007).

Table 24.4 Aurora homologues in different organism

Living organism	Type of aurora homologues	Other names
Saccharomyces cerevisiae	IPL 1	None
Schizosaccharomyces pombe	Ark 1	None
Caenorhabditis elegans	Aurora-A	AIR-1
	Aurora-B	AIR-2
Drosophila melanogaster	Aurora-A	Dm Aurora
	Aurora-B	IAL
Xenopus lavies	Aurora-A	Eq2
	Aurora-B	XAIRK2
Mammals	Aurora-A	Aurora2.AIRK1.ARK1.BTAK.STK6.STK 15.AYK1.IAK 1
	Aurora-B	Aurora 1. AIRK2.ARK2.IAL1.AIK2. STK12.AIM1
	Aurora-C	Aurora 3.AIRk3.AIE2.STK13.AIE1.AIK3

Table 24.5 Summary of Aurora classification and their features

Type of aurora	Chromosome location	Cell localization	Substrate-Aurora interaction effect	Substrate
Aurora A	20q13.2	Mitotic spindle centrosome	Spindle assembly Cytokinesis centrosome maturation and separation cytokinesis	pp1 p53 Cdh1 TPX2 RasGAP Ajuba
Aurora B	17p13.1	Centrosome central spindles chromosome arms	Chromosome alignment and segregation Cytokinesis microtubule dynamics	Histone H3 INCENP CENPA Desmin Rec8 Vimentin MCAK Survivin
Aurora C	19q13	Central spindles Chromosome arms?	Role in spermatogenesis possible role in regulation of chromosome segregation and cytokinesis	AuroraB INCENP

Aurora A (also known as Aurora-2, AIK2, AIR-2, ATA-1, AIRK1, AYK1, BTAK, Eg2, MmIAK1 and STK15), Aurora B) AIRK-2, ARK2, IAL-1 and STK12) and Aurora C (also known as AIK3) participate in several biological processes, including cytokinesis and segregation of deregulated chromosomes (Table 24.5). These important regulators of mitosis are overexpressed in various solid tumors (Bolanos-Garcia 2005).

Aurora B kinase as a chromosomal passenger protein plays multiple roles in regulating mitosis and cytokinesis (Table 24.5).whereas Aurora C expression plays a role in spermatogenesis at the time the cells assemble the two meiotic spindles and also cooperates with Aurora B to regulate the dynamics of the mitosis chromosomes in mammalian cells (Table 24.5). Aurora A has expressed in the thymus, testis and fetal liver and its low expression has seen in bone marrow, lymph node and spleen. Aurora B expression level is high in the normal thymus and fetal liver (Table 24.5) (Kollareddy et al. 2008).

Aurora dysfunction can cause aneuploidy, mitotic arrest, and cell death. Human Aurora Kinases A-C exhibit differential substrate affinity subcell localization and associated activities (Bolanos-Garcia 2005; Gautschi et al. 2008).

24.5.2 Structure

Human Auroras A-C are kinases ranging in size from 306 to 403 amino acid residues exhibiting a relatively high sequence divergence between species. The Aurora A-C kinases present a similar domain organization: an N-terminal domain of 39–132 residues in length, a protein kinase domain and a short C-terminal domain of 16–20 residues. The PEST-like motif has been identified in Aurora C. The mutation of this motif, found at the N-terminus, significantly negates Aurora C kinase activity (Fig. 24.1) (Bolanos-Garcia 2005).

24.5.3 Synthesis and Degradation

Human Aurora A is transferred (turned over) through the anaphase promoting pathway of complex/cyclosome—(APC/C–ubiquitin-proteasome. The degradation of Aurora A depends on hCdh1 in vivo and involves two different degradation motifs. The first corresponds to an N-terminal, D-Box-triggering motif (RxLxPS). This motif confers functionality to a second motif, a D-Box, which consists of the sequence RxxLxxG. The D-box is found in the C-terminal domain of the kinase and is the target of Fizzy-related proteins. While Aurora B has the same D-Box as Aurora A, it does not degrade by the same ubiquitin ligase. Instead, Aurora B undergoes degradation by binding to human C8 (HC8 proteasome subunit in a proteasome-dependent manner). Aurora A undergoes a cell cycle dependent regulation: it is inactivated or degraded when the cell proceeds in G1 phase and its maximum expression occurs in G2/M phases (Marumoto et al. 2005).

The destruction of Aurora-A and Aurora-B is mediated by APC/C Cdh1 Several domains have been identified within their degradation sequences, including KEN-box, D-box and A-Box. However, they are not all necessary for the degradation of Aurora-A and Aurora-B. The destruction of Aurora-A requires D-box and A-box but not KEN-boxes, whereas Aurora-B degradation requires intact KEN-box and A-box (Fu et al. 2007).

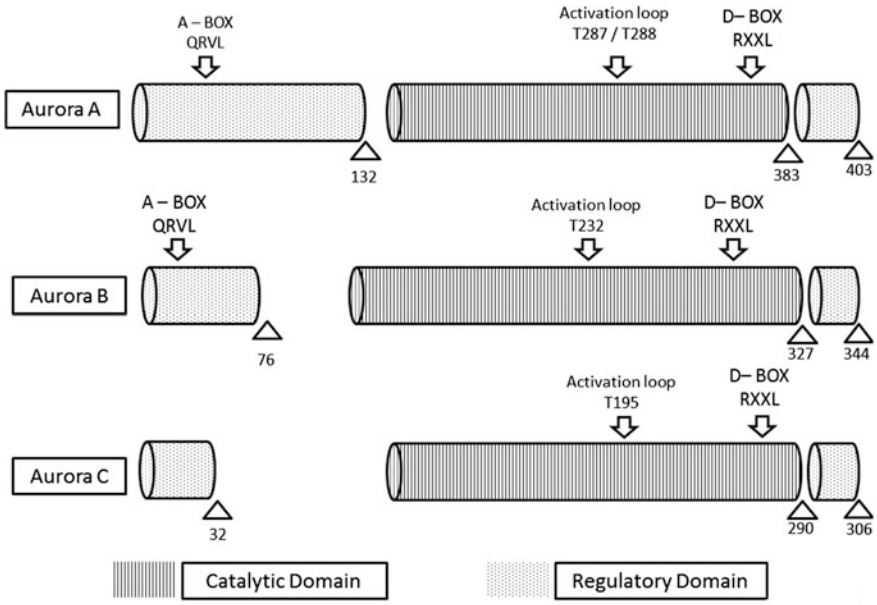

Fig. 24.1 Aurora kinases comprise mostly two domains: the NH2-terminal regulatory domain (dotted) and the COOH-terminal catalytic domain (*vertical lines*). The three homologues Aurora-A, Aurora-B, and Aurora-C have great homology in the catalytic domain yet differ in the NH2-terminal domain and a small sequence in the COOH terminus (*dotted areas*). Essential structures have shown by *arrows*. For kinase activity, phosphorylation at threonine within the activation loop is necessary. Besides, two boxes are identified as the recognition sites of APC/C^{Cdh1} and promoter of degradation: destruction box (D-Box) and D-Box-activating Box (DAD/A-Box), respectively. These features have been confirmed by experiments in both Aurora-A and Aurora-B; so far, the structure of Aurora-C is presumed by sequence alignment and seems to lack the A-Box (Fu et al. 2007)

24.5.4 Biological Function

24.5.4.1 Aurora A

Aurora A kinase activity depends on the state of phosphorylation of a threonine residue (T288) located in the enzyme "activation loop". This phosphate group is eliminated by protein phosphatase 1 or 2A (PP1/2A), which renders the Aurora A kinase inactive. The shift to the active form requires a number of cofactors, including the small GTPase and Ran protein Associated with microtubules TPX2 (Marumoto et al. 2005). Aurora A functions are (Fig. 24.2):

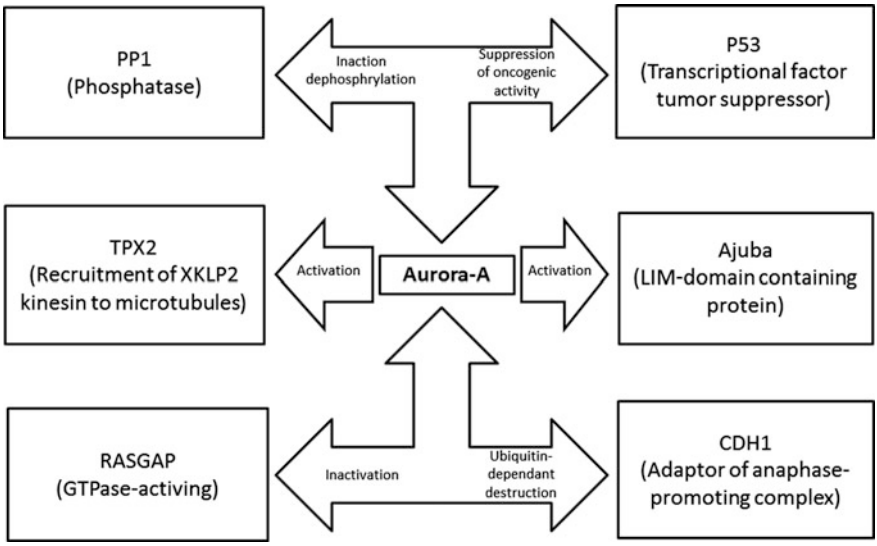

Fig. 24.2 Such of proteins that interact with Aurora-A. PP1, P53, CDH 1, TPX2, RASGAP, Ajuba are substrate proteins for Aurora-A. In this picture the role of each of the proteins listed in *brackets*. Aurora-A by interaction with these proteins regulate Spindle assembly, Cytokinesis, centrosome maturation and separation, cytokinesis in cell cycle

1. Centrosome maturation
2. Separation of centrosomes
3. Bipolar spindle assembly
4. Chromosomal alignment in the metaphase plate
5. Mitotic entry and regulation of the cell cycle
6. Cytokinesis.

24.5.4.2 Aurora B

Aurora B functions are (Vader and Lens 2008) (Fig. 24.3):

1. Condensation condensed
2. Consistency Coherence Column
3. Mitotic mounting spindle
4. Promotion of bi-orientation chromosome
5. Attachments of the syntelic chromosome
6. Merotelic chromosomal annexes
7. The control point of the spindle assembly
8. Anaphase and cytokinesis.

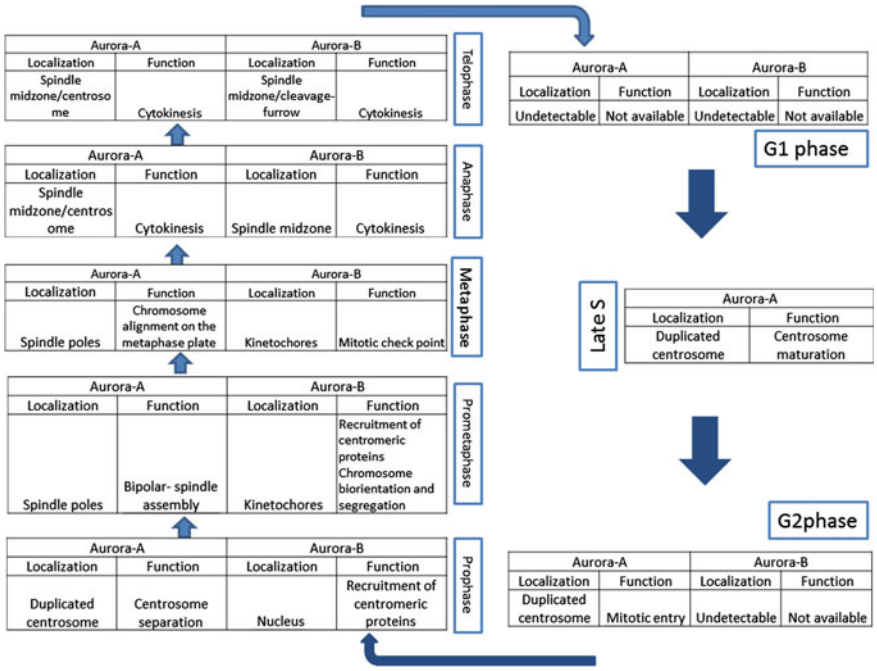

Fig. 24.3 Human Aurora kinases (A, B) localization and function in different cell cycle phases

24.5.4.3 Aurora C

The expression of the Auroras C and B proteins is highest during the G2/M phase but their expression profiles in synchronized cells reveal differential temporal regulation throughout the cell cycle. Aurora C, like Aurora B, interacts with the inner centromere protein (INCENP) at the carboxy terminal end that encompasses the conserved IN box domain. Competing binding assays and transfection experiments revealed that, compared to Auroras B, Auroras C have a lower binding affinity with INCENP (Bolanos-Garcia 2005).

Overexpression of the wild-type aurora-C kinase has been demonstrated to reduce the number of multinucleated cells that was initially Induced expression of the mutant kinase of aurora-B (Cheung et al. 2009).

Aurora C was first isolated from a testicular cDNA library. Aurora C is specifically expressed in the testis and plays a role in spermatogenesis. It was recently discovered that Aurora C also acts as a chromosomal passenger protein and could compensate for the loss of Aurora B function (Bavetsias and Linardopoulos 2015).

24.5.5 Potential Medical Utilization

Aurora A and B are overexpressed in a diversity of solid tumors and are aberrantly expressed in hematological malignancies, including acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), and acute lymphoblastic leukemia (ALL) (Ikezoe 2008).

The expression of Aurora in cancers usually related with genetic amplification, genetic instability, little histological differentiation and poor prognosis. Aurora B is often expressed at high levels in a variety of cancers, frequently in conjunction with Aurora A, and the level of expression was also associated with increased genetic instability and clinical output. In addition, the polymorphisms of the Aurora kinase gene are connected with an increased risk or early beginning of cancer (Gautschi et al. 2008).

First data to implicate this family of kinases in tumorigenesis came with the observation that Aurora A and Aurora-B are overexpressed in samples of primary breast and colon tumors (Keen and Taylor 2004).

Aurora kinase inhibitors by biochemical and cell-based assays to systematically profile a panel of 10 commercially available compounds with the selectivity reported for Aurora A (MLN8054, MLN8237, MK-5108, MK-8745, Genentech Aurora Inhibitor (de Groot et al. 2015) (Table 24.6).

24.5.6 Inhibitors of Aurora Kinase

The recent explanation of the biological function of Aurora kinases in normal and cancerous cells has led to the growth of inhibitors of small molecules. Both pre-clinical and preliminary clinical trial outcomes advise that this class of agent is hopeful for cancer treatment, although the molecular mechanisms by which Aurora kinase inhibitors induce growth arrest and apoptosis of cancer cells should be completely elucidated. In addition, a number of issues relating to this class of agent have not been addressed: for example, the best wax for the treatment of cancer is unknown. It is clear that more studies are needed to append this class of agent in the future cancer treatment plan (Ikezoe 2008).

