Cancer and Chemoprevention: An Overview

Summya Rashid

Springer

Cancer and Chemoprevention: An Overview

Summya Rashid

Cancer and Chemoprevention: An Overview

Springer

Summya Rashid
Department of Toxicology
Jamia Hamdard
New Delhi
India

ISBN 978-981-10-2578-5 ISBN 978-981-10-2579-2 (eBook)
DOI 10.1007/978-981-10-2579-2

Library of Congress Control Number: 2017946077

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Dedicated to My Loving Family

Acknowledgment

In the name of Almighty, the most Beneficient and the Most Merciful

Then which of the favours of your Lord will you deny? (Surah Ar Rahman, 14:34).

I first of all thank Almighty for His Unlimited blessings in every form and in every domain of my life.

One must be thankful to his fellow beings first and then he can be thankful to Almighty (Anonymous).

My heartfelt gratitude to my parents for their true love, noble guidance, and sincere efforts that shaped me to do something meaningful in my life. My brothers are the Noor of my eyes, the pillars of my strength, confidence, and determination. I thank my uncle Syed Zulfikar Ali and his family: Shahnaz baji; Musavir; my princesses Azra, Zara, Anusha, Zikra, Laiba; and Prince Mohammad; Ahmad; and Adeem. My family is an epitome of my strength. I feel lucky to avail this opportunity to thank them all for their unconditional love, care, and overwhelming support, for always believing in me, understanding me, and standing beside me as pillars of my existence. I owe them my everything.

In my life I believe "Behind Every Successful Daughter there is a father"

Last but not least, I must be thankful to my Uncle Mr. Syed Zulfikar and family for their love, compassion, and warmth and where I found my second home. Thank You Baba and Aunty and family for everything.

I feel happy to thank Dr. Sarwat Sultana, my Ph.D. mentor, for making me an independent researcher. Special thanks to my friends Dr. Iram Mushtaq, Dr. Sana Maam, Dr. Nayeema Maam, and Dr. Samina Bashir who serve as anti-stress agents in my professional and personal life. Special thanks to my junior Mr. Abul Vafa who is equivalent to a sibling to me for his help when needed.

I must thank Springer Nature for providing me this golden opportunity to write a book. I will never forget the cooperation and help from the Springer staff, namely, Dr. Bhavik and Ms. Saanthi for bearing with my prior commitments and warlike conditions in my hometown, Kashmir.

Lastly, I must mention my best friend and Sister Dr. Rafia who is a source of inspiration to me in terms of truth and purity she holds within.

They all are God's blessings in my life. May Lord grant them a peaceful, successful, and healthy life. Amen.

I thank everyone who I came across in my life for every person I met, I surely learnt a little from them.

Sumayah Rashid

Contents

1 Introduction 1
References 2

2 Hallmarks of Cancer Cell 3
Sustaining Proliferative Signaling 3
Evading Growth Suppressors 5
Resisting Cell Death 5
Enabling Replicative Immortality 6
Inducing Angiogenesis 7
Activating Invasion and Metastasis 7
Enabling Characteristics and Emerging Hallmarks 8
An Enabling Characteristic: Genome Instability and Mutation 8
An Enabling Characteristic: Tumor-Promoting Inflammation 10
An Emerging Hallmark: Reprogramming Energy Metabolism 10
An Emerging Hallmark: Evading Immune Destruction 11
References 11

3 Classification of Cancer 15
References 16

4 Factors Affecting Cancer Development 17
Age and Hormonal Status 17
Family Record 17
Genetic Predisposition 18
Extrinsic Factors 18
Radiation and Cancer 18
Viruses and Cancer 18
Role of Free Radicals 19
References 19

5 Carcinogenesis 21
Stages of Carcinogenesis 21
Initiation 22
Promotion 23
Progression 24
References 25

6 Molecular Mechanism of Carcinogenesis 27
Oncogenes 28
Tumor Suppressor Genes 28
References 29

7 Oxidative Stress-Induced Carcinogenesis 31
Oxidative Stress 31
Oxidative Stress-Induced Carcinogenesis 33
References 33

8 Major Mediators Linking Inflammation and Cancer 35
Cytokines 37
Tumor Necrotic Factor-α (TNF-α) 37
Interleukin-6 (IL-6) 38
Nuclear Factor Kappa-B (NFkB) 39
Cyclooxygenase-2 (COX-2) 41
Inducible Nitric Oxide Synthase (i-NOS) 42
References 44