Barasertib (AZD1152): Barasertib is a phosphate-based prodrug that is rapidly turning barasertib-hQPA in vivo. In clinical studies, barasertib has been evaluated in patients with solid malignant tumors and haematological cancers. The most frequent adverse events were stomatitis and febrile neutropenia (Bavetsias and Linardopoulos 2015) (Table 24.7).

AZD1152 a new and selective inhibitor of the aurora B kinase, induces growth stop, apoptosis and sensitization for tubulin depolymerizing agent or topoisomerase II inhibitor in human in vitro and in vivo (Yang et al. 2007).

Alisertib (MLN8237): Alisertib (MLN8237) is a selective inhibitor of Aurora-A kinase. Alisertib has been extensively characterized using in vitro and in vivo

Table 24.6 Alteration in Aurora kinase in cancer

Overexpression			Amplification			Polymorphisms		
Cancer type	Aurora kinases	Correlation	Cancer type	Aurora kinases	Correlation	Cancer type	Aurora kinases	Correlation
Breast	AURK A	Not addressed	Breast	AURK A	BRCA2 Tp53 mutation	Colon	AURK A	Cancer risk
		Chromosomal instability	Colon	AURK A	Not addressed			
		Centrosome anomaly	Brain	AURK A	Genetic instability			Aneuploidy
		Nuclear grade			Overexpression	Breast	AURK A	Cancer risk
Prostate	AURK B	Grade; proliferation	Bladder	AURK A	Overexpression			
Bladder	AURK A	Tumor recurrence	Head/neck	AURK A	Not addressed			
		Tumor T stage	Endometrium	AURK A	Survival		AURK B	Cancer risk
Liver	AURK A	Grade; stage			Aneuploidy; grade; outcome	Esophagus	AURK A	Cancer risk
					Not addressed	Lung	AURK A	Reduce cancer risk
					Not addressed	Gastric	AURK A	Risk of disease progression

preclinic models. It shows antiproliferative activity in a wide range of human tumor cell lines including lung, prostate, ovary and lymphoma cells. The most commonly observed adverse events ≥ 3 were febrile neutropenia, anemia, thrombocytopenia, neutropenia, and fatigue.

Danusertib (PHA-739358): Danusertib (PHA-739358 is a powerful inhibitor of the three isoforms of Aurora kinase. In addition to Aurora kinases, PHA-739358 has inhibitory activity against a number of other kinases with relevance as anti-cancer targets, such as ABL, RET and TRK-A. The most frequent grade 3–4 adverse events were anemia, diarrhea, and febrile neutropenia.

AT9283: AT9283 is a small molecule multiaim (multi targeted) kinase inhibitor with potent Aurora kinase. Other kinases inhibited by AT9283, with relevance as anticancer targets include JAK2, FLT-3 and ABL (T315I). Dosage-limiting toxicities included myocardial infarction, hypertension, cardiomyopathy, tumor lysis syndrome, and pneumonia.

PF-03814735: PF-03814735 is a potent, orally bioavailable inhibitor of Aurora-A and Aurora-B kinases and it also inhibits several other kinases. (E.g., FLT3, JAK2, TrkB, RET, MST3) in $\geq 90\%$ at a compound concentration of 100 nM. PF-03814735 exhibited antiproliferative activity against a number of human tumor cell lines such as HCT-116, HL-60, A549 and H125. Dose-limiting toxicities included febrile neutropenia and increased aspartate amino transferase levels.

AMG 900: AMG 900 is an orally bioavailable, potent and selective pan-Aurora kinase inhibitor. It inhibits Aurora-A, -B and -C with IC50 values of 5, 4 and 1 nM respectively and in cells shows a phenotype consistent with Aurora-B inhibition. AMG 900 showed potent antiproliferative activity against a range of human tumor cells including cells lines resistant to paclitaxel and some of the Aurora kinase inhibitors (Bavetsias and Linardopoulos 2015).

ZM447439 (ZM): is a potent and selective inhibitor of aurora-A and -B kinase with putative antitumor activity and induces apoptosis via mitochondrial. Li et al. (2010) ZM447439, a derivative of quinazoline, was identified through a high throughput campaign to be an interesting starting point for the development of aurora kinase, as it showed aurora-A inhibition. ZM447439 has been used to study the biology of aurora kinase in the initial stages of validation of aurora drug targets (Cheung et al. 2009).

24.5.6.1 Laboratory Studies of Aurora Kinases Inhibitor Effect

In a previous study, we investigate the expressions of Aurora (A, B, and C) kinases in newly diagnosed acute promyelocytic leukemia (APL) patients. We found overexpression of AURKB (Aurora kinase B) in 88% of APL patients. So, we investigated the effects of AZD1152 as a specific inhibitor of Aurora B on cell survival and DNA polidy in an APL-derived NB4 cell line. Our results showed that AZD1152 treatment of NB4 cells led to viability reduction, G2/M arrest and

Table 24.7 Inhibitors of aurora kinas

Inhibitor	Target	Best response
MK-0457	Aurora A Aurora B Aurora C FLT3 BCR-ABLT3151 JAK	Remission (leukemia) Stable disease (solid tumors)
AZD1152	Aurora B Aurora C	Stable disease (solid tumors)
PHA-739358	Aurora A Aurora B Aurora C FLT3 BCR-ABLT3151	Stable disease (solid tumors)
MLN8054	Aurora A	Stable disease (solid tumors)
AMG 900	Aurora A Aurora B Aurora C	
AT9283	Aurora A Aurora B BCR-ABLT3151 JAK2	Not available
PF-03814735	Aurora-A Aurora-B FLT3 JAK2 TrkB RET MST3	Such human tumor cell lines (E.g. HCT-116, HL-60, A549 and H125)
ZM447439 (ZM)	Aurora A Aurora B	

polyploidy induction. These giant polyploid cells displayed morphological evidence of mitotic catastrophe. It appears that inhibition of Aurora-B and polyploidy induction could be a novel anti-cancer treatment for APL that may be clinically available in the future (Ghanizadeh-Vesali et al. 2016).

Moreover, we show anti-cancer effects of AZD1152-HQPA, such as polyploidy induction, on androgen receptor-positive prostate cancer cell line-LNCaP (Fig. 24.4). In this study we showed the aneugenic action of AZD1152-HQPA via centromeric labeling by fluorescence in situ hybridization (FISH) (Zekri et al. 2015). In another study on neurological malignancy cell lines, we suggest a new resistance mechanism to AZD1152. We showed that status of p53/p73 could be a key regulator of sensitivity to AZD1152-HQPA (Zekri et al. 2016). Some of Aurora kinase inhibitors have progressed from preclinical to clinical studies and they are testing in different phases of oncology clinical trials.

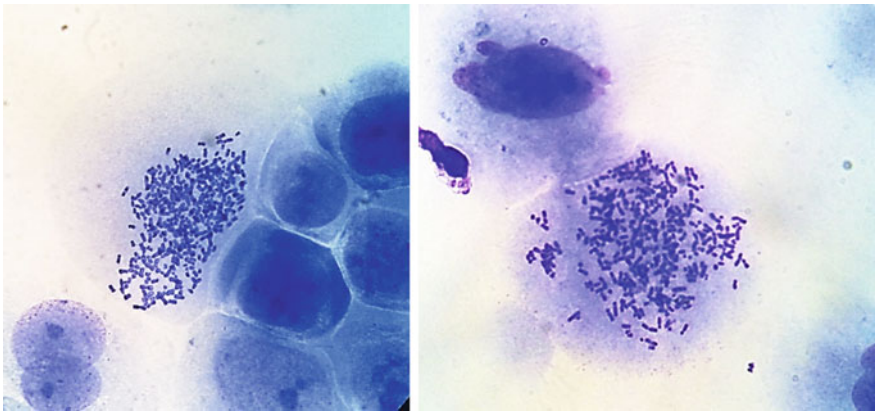

Fig. 24.4 Polyploidy induced by AZD1152. Metaphase spreads of AZD1152-treated LNCaP cells were shown here

24.6 Conclusion

Cell lines as in vitro models of disease; provide us a reliable source with the characteristics of original tissue. By use of cell lines, not only we can circumvent restrict challenges of human rights and ethics in researches, but also we able to investigate various biological aspects of disease. Moreover, we can run different probable treatments on cell lines. Today, cell lines are used in various fields including neurobiology, hematology, cancer and etc. The cancer cell lines as experimental models of cancer have special applications. Because of the cancer cell lines have characteristics of tumor origin, have an important role for recognition cellular and molecular biology of cancer, therapeutic agents and diagnostic of cancer. Similarities between tumor cell lines and their origin (i.e. Genetic heterogeneity) have resulted to use them to find a treatment for primary tumors or find mechanisms of drug resistance in recurrence of cancer. These capabilities have made cell culture techniques to be updated over the time, in order to be able provide the similar physiological conditions with special tissue in the body. All these benefits can make us hopeful for cancer treatment in the coming years.

24.7 Summary

Cancer as a challenging disease in recent century and a group of disease that including abnormal cell growth with the potential to invasion to other parts of the body and has attracted much attention. These efforts include identifying different aspects of cellular and molecular biology of cancer. Therefore, special techniques (i.e. culture of tumor cells) developed for evaluating of these aids. Under certain

conditions in a laboratory, Cancer cells keep dividing and growing over time. HeLa cell is the first human cell line successfully cloned. Using different cell line panels and other ways have seen in developmental history of evaluating anticancer agent by cell lines. Recently, Cancer cell lines are used in many researches to study the biology of cancer and to test cancer treatments. Using cancer cell lines is usually aimed at two purposes: detection of anticancer agent and evaluation of drug resistance in cancer. The systems biology approaches have been applied to panels of cell lines derived from a specific tumor type to determine significant molecular associates of sensitivity to drugs. Because of a main limitation and problem for the clinical profit derived from drug treatments of cancer is quickly acquired cancer's drug resistance. Thus, it is important to improve a better comprehension of the molecular mechanisms underlying the earnings of drug resistance.

Aurora kinases are family of serine/threonine kinases (consisting of Aurora-A, -B and -C) are an important group of enzymes that involved in some control pathways of the control point, including spindle assembly control point, alignment of metaphase chromosomes and chromosome biorientation. The aberrant expression of Aurora kinases could have a trigger role in development of tumors. As a result both preclinical and preliminary clinical trial outcomes advise that this class of agent is hopeful for cancer treatment.

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Chapter 25
Biostatistics Methods in Cancer Research: Cluster Analysis of Gene Expression Data

Fatemeh Bahreini and Ali Reza Soltanian

Abstract In recent years, gene chips have been widely applied in basic researches (e.g., identifying biomarkers/genes related to cancer). Therefore, it is important for biologists to understand the biostatistical methods used for analysis of biological data (e.g., gene expression level). There are many statistical methods to investigate factors associated with cancer. Gene mutation is one of the important factors in cancers. Microarray data is used to detect genes which have more expression in patients. Hence, modeling and classification of genes related to cancer is important. Clustering analysis is one of the capable biostatistical methods to classify genes based on gene expression level. There are many techniques for classifying genes into the clusters. These techniques have been established based on the distance between the paired observations (e.g., genes). In this chapter, we explain six distance similarity methods and two clustering algorithms.

Keywords Cluster analysis · Cancer · Gene expression

Abbreviations

FISH	Fluorescence in situ hybridization
IHC	Immunohistochemistry
HER2	Human epidermal growth factor receptor 2
p	*p*_value
DLBCL	Diffuse large B cell lymphoma
FL	Follicular lymphoma
CLL	Chronic lymphocytic leukemia

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Min Minimum
Max Maximum

25.1 Introduction

As mentioned in previous chapters, cancer is one of the main problems in the field of health care. Therefore, the identification of genetic and environmental factors associated with the disease can help preventing disease and stop its progress. Gene mutation is one of the reasons of how cancer develops; and also, genetic mutation is associated with increased gene expression (Mahdieh and Rabbani 2013). Microarrays technique is highly reliable and efficient in obtaining gene expression level.

A large number of genes are examined in microarray technique, which make modeling and classification of genes related to cancer is not easy. In this regard, a biostatistical method must be chosen by the researchers to provide valid results. Also, biostatistical methods should be accordance with aim of the study. For example, if we want to examine the effect of gene expression on patient survival, statistical methods such as Elastic Net and Lasso can be used (Jafarzadeh Kohneloo et al. 2015; Moslemi et al. 2016; Tibshirani 1997). As another example, in a meta-analysis for comparison between fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC) to detect HER2 alteration in breast cancer, it was shown that FISH is more valid than IHC (Bahreini et al. 2015). Therefore, it was clear that both genetic and statistical methods can have a significant impact on results. Hence, we explain common biostatistical methods such as cluster analysis to partition gene set.

Among the statistical methods, clustering analysis is an appropriate and powerful one in classifying genes (Johason and Wichern 2008). Cluster analysis usually obtains a pattern which can be used for partitioning observations, such as genes in distinct clusters. There are many techniques for classifying genes into different clusters (Luo et al. 2003; Shannon et al. 2003). The difference between such techniques is in calculating the similarity between the observations (i.e., dene expression) and the objective function (see Sect. 25.3.2).

In addition, most clustering techniques have been established based on the distance between the paired observations (i.e., genes). In the simplest case, when there are two genes for clustering (i.e., $p = 2$), we might be able to use the scatter plot for clustering, but, when the number of genes is greater than 2 (i.e., $p > 2$), the observations/genes must be plotted using principal component analysis (Rencher 2002).

As mentioned above, cluster analysis techniques have been used widely recently in various research fields, such as gene clustering based on gene expression

(Alizadeh et al. 2000; Baines et al. 2010; D'haeseleer 2005; de Souto et al. 2008; Kobayashi et al. 2015), genetic population clustering based on the alleles frequencies (Bahreini et al. 2012), etc.

25.2 The Goals of Clustering in the Cancer Genetics Studies

In recent years, gene chips have been widely applied in basic researches, such as identifying biomarkers/genes related to cancer. Therefore, it is important for biologists to understand the biostatistical methods used for analysis of biological data (e.g., gene expression level). There are many statistical methods to investigate factors associated with cancer, but each of them has different application areas and may act differently in determining the number of clusters (Smolkin and Hosh 2003); hence, jackknife (Yeung et al. 2001) and bootstrap (Kerr and Churchill 2001) methods were used to validate clustering in gene expression data analysis. In most microarray data set, the number of genes is much more than that of profiled samples. For this reason, we could not use conventional methods such as scatter plots to classify genes; hence, in the microarray studies, it is better if we apply a biostatistical technique for data reduction. In other words, a set of genes should be assigned to some cluster, so that the elements in each cluster become very similar. This is what biologists' researchers should be aware of.

25.3 Numerical Methods for Genes Expression Clustering in Cancer

One of the important applications of clustering analysis is genes grouping based on microarrays data. Microarray is a technique that obtains the expression level of a large number of genes within a number of different experimental samples (e.g., patients). Gene expression data are arranged in a data matrix with a few rows and columns, where each gene corresponds to a row and each sample corresponds to a column. For example, in a microarray data with n-genes and m-samples data matrix will be as follows,

$$\mathrm{M} = \begin{bmatrix} x_{11} & \cdots & x_{1m} \\ \vdots & \ddots & \vdots \\ x_{n1} & \cdots & x_{nm} \end{bmatrix}$$

where, each matrix element (i.e., $x_{ij}, i = 1, 2, \ldots, m$ and $j = 1, 2, \ldots, n$) in a gene expression matrix (i.e., M) shows the expression level of a gene for a sample. The M-matrix is unbalanced, because the number of genes (i.e., m) is much more than

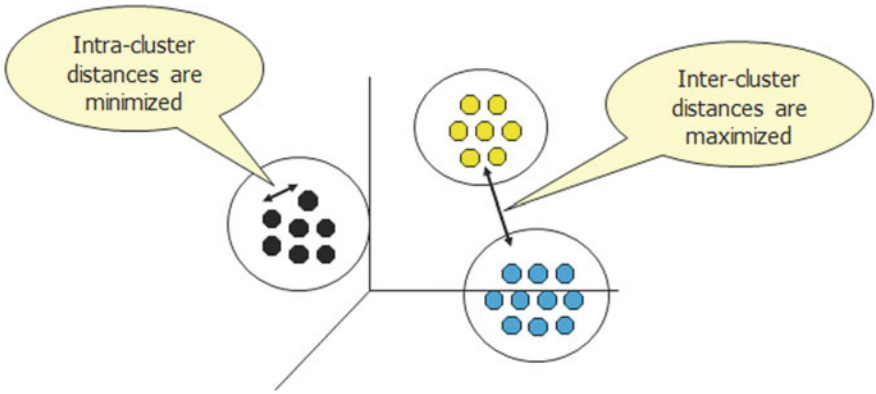

Fig. 25.1 Concept of Intra-cluster and Inter-cluster distance

the number of samples (i.e., n). It should be noted that the expression levels usually do not have normal distribution. For this reason, the logarithmic transformations may be used.

Cluster techniques classify genes into different clusters such that data within a cluster are similar (i.e., Intra-Cluster) and the clusters are separated (i.e., Inter-Cluster) (Everitt et al. 2011). Figure 25.1 shows schematically the concept of Intra- and Inter- cluster.

25.3.1 Distance Metrics for Genes Similarity

When trying to group similar genes in different clusters, we need a function that determines the distance between gene expressions. Small and great distance show similarity and dissimilarity, respectively. These obtained distances are placed in the distance matrix ***d*** which is a n-dimensional space, with n-row (corresponds to x) and n-column (corresponds to y). The distance matrix ***d*** shows the relation between x and y that has the following properties:

(i) *Symmetry*: The distance from x to y should be same as the distance from y to x (i.e., $d(x, y) = d(y, x)$)
(ii) *Positivity*: The distance between any two expression level, such as x and y should be at least equal to zero (i.e., $d(x, y) \geq 0$).
(iii) *Triangle inequality*: The distance between two expression level x and y should be shorter than or equal to the sum of the distances from x to a third expression level z and from z to y (i.e., $d(x, y) \leq d(x, z) + d(z, y)$)

For example, suppose that the following distance matrix ***d*** according to the expression levels of four genes (e.g., A, B, C and D) associated with prostate cancer

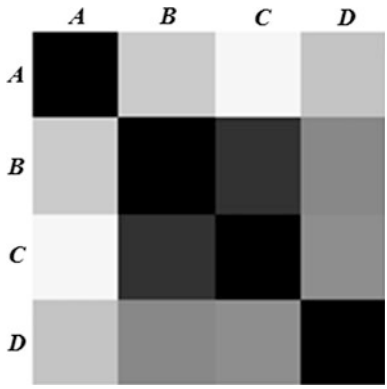

Fig. 25.2 The distance matrix schematically; where, *dark area* shows similarity (i.e., distance of 0) and the *white area* stands for dissimilarity (i.e., maximal distance)

is achieved. The method of calculating the distance between the two genes will be explained in the next sections.

$$\boldsymbol{d} = \begin{bmatrix} & A & B & C & D \\ A & 0 & 21 & 60 & 90 \\ B & 2 & 0 & 5 & 47 \\ C & 60 & 5 & 0 & 11 \\ D & 90 & 47 & 11 & 0 \end{bmatrix}$$

The distance matrix $\boldsymbol{d}$ can then be seen as a heat map which is shown below (Fig. 25.2).

25.3.1.1 Euclidean Distance

Euclidean distance or standard Euclidean distance is the simplest and most common method for calculating the distance between two genes. To calculate the Euclidean distance between the two genes at m-samples (i.e., patients), the expression of two genes have been measured.

For example, suppose two m-dimensional vectors $x = (x_1, x_2, \ldots, x_m)$ and $y = (y_1, y_2, \ldots, y_m)$ that x_i and y_i denote expression levels of A and B genes, respectively, for m-samples (i.e., patients 1, 2,…, m).

Hence, the Euclidean distance between A and B genes is,

$$\begin{aligned} d_E(x,y) &= \sqrt[2]{(x_1 - y_1)^2 + (x_2 - y_2)^2 + \cdots + (x_m - y_m)^2} \\ &= \sqrt[2]{\sum_{i=1}^{m} (x_i - y_i)^2} \end{aligned} \tag{25.1}$$

25.3.1.2 Squared Euclidean Distance

Squared Euclidean distance gives equal weight to all distances (i.e., $x_i - y_i$) while the standard Euclidean distance gives more weight to larger distances. Therefore, it is best using the former one when there are not outliers or large distances; otherwise, squared Euclidean distance and standard Euclidean distance are the same.

Similar to above mentioned example, here also suppose that two m-dimensional vectors $x = (x_1, x_2, \ldots, x_m)$ and $y = (y_1, y_2, \ldots, y_m)$ that x_i and y_i denote expression levels of A and B genes, respectively, for samples (i.e., patients 1, 2,…, m).

Therefore, the squared Euclidean distance between A and B genes is,

$$d_{E^2}(x, y) = (x_1 - y_1)^2 + (x_2 - y_2)^2 + \cdots + (x_m - y_m)^2 = \sum_{i=1}^{m} (x_i - y_i)^2 \quad (25.2)$$

25.3.1.3 Mahalanobis Distance

Mahalanobis distance has been also widely used. Mahalanobis distance between two n-dimensional vectors $x = (x_1, x_2, \ldots, x_m)$ and $y = (y_1, y_2, \ldots, y_m)$, where x and y denote the expression level of A and B genes, respectively, for samples (i.e., patients 1, 2,…, m) is,

$$d_{Ml}(x, y) = \sqrt[2]{(x - y)^T S^{-1} (x - y)} \quad (25.3)$$

where, S is the variance-covariance matrix between columns (i.e., patients) and $(x - y)^T$ is the transposition of $(x - y)$. In Mahalanobis distance, the weight matrix is obtained as the columns variance-covariance matrix.

25.3.1.4 Correlation Distance

Another technique to obtain the distance is the Pearson's correlation distance. Therefore, the Pearson's correlation distance between two m-dimensional vectors $x = (x_1, x_2, \ldots, x_m)$ and $y = (y_1, y_2, \ldots, y_m)$, where x and y respectively denote the expression level of A and B genes, respectively, for samples (i.e., patients 1, 2,…, m) is,

$$d_R(x, y) = 1 - r_{xy} = 1 - \frac{\sum_{i=1}^{m} (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^{m} (x_i - \bar{x})^2 \sum_{i=1}^{m} (y_i - \bar{y})^2}} \quad (25.4)$$

where, x_i and y_i are the expression level of A and B genes, respectively; $\bar{x}$ and $\bar{y}$ are average of x_i (i.e., the expression level of A gene for ith *sample*) and y_i (i.e., the expression level of B gene for ith *sample*), respectively.

25.3.1.5 Cosine Similarity

It is easy to show that the cosine distance is the Euclidean distance. This distance uses cosine angle between vectors instead of the gene expression; so, it seems appropriate when the data distribution is not normal. Therefore, cosine distance between two m-dimensional vectors $x = (x_1, x_2, \ldots, x_m)$ and $y = (y_1, y_2, \ldots, y_m)$, where x and y denote the expression level of A and B genes, respectively, for samples (i.e., patients 1, 2,…, m) is,

$$d_c(x, y) = \cos(\theta) \frac{x.y}{||x||.||y||} \tag{25.5}$$

where, θ denote the angle between two vectors x and y; $||x||$ and $||y||$ are the norm or the length of vectors x and y, respectively, and is calculated as follows,

$$||x|| = \sqrt[2]{\sum_{i=1}^{m} x_i^2} \tag{25.6}$$

The angle between the two vectors x (i.e., corresponding to the expression level of gene A) and y (i.e., corresponding to the expression level of gene B) schematically shown in Fig. 25.3.

25.3.1.6 Minkowski Distance

Minkowski distance is a generalization of Euclidean distance. Minkowski distance between two m-dimensional vectors $x = (x_1, x_2, \ldots, x_m)$ and $y = (y_1, y_2, \ldots, y_m)$, where x and y denote the expression level of A and B genes, respectively, for samples (i.e., patients 1, 2,…, m) is,

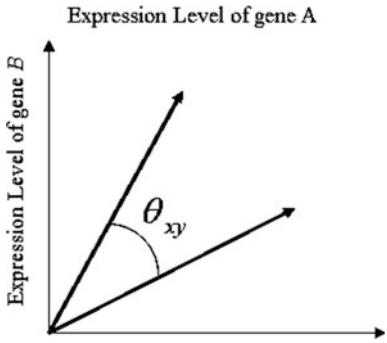

Fig. 25.3 The angle map between x and y vectors

$$d_{MK}(x,y) = \sqrt[k]{\left(|x_1 - y_1|^k + |x_1 - y_1|^k + \cdots + |x_m - y_m|^k\right)} = \sqrt[k]{\sum_{i=1}^{m} |x_i - y_i|^k} \tag{25.7}$$

For $m = 2$, the Minkowski distance reduces to Euclidean distance.

25.3.2 *Algorithm Approaches for Gene Expression Clustering in Cancer*

In cluster analysis, after use of the proper distance as explained in Sect. 25.3.1, selection of an algorithm for the iteration and clustering is required. Although there are several algorithms for clustering, only a few common algorithms are explained in this section. The clustering algorithms can be divided into three general categories, including partitional, hierarchical and model-based clustering. The k-means is explained as one type of partitional algorithm (Everitt et al. 2011) in Sects. 25.3.2.1. Also, hierarchical algorithm is explained in Sect. 25.3.2.2. To study model-based clustering see Everitt et al. (2011).

25.3.2.1 K-means Algorithm

In the partitional algorithm, the data set is usually partitioned to one or more categories, where each partition is called a *cluster* (McQueen 1967).

As shown is Fig. 25.4, the clusters should have the following conditions:

(i) Each cluster should have at least one member.
(ii) Each member should only belong to one cluster.

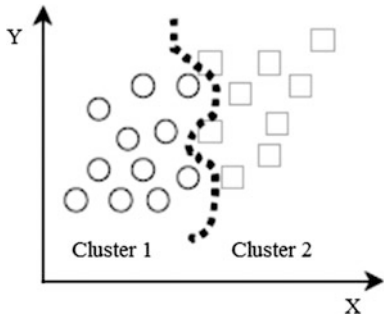

Fig. 25.4 Placement of members in each cluster schematically

As mentioned before, there are several algorithms for partitioning data into categories. K-means is one of the simplest partitional algorithm (Everitt et al. 2011; Shannon et al. 2003). In this algorithm, the first k set as the desired the number of clusters, and the second one make initial guesses for the k means (i.e., $k < n$, where k and n are the number of assume cluster and total observe, respectively). Each k is a centroid (i.e., center) of each cluster. It should be noted that determining the number of centroids (i.e., k) is difficult and one needs having enough experience. Yeung and Ruzzo have shown that principal component analysis can be applied to determine the number of clusters (Yeung and Ruzzo 2001); a deep explanation of this can be found in a study conducted by Rencher (2002). The next step is to take each point (i.e., gene expression level) belonging to a given data set and associate it to the nearest centroid. At this point, we need to re-obtain k-new centroids as average or center of clusters; and then, these points should be assigned to nearest centroid again and afterward re-formed clusters. This continues until the cluster centroids remain constant. Figure 25.5 shows a flowchart of the k-means algorithm process briefly.

Now, given a data set $x = (x_1, x_2, \ldots, x_m)$, where x_i is a m-dimensional real vector. The K-means clustering aims to partition the m observations (i.e., patients 1, 2,…, m) into k set, as called clusters. Therefore, the k-means algorithm tries to minimize the objective function as follows,

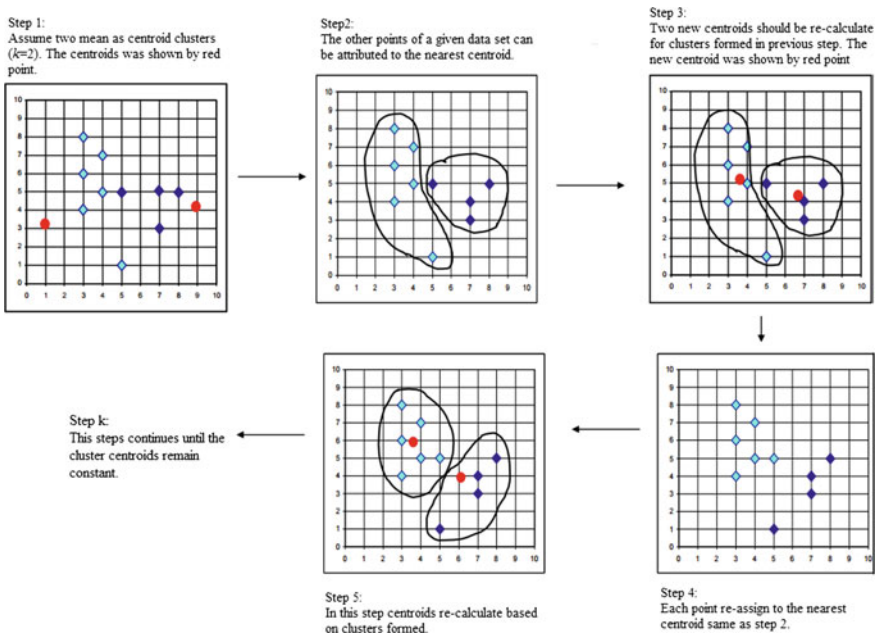

Fig. 25.5 A flowchart of the k-means algorithm

$$Q = \sum_{j=1}^{k} \sum_{i=1}^{m} \left\| x_i^{(j)} - c_j \right\|^2 \tag{25.8}$$

where, $\left\| x_i^{(j)} - c_j^2 \right\|$ is a chosen distance measured between $x_i^{(j)}$ as an observation and the cluster centroid c_j; and also, i and j represent the number of observations (i.e., patients 1, 2,…, m) and clusters, respectively.