9 Apoptosis 47
Intrinsic Pathway 48
Extrinsic Pathway 49
References 50

10 Chemoprevention 51
Characteristics of an Ideal Chemopreventive Agent 54
References 55

11 Classification of Chemopreventive Agents 57
Chemopreventive Agents 57
Blocking Agents 57
Suppressing Agents 59
Chemoprevention Encompasses Three Types 60
Tertiary Chemoprevention by Suppressing Agents 60
Metastasis Blocked by Phytochemicals 61
References 62

12 Preclinical Development of Chemopreventive Agents for Chemoprevention 65
Modulation of Phase I and II Enzymes by Chemopreventive Agents . . . 65
Antioxidant Activity of Chemopreventive Agents 66
Anti-inflammatory Action of Chemopreventive Agents 67
Modulation of Cell Signaling Pathways by Chemopreventive Agents . . . 67
Inhibition of NFκB Signaling Pathway by Chemopreventive Agents . 68
Inhibition of AP-1 Activation Pathway by Chemopreventive Agents . 68
Induction of Apoptosis and Cell Cycle Arrest 68

Inhibition of Sustained Cell Proliferation and Initiation of Apoptosis by Chemopreventive Agents 69
Modulation of Multidrug Resistance by Chemopreventive Agents 69
Modulation of Angiogenesis by Chemopreventive Agents 70
Regulation of Cell Cycle Regulatory Proteins by Chemopreventive Agents 71
Chemopreventive Agents as Chemosensitizers and Radiosensitizers. . . . 71
Possible Targets for Chemoprevention: MicroRNA 72
Nano-chemoprevention: Another Evolving Strategy 72
Translational Application of Chemoprevention 73
Outline. 74
References. 74

13 Modulation of Wnt/β-Catenin Signaling by Chemoprevention 79
Wnt/β-Catenin Signaling and Cancer 80
Wnt/β-Catenin Signaling and Melanomas 81
Wnt/β-Catenin Signaling and Colorectal Cancer 82
Wnt/β-Catenin Signaling and Prostate Cancer (PC). 83
Wnt/β-Catenin Signaling and Breast Cancer 83
Wnt/β-Catenin Signaling Targeting by Naturally Occurring Compounds 84
References. 87

14 Modulation of Akt/mTOR Pathway Signaling by Chemoprevention. 93
mTOR Signaling Pathway and Cancer 96
Akt/mTOR Signaling in Cancer 97
Role of Fisetin in Akt/mTOR Signaling Associated in Various Cancers 98
References. 101

15 Novel Chemopreventive Agents. 105
General Structure and Classes of Dietary Polyphenols 105
References. 107

16 Chemoprevention by Resveratrol 109
Resveratrol in the Chemoprevention of Colon Cancer 109
Resveratrol in the Chemoprevention of Skin Cancer 110
Resveratrol in the Chemoprevention of Breast Cancer. 110
Resveratrol in the Chemoprevention of Prostate Cancer 110
Resveratrol in the Chemoprevention of Hepatocellular Carcinoma (HCC) 111
Resveratrol in the Chemoprevention of Pancreatic Cancer 111
Resveratrol in the Chemoprevention of Gastric Cancer 111
Resveratrol in the Chemoprevention of Lung Cancer. 112
Resveratrol and Metastases. 112
References. 113

17 Chemoprevention by Genistein 115
Modulation of HCC Cells In Vitro by Genistein 117
Modulation of HCC In Vivo by Genistein 118
Modulation of Gastric Cancer by Genistein via Epidemiological Data 118
Modulation of Gastric Cancer by Genistein In Vitro System 118
Modulation of Lung Cancer by Genistein via Epidemiological Data 119
Modulation of Lung Cancer by Genistein In Vitro 119
Modulation of Colorectal Cancer by Genistein via Epidemiological Data 120
Modulation of Colorectal Cancer by Genistein In Vitro Data 120
Modulation of Colorectal Cancer by Genistein In Vitro 120
Modulation of Breast Cancer by Genistein via Epidemiological Data 121
Modulation of Colorectal Cancer by Genistein In Vitro 121
Modulation of Colorectal Cancer by Genistein In Vivo 121
Modulation of Colorectal Cancer by Genistein in Clinical Trials 122
References 122

18 Chemoprevention by Apigenin 129
References 130

19 Chemoprevention by Curcumin 133
Chemoprevention of Esophageal Cancer by Curcumin 135
Chemoprevention of Esophageal Cancer by Curcumin 136
References 136