The k-means algorithm is useful when a large database are available, because it does not have complexity in calculations. In addition, it is also useful to define the centroid of the cluster. However, it is not appropriate to be applied for the categorical data. It should be noted that k-means algorithm is not suitable for discovering clusters with complex shapes. One of the weaknesses of this algorithm is its sensitivity to outliers. In other words, outliers can easily affect the cluster centroids, and may not achieve the desired results.

For example, in a study conducted by Yeung and Ruzzo who tried to classify gene expression by the k-means clustering algorithm (Yeung and Ruzzo 2001). In addition, they were used correlation and Eucidean distance as similarity metrice. Figure 25.6a, b show the gene expression classify using the k-means algorithm based on correlation and Euclidean distance, respectively.

25.3.2.2 Hierarchical Algorithm (Graphical Method of Clustering)

The hierarchical clustering algorithm, which is the same as the k-means, is used to classify observations into k set. Unlike partitional strategy, the hierarchical clustering algorithm is gradually broken or bottom-up combined the data.

The hierarchical clustering analysis needs only similarity measures between the clusters; that has led to the k-means strategy have more acceptability among users. For this reason, this strategy has been accepted more than the k-means among the users. The hierarchical clustering algorithms shows a given data set in the form of a

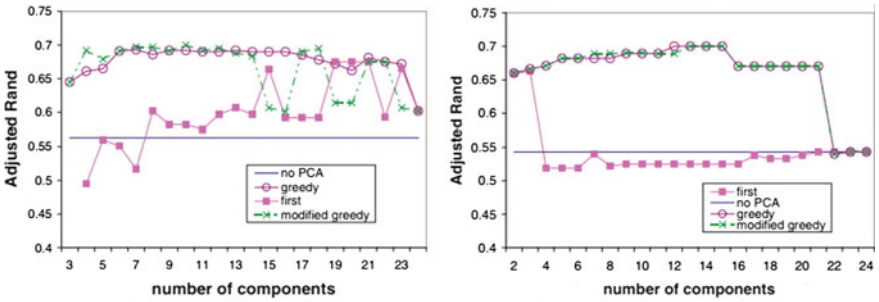

Fig. 25.6 K-means clustering algorithm

tree, which is called hierarchical tree or *dendrogram*. *Dendrogram* shows how the clusters are related to each other. If this figure is cut in the right place, we can obtain the number of clusters. The hierarchical clustering algorithms divided to two category, *agglomerative* and *divisive* approaches based on how dendrogram is formed (Everitt et al. 2011; Johason and Wichern 2008).

Figure 25.7 shows a flowchart for constructing bottom-up (i.e., agglomerative approach) and top-down (i.e., divisive approach). The agglomerative algorithm, which is a bottom-up approach, initially regards each observation (e.g., gene) as an individual cluster; while, divisive algorithm, which is called top-down approach, starts with all the observations (e.g., gene) in a single cluster, and at next steps splits a cluster using the k-means algorithm until only singleton clusters of individual objects remain (Fig. 25.7).

Agglomerative algorithm depends on different similarity distance function, that the most common of them are *single link* (i.e., distance between the closest neighbors), *complete link* (i.e., distance between the furthest neighbors), *average link* (i.e., average distance of all pattern in each cluster) and *central link* (i.e., distance of centroids).

For example, Alizadeh et al., for analysis of gene expression patterns in the three lymphoid malignancies (e.g., DLBCL, FL and CLL) used the hierarchical clustering algorithm based on similarities in genes expression (Alizadeh et al. 2000). Figure 25.8 shows that the matrix format of microarray output, where each row indicates all the hybridization results for a single cDNA element, and each column indicates the measure expression levels for all genes in a single sample (Alizadeh

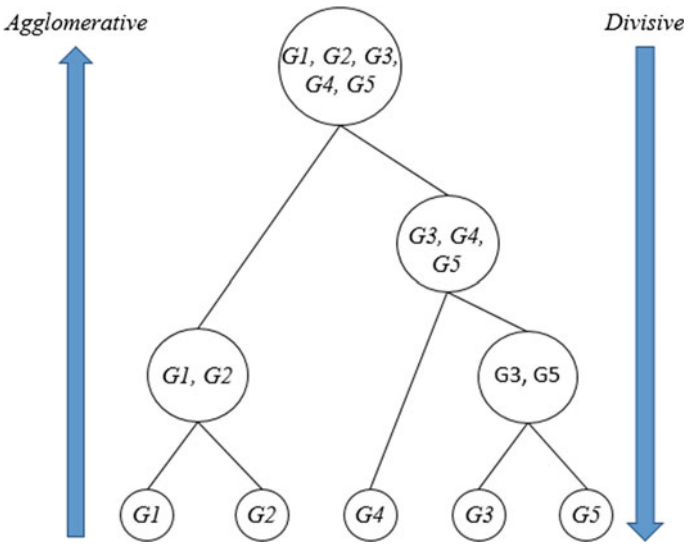

Fig. 25.7 A flowchart for constructing *bottom-up* (i.e., agglomerative approach) and *top-down* (i.e., divisive approach)

et al. 2000). In this figure, red and green color was determined based on each gene expression level is greater or less than the mean expression of all samples, respectively.

The agglomerative hierarchical approach combines the two closest clusters at each step. Therefore, we must consider the similarity or dissimilarity function for two clusters. Rencher and Johanson show different approaches to measuring similarity (or distance between clusters), such as single linkage (i.e., nearest neighbor), complete linkage (i.e., farthest neighbor), average linkage, centroid median, ward's method, and flexible beta method (Johason and Wichern 2008; Rencher 2002). We explain three common methods, including single, complete linkage, and average linkage.

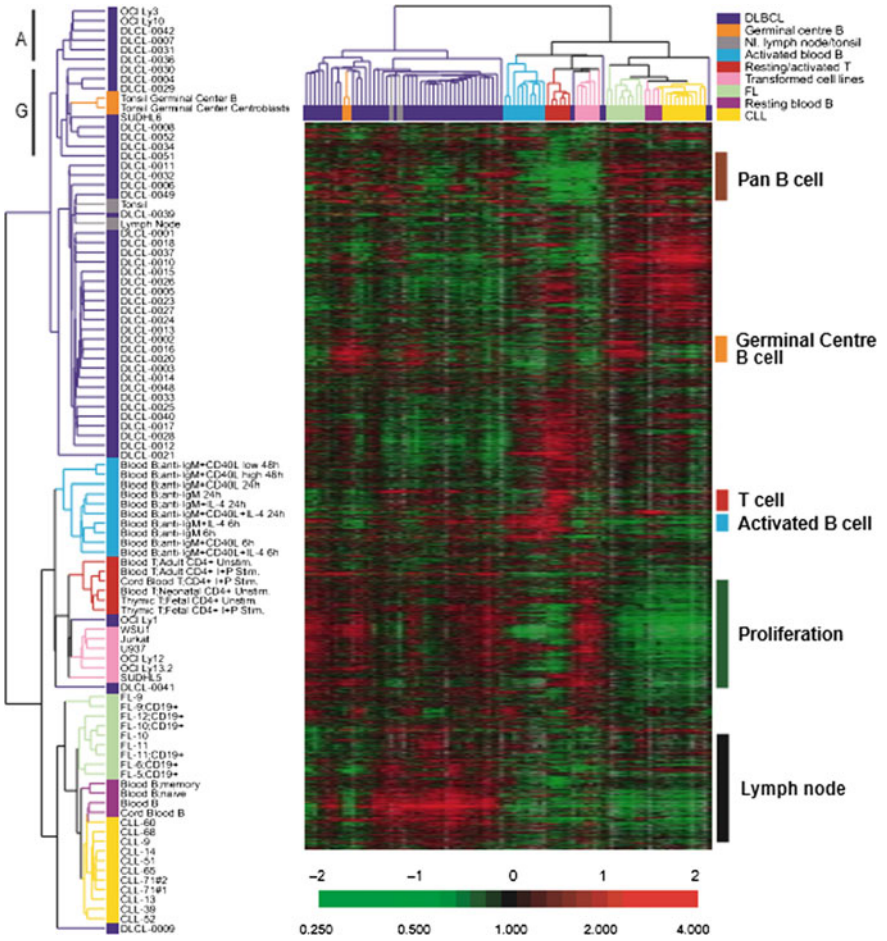

Fig. 25.8 Hierarchical clustering of gene expression data for analysis of gene expression patterns in the three lymphoid malignancies (i.e., DLBCL, FL and CLL); taken from Nature February 2000 by Alizadeh et al. (2000)

Single Linkage (Nearest Neighbor)

In the single linkage, the distance between two closest clusters C_1 and C_2 is defined as *the minimum* distance between elements (i.e., patients) in the clusters as follows:

$$D(C_1, C_2) = \min\{d(y_i, y_j)\}, \text{for } y_i \text{ in } C_1 \text{ and } y_j \text{ in } C_2 \tag{25.9}$$

where, $d(y_i, y_j)$ is the distance (e.g., Euclidean) between the vectors y_i and y_j. At each step in this method, the distance is found for every pair cluster, and then two closest clusters are merged. Two clusters have been merged would consider as a new cluster, and the procedure is repeated for the next steps.

In other word, the single linkage method computes the distance between each object pair (*i, j*); where object *i* and j are in clusters *C* and *Ć*, respectively. The distance between them is given by the measure of the smallest link between the clusters, which is shown Fig. 25.9.

The *single linkage* method is an agglomerative algorithm. The results of single linkage (i.e., *nearest neighbor* method) can be plotted using *dendrogram*.

For example, we use a part of prostate cancer data set in 2002 (Singh et al. 2002). For this purpose, we select the first 51 genes from the data set and apply the *single linkage* method to classify genes into different clusters with Euclidean distance as a measurement interval index. Note that z-score transformation was used on gene expression level before clustering. The single linkage method can classify

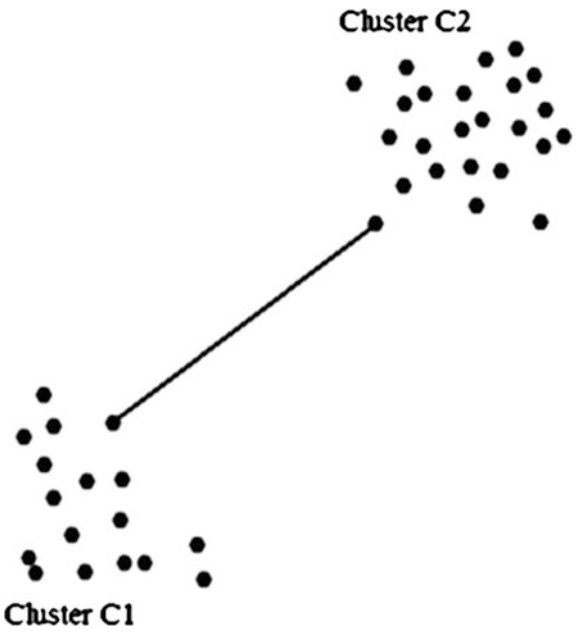

Fig. 25.9 The smallest link between the clusters *C1* and *C2*

51 genes into two clusters, as can be seen from Fig. 25.10. As shown in this figure, smallest distance is between gene 1173_at and gene 1288_s (i.e., numbers 23 and 46, respectively). Thus, genes 23 and 46 will form as a new cluster, namely, C_1. In the next step, single linkage will be conducted on C_1 and other genes (i.e., 49

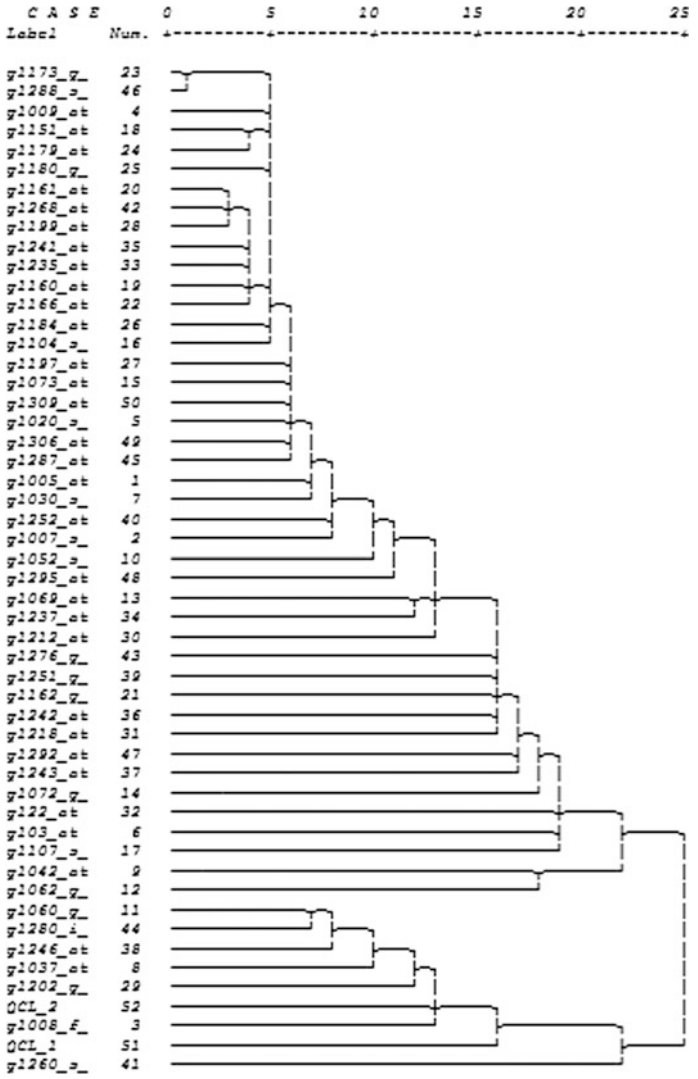

Fig. 25.10 Dendrogram of clustering process using single linkage methods with Euclidean distance. "*Label*" denote the gene's name, and "*Num.*" denote number of genes in prostate data set

genes). The procedure is repeated until the number of clusters is reduced by one cluster.

Complete Linkage (Furthest Neighbor)

The complete linkage approach, furthest neighbor, is one of the agglomerative hierarchical clustering algorithm. The complete linkage approach is based on *the maximum* distance between elements (e.g., genes expression) as follows,

$$D(C, \acute{C}) = \max\{d(y_i, y_j)\}, \text{for } y_i \text{ in cluster } C \text{ and } y_j \text{ in cluster } \acute{C} \qquad (25.10)$$

where, $d(y_i, y_j)$ is the distance (e.g. Euclidean distance) between the vectors y_i and y_j. At each step in te complete linkage method, the distance is found for every pair cluster, and then two furthest clusters are merged. Two furthest clusters have been merged would consider as a new cluster, and the procedure is repeated for the next steps. In other word, the complete linkage computes the distance between each object pair (*i, j*), where object *i* and j are in cluster *C* and $\acute{C}$, respectively; and also, the maximum value of these distances will be considered as the distance between clusters *C* and $\acute{C}$. The distance between these clusters is given by the value of the longest link between the clusters, as shown in Fig. 25.11.

Now, we use the previous data (Singh et al. 2002), and apply the complete linkage method to classify genes into different clusters with Euclidean distance as a measurement interval index. It is noteworthy that the same z-score transformation

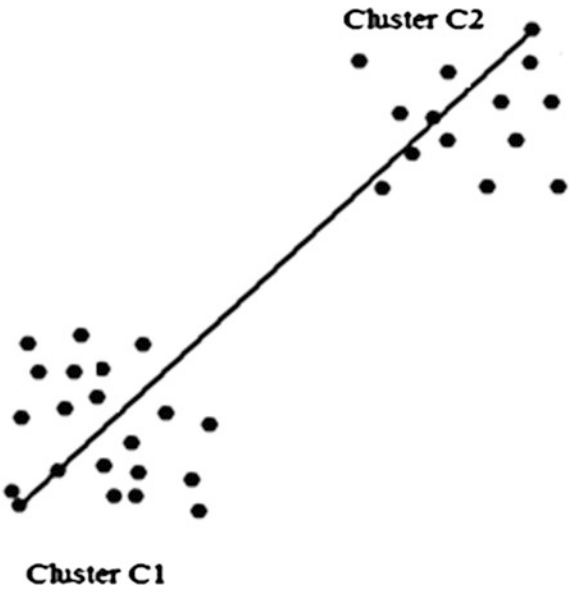

Fig. 25.11 The longest distance between the clusters *C1* and *C2*

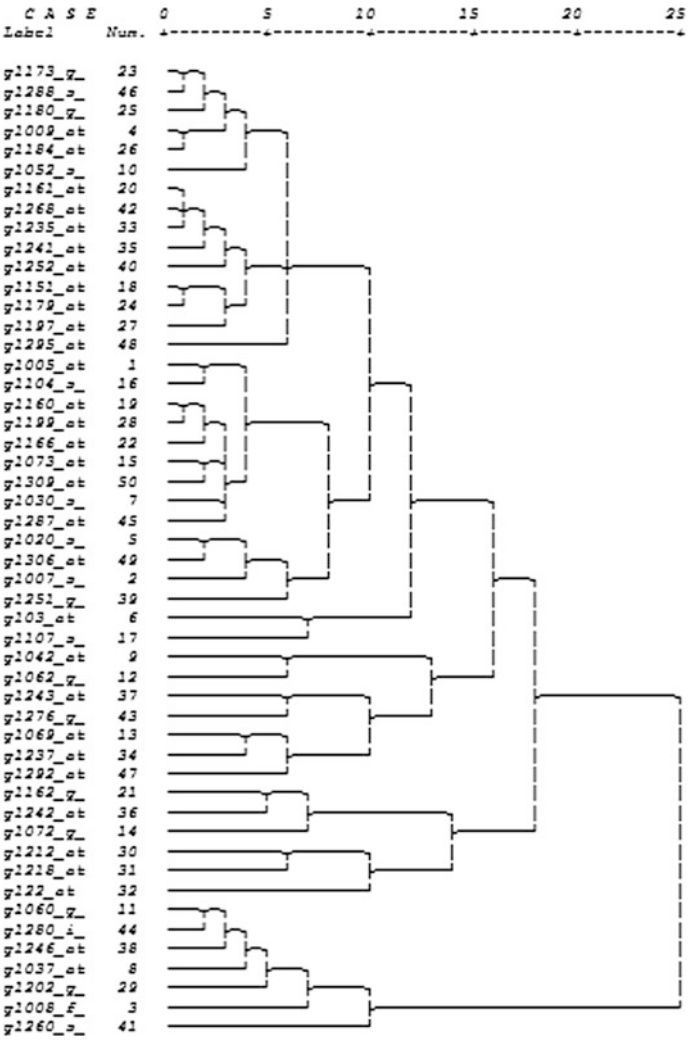

Fig. 25.12 Dendrogram of clustering process using complete linkage methods with Euclidean distance. "*Label*" denote the gene's name, and "*Num.*" denote number of genes in prostate data set

of gene expression was also used here. Figure 25.12 shows *dendrogram* for the complete linkage of the first 51 genes in prostate data set (Singh et al. 2002).

The dendrograms in Figs. 25.10 and 25.12 were set on the same data, but there are some differences between the single linkage and the complete linkage approaches. To select the appropriate method refer to Johason and Wichern (2008) and Rencher (2002).

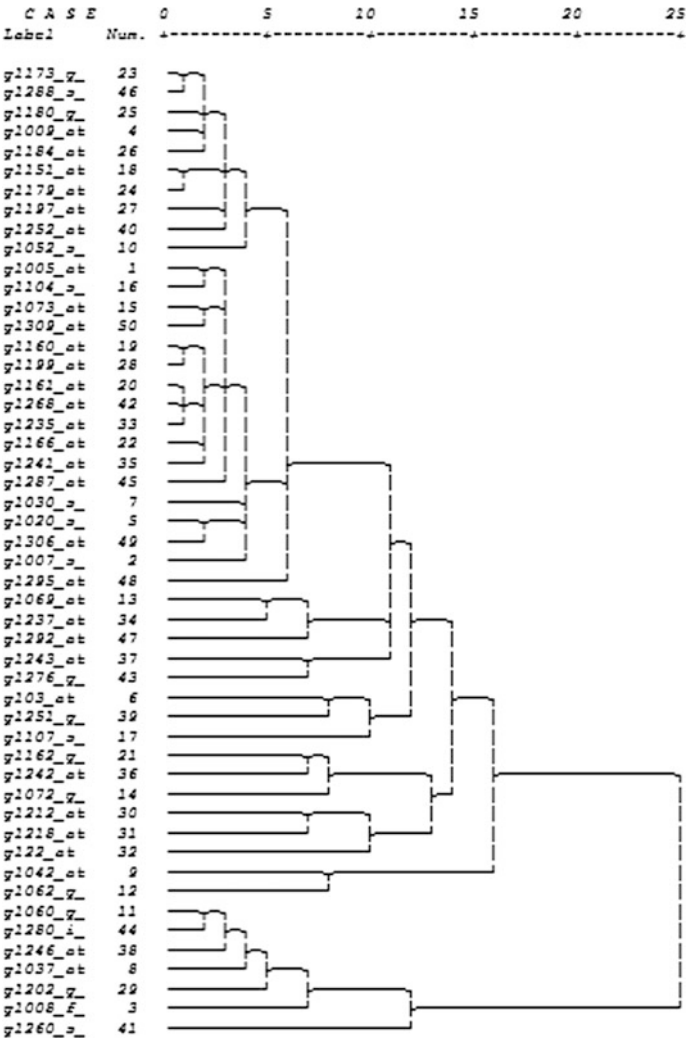

Fig. 25.13 Dendrogram of clustering process using average linkage methods with Euclidean distance. "*Label*" denote the gene's name, and "*Num.*" denote number of genes in prostate cancer data set

Average Linkage

The average linkage approach, same as the single and the complete linkage, is one of the agglomerative hierarchical clustering algorithm. The average linkage between two clusters C and $\acute{C}$ is the average distance between each point in C (i.e., y_i) and each point in $\acute{C}$ (i.e., y_j) as follow,

$$D(C, \acute{C}) = \frac{1}{n_C n_{\acute{C}}} \sum_{i=1}^{n_C} \sum_{j=1}^{n_{C\acute{C}}} d(y_i, y_j) \tag{25.11}$$

where, n_C and $n_{\acute{C}}$ denote elements in C and $\acute{C}$, respectively.

As the two previous sections, we used prostate cancer data (Singh et al. 2002) to determine gene clusters based on genes expression level. Procedure of the average linkage was presented in following *dendrogram* (Fig. 25.13).

25.4 Conclusion

As we explained, cluster analysis is a useful technique to classify genes in different clusters. Therefore, researchers will discover similar genes (e.g., genes set) which related to the cancers by cluster analysis. The method of computing the similarity distance between genes and merging algorithm of clusters to gather is different. Each of them is appropriate for certain conditions. Note that same data clustered with squared Euclidean distance compare to the standard Euclidean distance, might appear more cluster sparse.

Pearson's correlation distance is not sensitive to the differences between gene expressions. In addition, the distance also considers the trend of change. This distance can be used when the joint distribution of data is normal. Cosine distance only takes into consideration the angle between vectors and not the magnitude.

Sometimes the type of variables may be different. In this case, we propose the use of Mahalanobis distance to determine the distance between genes. Mahalanobis distance considers variance-covariance matrix as an optimal weight and this was adjusted the effect of dispersion between differences of gene expression level (e.g., x and y).

Note that genes clustering strongly depends on the distance metric; and also, various clustering algorithms applied to the same data set may produce different results. Therefore, we propose a principal component analysis to be applied in obtaining the hypothetical number of clusters before cluster analysis, so it can determine the number of clusters with minimal error.

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Chapter 26
Horizon of Cancer Genetics and Psycho-Art

Parvin Mehdipour

Abstract The bridging system through human body, by considering cancer as an unexpected event, psycho-oncogenetic and cultural heritage was briefly provide in this chapter. The vantage points of the provided chapters by contributors were highlighted which was aimed to reflect the cascade/clustering/complementary (CCC) insight. Such manner has linked the ethics as 'paving the ways' through different elements including the Scientific and Clinical channels, the Psycho-art, Psycho—Oncology, psychotherapy, Cancer Genetics, Genetic counselling, Pharmacogenetcs, and the challenging facts in different organs. The basic paradigms were also provided which include "Non-linear-system and entropy method", impact of cell line based research in evaluation of the innovative cancer drugs, and biostatistics. Furthermore, the alphabetical facts in cancer stem cells and the concerns regarding diagnostic and therapeutic application are highlighted. Besides, alternative or complementary medicine, nutrigenomics, epigenetics and pain in cancer were explored. Breast cancer metastasis as a systematic health care concern, and the major/global cause of lethality in women was also presented which require the routine and systematic personalized cancer management by considering the supportive cares.

Keywords Cancer genetics- pedigree-psycho-oncogenetic · Personalized pharmacogenetics- pharmaco-psycho-genomics · Pharmaco-psycho-somatic · Pharmaco-psycho-kinetics

Abbreviations

3D NLS	Three-dimension non-linear system
BC	Breast cancer
BCM	Breast cancer metastasis
BCBM	Breast cancer brain metastasis

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CCC	Cascade/clustering/complementary
CRC	Colorectal cancer
MCC	Molecular and cellular characteristics
MCM	Molecular and cellular machinery
PC	Prostate cancer
PPG	Personalized pharmacogenetics
PPG	Pharmaco-psycho-genomics
PPS	Pharmaco-psycho-somatic
PPK	Pharmaco-psycho-kinetics

26.1 Introduction

Beginning of a scientific aim may be the end of the previous task, and an end of the previous aim and task may be the beginning of another plan. But we need to remember that the NATURE has its own rules and restrictions.

The machinery of human body and interaction between psychological platform and different organs is not totally unmasked. Does the brain govern the human body or the heart, or both? It depends on our health status, especially when an individual is in the middle way for being affected with cancer; or in an individual who is already affected with cancer. *Cancer, Culture, and Psycho-Art* form the *Ordering* and *Re-ordering* of the natural spirit. As a matter of fact, cancer, miss-organizes the patients' soul and body. However, culture is aimed to balance the miss-ordered corners of the cancer patients' life, and psycho-art assists to harmonize and give a convincing shape to the broken pieces of the cancer patients' soul. Now the question would be 'How strong is the personalized bridge between an individual's body and soul?'

By considering privacy, cancer has its own culture and rights, therefore, requires a supportive cultural characterizations which is rather difficult to provide a global, unique and general classification and definitions. In addition, cancer as a natural complication has its own rules and regulations. Besides, cancer culture is rather diverse within different populations, so has its roots in the inherited genetic predisposition factor(s), beliefs, social and cultural habits through the multi-generations. In addition, cancer has territorial restrictions, and is also characterized with a multi-potential behaviors with an extraordinary heterogeneity and diversity. Besides, cancer relies on the vulnerability with commendable potential. But, as a matter of fact, cancer could pave its ways towards the development and progression by ignoring the natural rules. However, cancer is the unsolved puzzles which absconds from the ongoing medical managements. Therefore, the complementary and deep maneuvering in the lands of cancers, by considering the scientific and clinical strategies, is prerequisite.

Psycho-Oncogenetics is an insight for research, education, and the translational cancer medicine that pave the ways towards an appropriate social life style with a personalized management for cancer patients.

Now, is there any correlation between Cancer Genetics and Psychotherapy? This may be the first proposed question by readers. In this regard, the editing of this book was aimed to provide two different arenas of Medical Sciences, besides each other, hands in hands, as the complementary insights, for proposing questions/problems and discussing about the alternative solutions and responses. My hope was to create an atmosphere for further explorations in this dual paradigms.

26.2 Viewpoints and Highlights of Book Chapters

This section is reflective of all provided chapters at a glance with some complementary information.

This book delivers 27 chapters beginning with an introduction to pave the ways by emphasizing on Medical ethics. Chapters 1–4 deal with psychology and psychotherapy. Chapters 5–14 provide genetics, clinical and histopathological aspects in different cancers. Chapter 15 focusses on cancer stem cells. Chapters 16–17 presents the importance of immunotherapy and endocrine therapy respectively. The focal points of Chap. 18 include genetics, immunology, treatments, and psychological attention in skin cancers. Chapters 19–20 are reflective of the alternative or complementary medicine in west and east. In Chap. 21, nutrition, epigenetics and pain in cancer is explored. In Chap. 22, the metastatic breast cancer with two destinations is illustrated. In Chap. 23, cancer diagnosis by bio-resonance is provided. Chapter 24 reflects the potential of human cancer cell lines in valuing the anticancer agents. In Chap. 25, the importance of biostatistics methods in cancer research is highlighted. This chapter as a final station of this book, presents an impression of provided "An Introduction to Pave the Ways", and "Chaps. 1–25 and this chapter", which are accompanied by further complementary discussion and conclusive insights in cancer genetics and psycho-art:

An Introduction to pave the ways

Ethics, as a vital and essential issue has been presented as 'An Introduction to pave the ways'.