20 Chemoprevention by Fisetin 139
References 141

21 Chemoprevention by Epigallocatechin-3-Gallate (EGCG) 143
References 149

22 Chemoprevention by Graviola 153
References 155

23 Cancer Chemoprevention: Hurdles and Future Prospects and Considerations 157
Future Prospects 159
References 160

24 Clinical Trials of Chemoprevention 163
Important Negative Trials 165
References 166

25 Future of Chemoprevention 169
References 172

List of Abbreviations and Symbols

5-FU	5-Fluorouracil
ABCG2	ATP-binding cassette subfamily G member 2
AhR	Aryl hydrocarbon receptor
ANOVA	Analysis of Variance
APAF-1	Apoptotic protease activating factor 1
APC	Adenomatous polyposis coli
ARE	Antioxidant response element
ATM	Ataxia telangiectasia mutated
b.wt.	Body weight
BMDCs	Bone marrow-derived dendritic cells
CaP	Prostate cancer
CAPE	Caffeic acid phenethyl ester
Cdc2	Cell division cycle protein 2 homolog
CDKs	Cyclin-dependent kinases
CDKIs	CDK inhibitors
CENPF	Centromere protein F
COX-2	Cyclooxygenase
CRC	Colorectal cancer
CYPs	Cytochrome
DEN	Diethylnitrosamine
dFMGEN	7-Difluoromethyl-5,49-dimethoxy genistein
DIABLO	Direct IAP binding protein with low pI
DIM	3, 3-Di-indolylmethane
DMBA	7,12-Dimethylbenz(a)anthracene
DNA	Deoxyribonucleic acid
DNA-PK	DNA-dependent protein kinase
EC	Epicatechin
ECG	Epicatechin-3-gallate
ECM	Extracellular matrix
EGC	Epigallocatechin
EGCG	Epigallocatechin-3-gallate
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EMT	Epithelial–mesenchymal transition

ER	Estrogen receptor
ERK	Extracellular signal-regulated protein kinases
ERK	Extracellular signal-regulated protein kinases
FAD	Flavin adenine dinucleotide
FAK	Focal adhesion kinase
FoxM1	Forkhead box protein M1
FRAP	FKBP12-rapamycin-associated protein
G3BP1	Ras-GTPase-activating protein SH3 domain-binding protein 1
GCSCs	Gastric cancer stem cells
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Reduced glutathione
GSK-3b	Glycogen synthase kinase-3b
GSSG	Oxidized glutathione
GST	Glutathione S-transferase
GST-pi	Glutathione S-transferase (placental type)
GSTs	Glutathione S-transferases
GTPs	Green tea polyphenols
H. pylori	*Helicobacter pylori*
H_2O_2	Hydrogen peroxide
HGF	Hepatocyte growth factor
HO-1	Heme oxygenase-1
HSP	Heat shock protein
i.p.	Intraperitoneal
I3C	Indole-3-carbinol
IFN-γ	Interferon gamma
IFS	Isoflavone synthase
Ig	Immunoglobulin
IGF-1	Insulin-like growth factor
IKK	Inhibitory kappa B kinase
ILs	Interleukins
ILK	Integrin-linked kinase
I-NOS	Inducible nitric oxide synthase
IRF-1	INF regulatory factor-1
ISRE	IFN-stimulated response element
JNK	JUN-terminal kinases
KIF	Kinesin-like protein
KIM-1	Kidney injury molecule-1
LDH	Lactate dehydrogenase
LMB	Leptomycin B
LOX	Lipoxygenase
LPO	Lipid peroxidation
M	Molar
mab	Monoclonal antibody
MAPK	Mitogen-activated protein kinases

mAR	Membrane androgen receptors
MDA	Malondialdehyde
MDR	Multidrug resistance
MITF-M	Microphthalmia-associated transcription factor-M
mM	Millimol
MMP	Matrix metalloproteinases
MPO	Myeloperoxidase activity
mTOR	Mammalian target of rapamycin
n mol	Nanomole
NADPH	Nicotinamide adenine dinucleotide phosphate reduced
NAG-1	NSAID activated gene-1
NFκB	Nuclear factor kappa B
NHBE	Normal human bronchial epithelial
NO	Nitric oxide
NOS	Nitric oxide synthase
NQO1	NAD(P)H:quinone oxidoreductase-1
NSAID	Nonsteroidal anti-inflammatory drug
NSCLC	Non-small cell lung carcinoma
NTA	Nitrilotriacetic acid
O_2	Superoxide radical
OH	Hydroxyl radical
PAK-1	p21-activated kinase 1
PCNA	Proliferating cell nuclear antigen
PDGF	Platelet-derived growth factor
PDK	Phosphoinositide-dependent kinase
PI3K	Phosphatidylinositol-3-kinase
PI-9	Proteinase inhibitor 9
PKC	Protein kinase C
PPAR	Activating peroxisome proliferator activator receptor
PSA	Prostate-specific antigen
PTEN	Phosphatase and tensin homolog
r.p.m.	Revolutions per minute
Rb	Retinoblastoma
RCC	Renal cell carcinoma
ROS	Reactive oxygen species
RTKs	Receptor tyrosine kinases
S.E.	Standard error
SCF	Skp1-cullin 1-Fbox protein
SCLC	Small cell lung cancer
SGK1	Serum and glucocorticoid-regulated kinase 1
SITEP	Short-term intermittent therapy to eliminate premalignancy
Skp2	S-phase kinase protein 2
Smac	Second mitochondria-derived activator of caspase
SOD	Superoxide dismutase
STAT	Signal transducer and activator of transcription