Ethics, as a directory channel, is the ***Breathing Gate*** for all the tasks and aims of clinicians and scientists.

In this introduction, the author has conclusively focused on (1) the traditional nature of ethics; (2) the application of Medical Ethics related to the broad spectrum of diseases including cancer, the experimental-based approaches, and other aspects of life including health and malady; (3) the importance of life style which has been influenced by "alternative medicine"; and (4) the impacts of social- and health-organizations on the medical managements of patients.

However, the applicability of medical ethics in term of rules, traditional aspects, awareness, sensitivity and other concepts of different individuals, is more

convincing to be relied on the population-based manner. So generalization of ethics' rules for all nations remain to be clarified.

Author has also emphasized on different aspects of medical ethics including the interaction between physician and patient with highlighting the personality of patients and to what they believe.

Chapter 1 "Psycho—Oncology: The Relationship Between Psychology, Personality and Cancer" presents a crossroad between psychology, personality and cancer which is directly related to variety of other medical/non-cancerous complication(s) as well.

As the matter of fact, diverse characteristics of population, cultural/social issues, environment, habits, beliefs and the applied methodological techniques lead to some limitation for providing a fruitful and supportive managements for cancer patients. In this chapter different applicable insights including "prevention, aetiology, diagnostics, treatment, and rehabilitation of cancer" were highlighted. Importantly, the standard life quality and the optimistic behaviour of cancer patients could be considered as an influential impact on the therapeutic management of cancer patients. However, better understanding of aetiology lead to achieve the directions for translating the personalized management to psycho-oncology.

Chapter 2 is reflective of the key facts about cancer psychotherapy by considering an interactive manner between the most relevant factors involved in psychotherapeutic aspects in cancer patients. They have also emphasized on the role of non-governmental organization, as the beneficial supportive bodies, in diverse populations including the Middle East and Europe. Besides, the authors have highlighted the alternative medicine, as a popular choice, among the cancer patients. The challenging applications such as internet territories are also introduced as the highpoint and popular contact with psychotherapist. However, any movements in the cancer psychotherapy would pave the ways towards improving the life style of patients who seek advice, support and assistance.

The end destination of translational strategy is capability of providing the appropriate management to the clinic. This matter was the message of Chap. 3, by focusing on the key psychological factors in cancer patients and decreasing the side effects of routine clinical managements including different treatments offered by oncologists. However, the main goal of Bridging system between psychology and oncology is a matter of harmonizing the interaction between body and soul which is rather challenging and requires an absolute complementary and systematic strategies.

Chapter 4 "Psychiatric and psychological aspects of breast cancer: Diagnosis and treatments" explores the epidemiological and clinical aspects of breast cancer by considering the psychological disorders in breast cancer (BC) patients and its impact on the patients families in depth. Regarding the therapeutic strategies including the spectrum of surgery and cosmetic surgery of breast, serious concerns by psychologists, is highlighted in this chapter. Classification and pathophysiology of depression, by highlighting its impact on the clinical managements of BC patients are also presented. Most importantly, the life style in BC patients due to different therapies either against cancer or the psychological disorder, and the

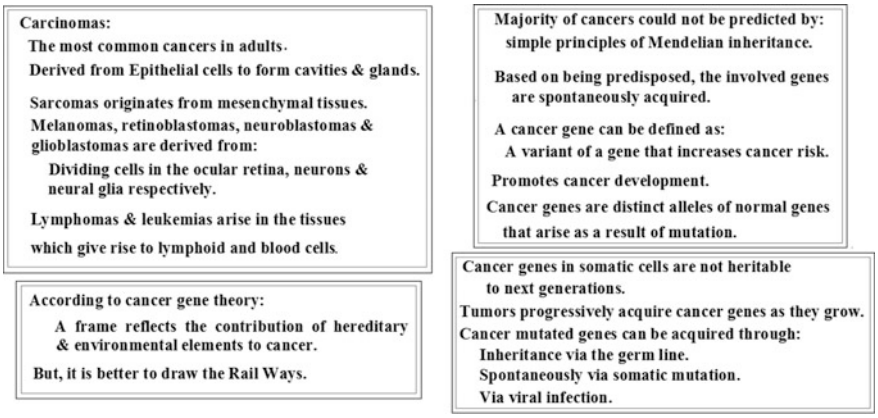

Fig. 26.1 Basic facts about cancer

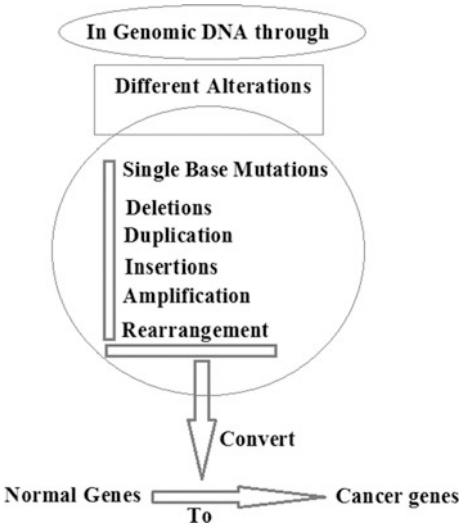

Fig. 26.2 Genes' conversion machinery in cancer

patients' clinical symptoms are explored in details. The authors have emphasized on the teamwork therapy for BC patients with the psychological disorder. In this regard, interaction between psychiatric and standard cancer therapies including hormone based drug in BC patients; and cognitive behavioral therapy are also included in this chapter. Supportive care is considered as a goal for the therapeutic aspects in BC patients by highlighting an appropriate clinical and social management to improve the patients' health and life style.

Chapter 5 focuses on "Cancer Genetics at a glance: The comprehensive insights". Cancer globe is fascinating with a heterogeneous architecture. Cancer is, fundamentally, formed from a single cell harboring a computerized programming

and capable of uncontrolled behaviors. This single cell is fertile which could produce lines of cells either similar or diverse to it. Besides, cell colonization is the source of evolution and heterogeneity. These cascade events are accompanied by different molecular and cellular scenarios through which the final influential and productive events occur. Through such alterations, the functional step would guarantee the occurrence of further evolution(s) as hit at both genomic and somatic level (Mehdipour et al. 2008).

In this chapter, the brief definitions about basic items are presented (Fig. 26.1). Besides, the involved machinery for converting normal genes to cancer genes are also provided (Fig. 26.2). In this chapter the genetic, miRNA, epigenetic etiology of different malignancies including Brain & CNS, Breast, Alimentary system, oral, and Hematopoietic disorders were explored. Besides of the role of non-coding RNAs, it also affect the gene expression including transcription, mRNA stability and mRNA translation. The importance of long noncoding RNAs is linked to cancer phenotypes. In addition, the abnormal mechanistic role of epigenetic tissue-specific gene expression patterns, and malignant cellular transformation was highlighted. The authors have classified the key cancer genes and miRNAs involved in cancer pathogenesis of mentioned cancers as Tables. Retinoblastoma and colorectal cancer with the remarkable fundamental characteristics were also highlighted. The accessibility of colorectal tumors at different stages of disease led to unmask more genetics facts by new diagnostics tools such as large-scale sequencing. However, still, more complementary/functional investigations are required to apply the personalized insight in diagnosis and clinical managements of different neoplasms. Cancer treatment is governed by many factors of those the key domain is Pharmacogenetics. This branch of Medical Genetics has also its roots in pharmacology. However, all the mentioned insights need to be accompanied by the pedigree based analysis, to present the importance of an informative pedigree for an appropriate scientific and clinical management, so, two sample pedigrees are provided (Fig. 26.3a, b). Distribution of 6 patients affected with BC through three generations including three to 5 could be considered as an informative guide to offer the genetic test for BRCA1 and/or BRCA2. As Fig. 26.3b demonstrate, the pedigree is apparently a low risk family in which only one family history as the proband's uncle was affected with gastric cancer (generation IV/3). The proband (V/14) was affected with astrocytoma. As another example, three-hit hypothesis was discovered at her genomic and somatic levels of a patient affected with astrocytoma (Fig. 26.3b). So, in specific pedigree, even with low number of family history could not reflect of non-invasiveness or severity of the molecular alteration (s), unless the required follow-up and complementary tests are performed. As this pedigree illustrates, three-hi hypothesis is reported in the proband (generation V/14) and the only family history is revealed to be the proband's uncle affected with gastric cancer (VI/3). Due to the specific molecular alterations in proband, any possible predictive test could be offered, at least to the proband's first relatives, or even to other family members at generations V to VII, through an appropriate genetic counselling by considering standard' s approaches.

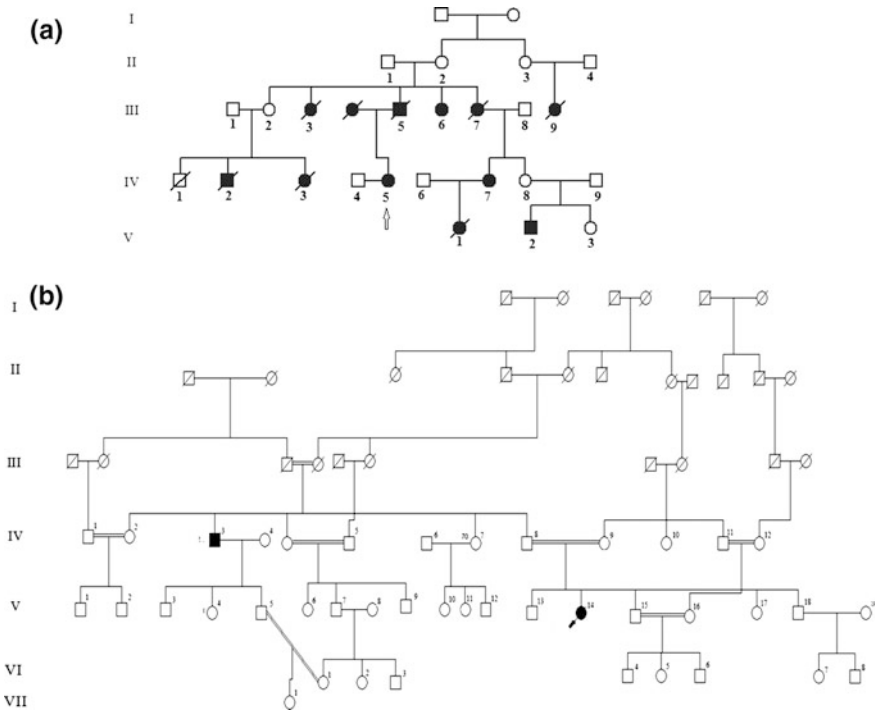

Fig. 26.3 **a** Pedigree of a high risk breast cancer family. Legend for Fig. 26.3a. III/3: affected with breast cancer at age of 55 year. III/3: affected with breast cancer at age of 62 year. III/6: affected with breast cancer at age of 31 year. III/9: affected with breast cancer at age of 34 year. IV/5: affected with breast cancer at age of 48. IV/7: affected with breast cancer at age of 50. *Arrow* is indicative of proband. This figure is adopted from P. Mehdipour's archive and Atri et al. (2002). **b** A low risk pedigree with a proband affected with astrocytoma. This pedigree is apparently a low risk family. The proband (V/14) was affected with astrocytoma. Three-hit hypothesis was discovered at her genomic and somatic (brain tumor) levels. The proband's uncle (generation IV/3) was affected with gastric cancer. *Arrow* is indicative of proband. This figure is adopted from Parvin Mehdipour's archive

Regarding the presence of multi-cancer family in a pedigree, named as BUTTERFLY, an extremely high risk family history is created by the grand-father in 2nd generation (II/2) who was a real supper carrier, but not affected with any neoplasms! And he was always supposing himself as an absolute healthy individual, led him to an unbelievable polygamy, and hoping for having more healthy births in his family (Fig. 26.4). Family history of breast cancer (BC), colorectal cancer (CRC) and prostate cancer (PC), is indicative of a typical clustering of cancers for tracing BRCA1 and BRCA1 genes. However, screening test, surprisingly, revealed to be negative for both genes. However, to clarify the nature of genetic alterations, the whole genome analysis is required to be performed. This pedigree is rather rare, but the remarkable message is to consider all standards follow-up study including genetic counselling and performance of essential

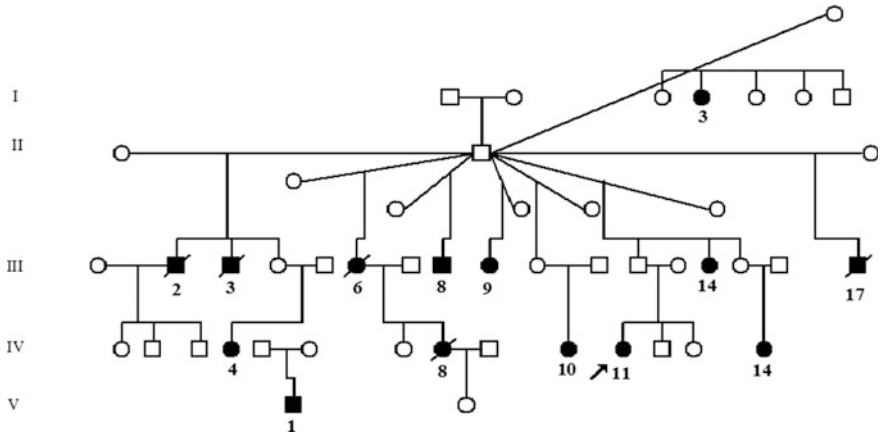

Fig. 26.4 A high risk pedigree with a BC proband and multi-cancers family history. *BC* breast cancer. I/3: affected with breast cancer. III/2: affected with acute leukemia. III/3, 17: affected with prostate cancer. III/6, 9, 14: affected with breast cancer. III/8: affected with colorectal cancer. IV/4, 8, 10, 11, 14: affected with breast cancer. V/1: affected with lymphoma. *Arrow* is indicative of proband. This figure is adopted from Parvin Mehdipour's archive

diagnostic, preventive, and predictive test(s) for target family members who are at risk of being affected with cancer.

Chapter 6 is reflective of a comprehensive exploration on the applicability of Pharmacogenetcs. The authors have emphasized on; (1) the importance of genetic complications that direct the molecular and cellular events which are directive behaviors toward clinical managements including therapy, (2) the target-based therapy with the personalized approach, the benefits of targeted- to the standard-therapy, (3) the advantage of antimutagenic ingredients, (4) gene therapy, and (5) in vivo and in vitro strategies. However, they have highlighted the fact of combination cancer therapy for fighting against cancer.

Chapter 7 provides the key elements and the required basic information about genetic counselling, molecular diagnostic test as a causal inherited susceptibility gene in the proband (propositus) who is the referral/affected patient with cancer and his or her relatives who may be candidate for performing the molecular predictive test. The author has contributed the essential information on pedigree analysis, risk assessment and further clinical interference such as prophylactic surgery. The level of risk factor and the process of pedigree analysis through the genetic counselling, by considering the ethic issue, have provided. The author has also emphasized on the basic exceptional molecular status such as "rare high penetrance gene mutations and less common variants of low penetrance", and the influential factors including style of life under environmental territories. I would like to state that environment play an significant role in carcinogenesis, as a parallel partner of genetic factors which is active through different periods of individuals 'life. Therefore, everybody may be affected with cancer, but the individuals having predisposing genetic factor

have the remarkable risk and are within the half way through the road for being affected with cancer. However, besides the availability of informative pedigree, and multi-genetic counselling through different stage of the proband and the relatives, a systematic bridging system would be created to improve the *Health Cancer Genetic* (HCG) guidelines for the benefit of cancer families and the health care systems. Besides, by accessing such system in different populations, a global bridging machinery would be created through which the interactive analyses would lead to design different meta-analysis by considering *Population Cancer Genetics* (PCG). In fact, the accessible PCG data would be the key to unmask the origin of genetic alteration and the manner of *Developmental Cancer Genetic* (DCG) through the human generations from past to present. Furthermore, the way to the future will be built up.

Chapter 8, has focused on the cancer psychotherapy and the focal point is the Airway which, dramatically, affects the patient' life style. The author has, systematically, presented a cross talk between genetic territory and psychotherapy for a possible elusive applicability for the cancer patients. Furthermore, he has emphasized on the impact of "Psychological interventions on anxiety, depression and mood disturbance".

Chapter 9 presents the bridging between gynecological cancers and the impact on the psychosocial milieu. This chapter also deals with epidemiological, histopathological, clinical aspects, diagnostic and therapeutic issues. Prevention of the reproductive system as a highlight point includes genetic counseling, screening of hereditary cancer susceptibility, HPV vaccination, performance of the routine cervical cancer. The special issue in this chapter covers "Psycho-neuroimmunological" and impact of psychosocial aspects on cancer risk and survival.

The mutation-based classification of the target cancer patients has a remarkable influence on the therapeutic strategy and clinical managements such as chemoprevention, and/or prophylactic surgery. Pregnancy and gynecological cancers, as the most complicated issue is also deliberated in which the therapy is a serious concern for the patient and physician.

The challenging matter is reflective of the impact of different therapeutic strategies during the pregnancy on the neonatal period and post-natal periods of offspring's health system.

Chapter 10 "Cancers of the endocrine system" has explored pathological/clinical classifications, Genetics diagnostic and screening aspects in endocrine system. The authors have highlighted the key facts on genetic counselling and individualized medicine as well. They have also focused on the application of next-generation sequencing by emphasizing on capability of such new tools in providing new sub-classifications of these tumors.

In Chap. 11, the authors presented an exploration on head and neck cancer (HNC) in which different aspects of diagnosis, clinical managements including regional metastasis, new advances in the field of diagnosis aids and treatment modalities, targeted chemotherapy and the interactions of practice aspects with psychosocial field with aim of improving patients' survival. This chapter also provides the brief classifications and key points of clinical and histopathological

features of HN cancers. An interactive cooperation by different specialties and considering the style of life was the main road of this chapter. The authors have provided the complementary approaches to reflect the importance of patients' education, the most appropriate care system for the patients affected with different types of HNC by highlighting an early detection, based on most informative diagnosis and most influential therapeutic strategies.

Chapter 12 "Gastrointestinal cancers" deals with classification, pathophysiology, genetics, diagnostic, counseling and management including individualized medicine in gastrointestinal (GI) cancers including esophageal, gastric and colorectal. The authors have emphasized on the application of next-generation sequencing in both research and diagnostic purposes which may lead to the molecular-based classification of patients affected with GI. Such strategy may improve the clinical managements of these patients and providing new guidelines through cancer associations.

Chapter 13 "Genetic complexity of brain tumors" presents an exploration on both molecular alterations and functional behaviors in patients affected with different types of brain tumors. Besides, the impact of incorporative and supportive psychological treatment on the overall health status and its possible interaction with efficacy of chemo- and radio- therapies were considered in this chapter. However, an urgent complementary and multidisciplinary insights are required to overcome the high rate of mortality and very low level of life expectancy in brain tumor patients under protection of *Neuro-Oncology*. Finally, protein expression is a crucial and complementary paradigm which is capable to confirm the molecular alteration either at genomic or at somatic levels. Such strategy would improve the standard of clinical management including therapeutic aspects in patients affected with brain tumors.

Chapter 14 "Genetic, Hematologic and Psychological Aspects of Leukemias" concentrates on the progressive era of fundamental genetic facts and clinical aspects of leukemias.

The authors have highlighted leukemia as a favorite domain of malignancies. I would like to accomplish their statement by emphasizing on the fact that the genetic/molecular alterations are limited in leukemia that would lead to the more reliable therapeutic aspects of this category of malignancy than solid tumors.

They have emphasized on the hematologic, molecular, cytogenetic and psychological aspects in leukemic patients by considering the importance of classification and therapy including bone marrow transplantation. The importance of an interactive panel between the nature of alterations at molecular and chromosomal levels for the therapeutic decision making is also highlighted. They have also focused on the complicated events including different therapeutic, social, and psychological disorders in leukemic patients. Interaction between psychotherapeutic managements and cancer therapies is also highlighted in this chapter. They have also highlighted the "psychological screening, education and psychosocial interventions" in leukemic patients through different stages of disease. They have emphasized on the critical role of Psycho-oncological management which, in fact, would be considered as an influential supportive care.

Chapter 15 discuss the presence of rare cancer stem cells (CSCs), as the minor malignant heterogeneous cells capable of both symmetrical and asymmetrical proliferation and tumor initiation, and self-renewal. These cells are also involved in different invasive behavior such as tumor metastasis through cooperation of "microenvironment and metabolic activity". Author has also emphasized on the chemoresistance and recurrence as two remarkable characteristic of CSCs. However, there are some concerns regarding the diagnosis of and the therapeutic aspect of CSCs.

In Chap. 16 the impact of immunotherapy in cancer patients is highlighted. Immunotherapy, as a double edged sward may influence on the health of patients' mental behaviors leading to undesirable life style. However, the chain of factors control the manner of immunotherapeutic efficiency in cancer patients. In this regard, examples are provided including Interleukin-2 and IL-4 which lead to different degrees of depression. Another available therapies include "T-cell directed immunotherapies and dendritic cell vaccine and cytokine-induced killer cell" which lead to damaging and improving the quality of patients' life respectively. However, correlation between immunotherapy and status of mental health is a challenging matter in cancer patients. The question is 'to apply or not apply'.

Chapter 17 reflects the impact of endocrine therapy in the patients affected with breast cancer and an overview of the psychotherapeutic strategy in BC patients who have received such therapy. The basic aspects of estrogen including the involved mechanisms in cell proliferation and the outcome of estrogen therapy, the molecular based resistance to endocrine therapy are also discussed in this chapter. In this regard, the involved mechanism of Tamoxifen resistance is highlighted as "over-activation of cytoplasmic cascades". However, the authors have concluded that "over—activation" of the key functional targets are the fundamental reason for the endocrine resistance.

In Chap. 18, genetics, immunological aspects, psychological complications and treatments are explored in patients affected with different types of skin neoplasms. The most known risk factors is found to be sun exposure and positive family history of cancer. The causal gene mutations including oncogenes or tumor suppressors, as well as complications such as immunosuppression due to organ transplantation were also highlighted. Besides, the functional mechanisms of these genes were found to be the main cause of the syndromes related to the skin cancers. Furthermore, the Epigenomics alterations at DNA and RNA levels, immunologic elements and many environmental hazards such as viral infections and immunosuppressive agents are also amongst the pathogenic factors of skin cancers. Regarding the clinical managements of skin cancers, the FDA approved targeted based therapies for the patients with the NMSCs and melanoma are described. In contrast, the psychological misery lead to an undesirable status of the patients' life style including the survival rate. The social discernment and isolation are another side of psychological scenario in cancer patients.

As the final words, the patients affected with cancer including skin cancers are deserved to receive the most effective and appropriate clinical managements. Besides, in case of having any sign of genetic predisposition factor through the genetic counselling, providing the adequate information regarding the most

advanced prognostic, preventive, and predictive strategies to the patients' unaffected relatives who may be within a road of being affected with cancer, which is an essential step in cancer management.

Chapter 19 deals with the history, definitions of alternative or complementary medicine and its interaction with the key relevant aspects from 19th century. Due to their inconsistent principles, the comparison with academic medicine is quite challenging item which is provided in details. The pioneer of traditional medicine and its development is also described in this chapter. The link and common element between "tradition of religious medicine" and alternative medicine is highlighted as "spiritual healing methods". Besides, the concepts of philosophy and early modern natural sciences were discussed. Furthermore, the healing insights in Asia (India and China) was later considered as an element of complementary medicine in the European health market. Author has emphasized on initiating a global link between academic (Western) and alternative medicine.

Pharmachotherapy based on traditional medicine is reflective of trusting the Nature.

Chapter 20 has focused on historical aspect and application of natural sources of anticancer agents in the field of pharmacotherapy and emphasizing on the Persian traditional medicine (TPM) in cancer. It was highlighted that complementary and alternative medicine is considered as "adjuvant therapy in line with the standard chemotherapy protocols". However, safety and efficacy of these natural agents' is two key requirements in this paradigm. The authors have referred to the TPM as an ancient medical application written by Avicenna in 11th century and by Razi in 10th century. In addition the complementary information and the anticancer agents' prescription of TPM was provided in this chapter. The preparation of medicinal plants was classified as simple or multicomponent production. Furthermore, they have considered mechanistic studies to evaluate the level of effectiveness and safety of the new drug products.

Chapter 21 "Nutrigenomics, Epigenetics and Pain in Cancer" is reflective of a comprehensive insight on pain in cancer which is a critical complication for both cancer patients and their families. The author has provided information on different involved target and mechanisms including cellular, Physiological, central nervous system and chemical based elements. Besides, the important role of epigenetic and nutrients/phytochemical in pain is highlighted. As an example, the author has highlighted the "targeting capability of Drugs or nutritional manipulations of different pathways and signaling to relive pain". The author has underlined the critical role of Curcumin by upregulation of the "antioxidant transcription factor" in order to healing the neuropathic inflammatory pain. I congregated that, the main aim of author was to drew bridges between the existing natural elements for a possible healing system against pain in cancer patients by considering the key facts in cellular/molecular biology and nutrigenomics.

By focusing on Chap. 22, breast cancer metastasis (BCM) is: (1) a systematic health care concern, and (2) globally, the major cause of lethality in women. A key problem is detection of these rare metastatic cells. Besides, due to the diverse molecular and cellular behaviors either in different tumors of the same patients, and

also in the same tumor of different patients, early detection of metastatic cells is difficult. Therefore, the personalized diagnosis, and clinical management including therapy is required. Cancer metastasis is reflective of a successful journey of cancer cells' from a primary to far distant organ. The metastatic process is derived from the interaction between multi-potential/diverse/cascade-scenarios which involves generations of cells. In this chapter, the involved machineries and the interactive processes between different territories in metastasis are, mainly, explored by illustrations. The focal points included breast cancer brain metastasis (BCBM) and breast cancer bone metastasis. The contents include the road map in metastatic BC which is supported by complementary information including the essential managements, predictive factors, interaction between the key genes, impact of stem cells and growth factors, circulating cancer cells. The significant correlations between the involved cellular and molecular targets with prognosis, prediction, and survival in BC-brain and BC-bone metastasis are highlighted. Furthermore the diagnostic application of miRNA, experimental models and strategies in cancer metastases, and target based therapy are also provided. Finally, the rapid and personalized base updates are required for managing the BCM including; (1) Classification of the cellular/molecular characteristics, (2) Bridging and unmasking the gaps at genomic and somatic levels, and (3) Innovating the most influential personalized therapy to the metastatic patients.

In Chap. 23, the importance of "Non-linear-system and entropy method" for specific evaluation of interaction between body and environmental factors. They have provided the definitions by considering the thermodynamics state for the organisms. The key points are found to be; (1) harmonization of internal targets of human body to reach a desirable health status, and (2) Continuous interaction between biological machinery and its environment. It was also highlighted that "deviation of hemostasis due to abnormalities in information itself is the primary cause of malignancy". However, they have stated that three-dimensional NLS bio-feedback system may be facilitate a non-invasive application in diagnosis and therapy of cancer. Besides, the author highlighted the preferential application of three-dimension non-linear system (3D NLS) in comparing with two-dimensional NLS at research level.

Chapter 24 highlights the impact of cell line based research in evaluation of the innovative cancer drugs. The authors have also emphasized on; (1) the developmental period of the application of different cell lines, (2) investigating the biological fact, (3) evaluation of the efficacy of therapeutic drugs against cancer, and (4) unmasking the molecular involvement related to the sensitivity of new drugs. However, the drug resistance is still an unsolved problem in cancer therapy. In final section of this chapter, the importance of Aurora kinases as a key enzyme involved in tumor development, is highlighted as the target for cancer therapeutic research.

The importance of biostatistics in biology including the functional alterations was the focal point in Chap. 25. The modeling and classifying the involved genes in cancer are the challenging items in cancer. Besides, in order to classify genes to different groups, clustering analysis is a suitable statistical method. The authors by providing different examples, have clearly explained which method is suitable to be used and how to discuss the diverse outcome of analysis.

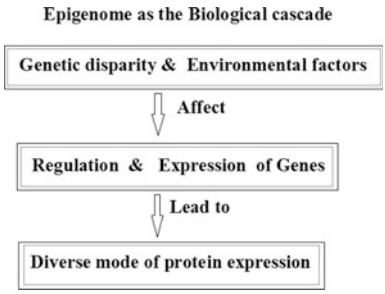

Fig. 26.5 Epigenome as a programmed cascade in cancer

26.3 Inclusive and Interactive Words and Messages

26.3.1 Encoding of the Fundamental Biological Steps

Epigenome could be considered as the Biological frame which is reflective of the programmed cascade (Fig. 26.5). As a matter of fact, environmental factors can potentially alter the epigenetic pattern of the genome through the critical periods of development, and may be at later stage of the individual's' life. Such alterations may alter the gene expression and affect the function of neural system (Fagiolini et al. 2009).

As it was previously highlighted (Franklin et al. 2010; Dietz et al. 2011; Rodgers et al. 2013; Hodes et al. 2011), the question is that whether the epigenetic characteristic due to the environmental factor would be inherited to the next generations or not. This issue was explored by us through the pedigree-based investigation in the patient affected with brain tumor and the offspring. In this study we could trace the methylation of ATM, p53 and MCPH1 genes in two generations (Karami et al. 2015).

Due to an inefficient target-based therapy for the patients with metastatic colorectal cancer (CRC), the authors have applied the combination of flavonoid morin and telomerase inhibitor MST-312 to decrease the cancer stem cell function in cell lines of CRC (Chung et al. 2016). Based on the achieved data, they have suggested that flavonoid morin and telomerase inhibitor MST-312 may be applied in cancer prognosis.

26.3.2 Therapeutic Issue

It is essential to aim for an appropriate cancer therapy which relies on many facts and factors.

Balancing and harmonizing the natural and defected different molecular and cellular characteristics (MCC) may lead to an influential and relatively harmless cancer therapy. Furthermore, the interaction between different molecular and cellular machinery (MCM) play the crucial role in the cancer therapeutic decision making.

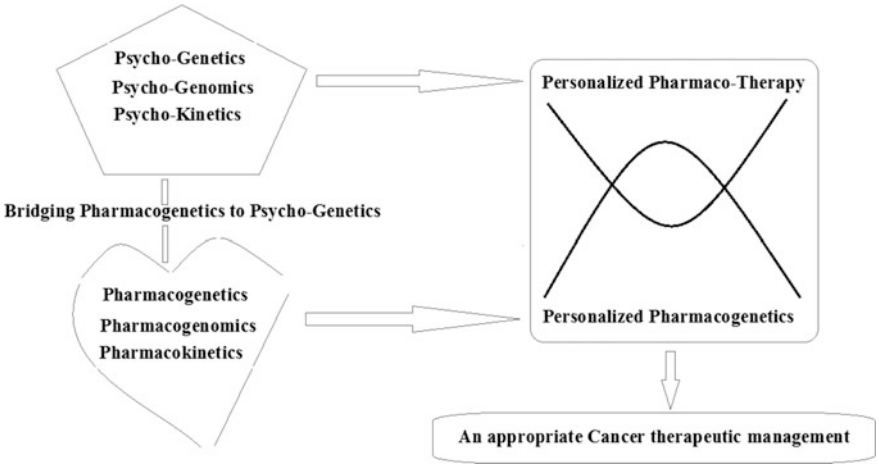

Fig. 26.6 Bridging pharmacogenetics to psycho-genetics

In this regard, it is also important to consider the psychological status of cancer patients. So, the Personalized Pharmacogenetics (PPG) Strategy, as the core, could pave the ways to design the most harmonized cancer therapy within the natural instinctive pattern of pharmacogenetic for each or at least the most homogenic and well defined and characterized group of patients in each population. So, there is a hope that cancer therapeutic manufactures' productions would be designed according to the personalized MCM- and MCC-based. The involved paradigms in pharmaco-psychogenetics is summarized (Fig. 26.6). Such considerations would be the aim of taking care for an appropriate cancer therapy.

In addition, a triangle supportive strategy including Pharmaco-Psycho-Genomics (PPG), Pharmaco-Psycho-Somatic (PPS), and Pharmaco-Psycho-Kinetics (PPK) paly the influential roles in cancer drug innovation (Fig. 26.7). It's also worth to emphasize on the fundamental point of views, the pharmaco-psycho-Genetic strategy including the elements which play key roles in the road map of diagnosis and therapy. This panel include the key elements for characterization of MCM and MCC which are two complementary cores in cancer programming. Besides, the machinery and characteristics of molecular and cellular targets may be defined as two diverse destination in cancer research. In fact the expectation of behavioral characteristics are not, necessary, reflective of the programmed and manner of machinery in different types of cancer. So, the machinery may be unable to dictate the programmed message(s) to the cellular target(s). In this case the further cellular decision relies on the clonal evolution and the pattern of heterogenic cell population(s). In addition, the micro- and macro-environmental factors have the crucial impacts on the molecular and cellular behaviors through the embryonic, fetal and the normal post-birth durations of the individuals which may be, gradually, reflective of the initiation and development of the neoplastic cells. The scenario of evolution relies on the individual's genetic makeup, inherited

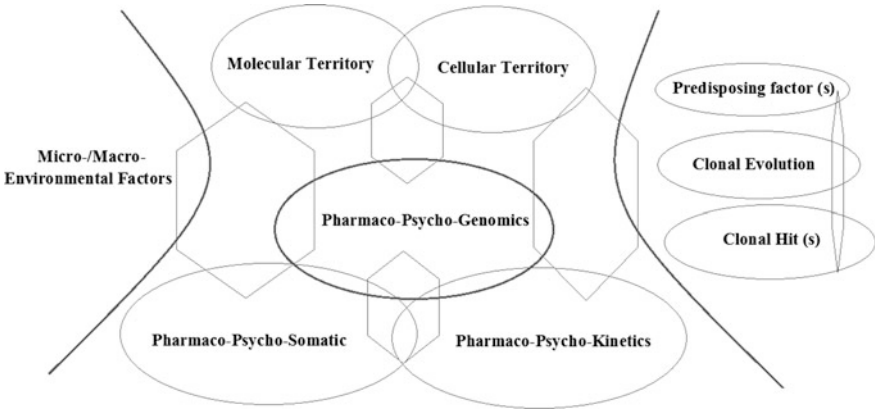

Fig. 26.7 Pharmaco- and psycho-strategy. *PPG* pharmaco-psycho-genomics; *PPS* pharmaco-psycho-somatic; *PPK* pharmaco-psycho-kinetics

Fig. 26.8 Psychological supportive care and cancer therapy

predisposing factor(s), and rarely may be followed by the complementary event(s), known as hit(s) which could be occurred at the early embryonic period (before differentiation of blood system) and/or at somatic level upon triggering the programmed destination of specific organ (Mehdipour et al. 2008). However, the events in this supportive model are characterized with the interactive system that facilitate an innovative road map of the personalized therapeutic innovation, not even in neoplasms, but in non-malignant diseases (Fig. 26.7).

The cross-talk between psychological and cancer genetics managements is rather complicated. Therefore a combined strategy for the most influential care of cancer patients who are also facing psychological disorder is essential. In this regard an illustration is provided (Fig. 26.8).

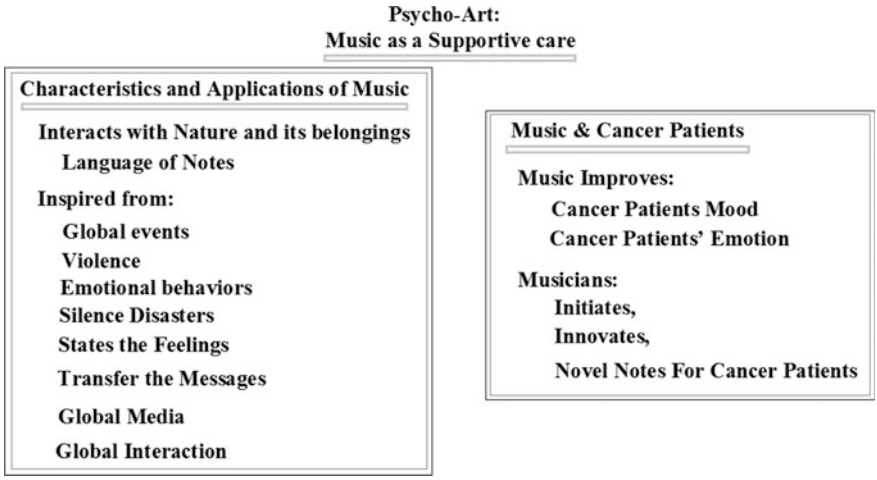

Fig. 26.9 Characteristics of music therapy

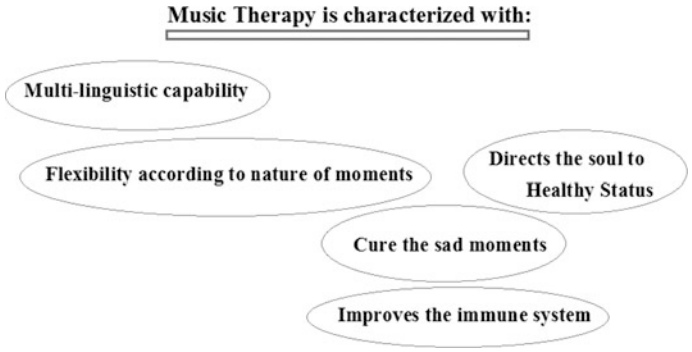

Fig. 26.10 Music as psycho-art and a supportive care

26.3.3 Music Therapy

Music as the language of "NOTES" originated from all kind of sounds and does not require any languages to be understood. Music is the only territory which has no limitation, barrier and welcome any individual from all over the world, without any restriction or rules or license to listen to it. Besides, its influential impact is globally irreplaceable. The characteristic of music and its role as a supportive and magic Art-Therapy, not only in cancer patients, but even for non-cancerous individuals is provided in an illustration (Figs. 26.9 and 26.10).

26.4 Viewpoints

Cancer world harbors the most complicated chains of events that gradually have created multi- islands which are not totally discovered. The scenarios of cancer initiation, progression, and recurrence seem to be cooperative and supportive and therefore, even the response to target- based therapy are not adequately satisfactory. Diverse cellular and molecular mechanism restrict the responsive mood of cancer patient following the application of either classic- or the innovative-therapy. So, generalization of diagnosis, and clinical managements including therapy is required to be replaced by the personalized approach. Such insight will lead to unmask the key facts involved in diverse cancer development, metastatic event, and the possible responsiveness to therapy. Through this channel, the step by step exploration enable the scientists to classify the relevant cellular and molecular behaviors for diagnostic and therapeutic aspects in different cancers by personalized approach. In this direction innovative bio-markers would facilitate the way to combat against neoplasms.

Cancer Psychology deals with the complicated lines and layers forming a spiral shape. When the psychologists enter to this atmosphere, they carry on and suddenly they found themselves at the closed section of this spiral. Then, the journey begins. There is no way to discontinue and the concerned moments keep on going with a slow motion towards the successful moments. Besides, it is important to consider the cancer family history and the genetic makeup of cancer patients for taking psycho-therapeutic into consideration.

26.5 The Further Aims and Tasks in Cancer Research Are to

- Consider traditional insight and modern technologies.
- Explore the natural history, causes, mechanisms, and related elements in different cancers.
- Cover a wide range of applicable disciplines.
- Target the unmasked interactive facts.
- Spread the standard- based data in a broader sense.
- Take the available and remarkable opinions into consideration.
- Share the expertise triumphs for developing and/or enhancing the intelligent capability of researchers amongst young generations.
- Welcome new thoughts, ideas, findings, experiences and hypotheses.

As final words, these opinions may be the fundamental vision for the distinction and excellence of quality in cancer research.

26.6 Supportive Elements

By considering music as a supportive tool for cancer patients, the most influential music for minimizing the senses' gaps and improving the feelings of cancer patients, seems to be an appropriate music selection by considering: (1) Cancer patients' characteristics including the basic/past psychological mood, (2) Cancer type/clinical features, (3) Population/traditional based, (4) Believe based, and (5) Personalized/innovative based.

26.6.1 Tails

Reality or as a tail, the following texts are reflective of thoughts and believes of individuals who are in the middle of being affected with cancer or from those who are diagnosed as the cancer patients. However, the tails may be useful as the experiments in the field of Psycho-oncology.

26.6.1.1 Episode 1

Surprise includes misery and joy, either occurs, that is *SURPRISE* and we have to tolerate it. By the way, toleration is a powerful tool against all events which occur through our life. Since my childhood I had experienced many surprising moments with the positive impacts on my memory. I had the opportunity to spend hours on the top of a Quince tree to play with my thoughts. It was a wonder land for me in which I would be able to develop my dreams and plans for a cloudy future. There was no disturbing moments in my sweet life, no concern, no anger, and no misery. The curiosity moments were always persuasive which made my life; an important question was about the roots of my tree, I was wondering how far the tree's root move forward and backward? Are they frightening? Do the roots disturb the earth or beyond its territory? In fact, I have frequently dreamed about the roots within a huge atmosphere covered by these roots, it was somehow fascinating. The time passed and I had to visit a doctor to check my breast for an uncomfortable feeling in my both breast at age of 25 years old. Immediately, my childhood memories, about the roots of my tree, was refreshed and I tried to cooperate with this undesirable and frightening event in my life. I tried to be optimistic and occupied myself with desirable memories of my childhood. *The growth of roots are not always frightening!*

26.6.1.2 Episode 2

That was a sunny day and suddenly I have realized that there is a spring in my left breast, then I remembered this phrase: "I Spring, like spring, in spring, at spring".

Day have passed and I gradually learned that I must get used to this situation, it was just a drop of liquid similar to water which surprised me every day at age of 38 years! Then I decided to arrange an appointment with a clinic, they passed me to a surgeon and the story began. However after performance of different tests and all essential clinical assays, diagnosis has been announced as' Cancer' which was a misery for my brain and heart. Till that moment I had no idea what so ever that brain and heart could be so close friends. However, I let my brain to govern my heart which was a beneficial sympathy and great moral support for me to understand the whole scenario and coop with this gloomy situation.

Besides, I have decided to keep this event as a secret which was the worse choose in my life. It is so hard not having the relatives' supports. By passing two months, I did change my mind and informed my family, close relatives and friends. Since then, I felt happier and stronger to fight against cancer, the event which made me more flexible. I tried to improve my life style by choosing the appropriate alternatives, considering the available standards in nutrients and avoiding the environmental hazards as much as possible; so the message is 'Pay attention and read careful, calculate properly, and then decide to use the products'.

26.7 Conclusions

As the final words, everybody irrespective of being apparently healthy or is in the middle of the way for being affected with any type of neoplasms, or the individuals who are already affected with cancer, are required to EDIT their style of life and habits including nutrition. Revising the life style is a key message for living healthy.

The message of present chapter is not just reflective of the facts about Science and Medicine, but it rather highlights the essential parallel elements in the patients' routine life as well. For instance the importance of ART at all corners of the cancer patients' atmosphere, their relatives and friends. It seems that there is a deep interaction within the galaxy, or even, beyond this territory. So, the magic healing elements are hardly achievable. How would we find disturbing elements which restricts our way towards a wonder world without cancer? And, if we unmask these facts, would we be capable to combat against these elements? Upon this frame, it was my aim and task to invite contributors with diverse expertise to provide an architected information by possible fulfilling the gaps in cancer genetics and psychological aspects.

Regarding genetic counselling and clinical managements, the optimal aim and task is not just to apply the most influential clinical managements for the cancer patients, but is also to consider the predictive and preventive strategies, most importantly, an early detection within the probands' pedigrees, at least, for the first degree relatives. The final concern is whether pinnacles on the ladder of cancer care accessible? May be, through the global sharing movements and applicability of the translational outcomes to the cancer clinics. So, global aiming in cancer, requires personalized and bridging strategies in research and clinical territories.

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