TBA	Thiobarbituric acid
TCA	Trichloroacetic Acid
TCF	T-cell factor
Lef	Lymphoid enhancer-binding factor
TGF-β	Transforming growth factor-β
TNFR-1	TNF receptor 1
TNF-α	Tumor necrosis factor-α
TPA	12-O-Tetradecanoylphorbol-13-acetate
TRAMP	Transgenic adenocarcinoma of the mouse prostate
TSC	Tumor suppressor complex
uPA	Urokinase plasminogen activator
VEGF	Vascular endothelium growth factor
α	Alpha
β	Beta
γ	Gamma
μg	Microgram
μM	Micromole

About the Author

Dr. Summya Rashid did her PhD from the Department of Medical Elementology and Toxicology at Jamia hamdard University, New Delhi, India, and currently, she is working as a research associate at the same place. Dr. Rashid has primarily worked on renal cell carcinoma and its various aspects. She possesses more than 5 years of research experience in the field of cancer biology and has contributed in 23 publications with h-index of 7. Dr. Rashid has attended various national and international conferences and workshops. In 2015, she was invited as honorary speaker at the "International Conference on Antimicrobial Agents and Chemotherapy" at Valencia, Spain.

In her short scientific career, she has received various awards and fellowships. She is also the recipient of the prestigious "Meritorious Research Fellowship" by University Grants Commission-Basic Science Research (UGC-BSR) at the Molecular Carcinogenesis and Chemoprevention Laboratory, Jamia hamdard University, India, in April 2011.

Introduction 1

Cancer is a cluster of diseases which involves variation in the status and activation of multiple genes that impart an advantage to survive and unexhausting proliferative potential to somatic or germinal cells (Cho 2007). The three main classes of genes altered primarily are proto-oncogenes, tumor suppressor genes, and DNA repair genes. Together they contribute to the growth of cancer phenotype and genotype to defend against the natural and inherent death mechanisms ingrained in cells as apoptosis and like processes, associated with deregulation of cell proliferation events. Apart from this, there is also an escalating evidence to recommend that cancer is also driven by epigenetic changes like DNA methylation and transformed patterns of histone modifications resulting in variation in chromatin condensation status, hence regulating activation of certain set of specific genes (Ediriwickrema and Saltzman 2015; Bayli and Ohm 2006). Most cancers are named according to their origin of sites in which they begin like cancer that originates in the renal proximal cells is called renal cell carcinoma and renal pelvis carcinoma is the cancer that originates in the center of the kidney where urine collects. Wilms tumor usually develops in children under the age of 5 (Bhatt et al. 2010).

Hippocrates, a Greek physician, was the first to give the term cancer, and he used Greek words, carcinos and carcinoma, to illustrate tumors; thus he named cancer as karkinos. Karkinos is a Greek term used to describe a crab, and according to Hippocrates, the tumors resembled it. In 1902, Theodor Boveri documented the genetic basis of cancer as chromosomal mutations that can generate indefinite growth which can pass on to the progeny. Cancer may be caused by radiation and physical or chemical exposure also (Weiss 2000).

It has been estimated that more than 11 million people are diagnosed with cancer every year and there will be 16 million new cases every year by 2020. It accounts for around 13 % of all deaths in the world; more than 30 % of cancer deaths can be prevented by modification or avoidance of chief risk factors (World Health Organization 2009). It is still a big threat to our society despite good development for diagnosis and treatment. It is the second most common disease after

S. Rashid, *Cancer and Chemoprevention: An Overview*,
DOI 10.1007/978-981-10-2579-2_1

cardiovascular disorders worldwide accounting for about 23 % and 7 % of deaths in the USA and India, respectively. It is expected that the world's population will be 7.5 billion by 2020 and 15 million new cancer cases will be diagnosed with deaths of about 12.0 million cancer patients. About 70 % of cancer cases have been diagnosed and treated, with few patients surviving during the last one decade. It is predicted that in the developing and underdeveloped countries, in the near future, the number of cancer patients will increase which may rise up to 70 %, which is a serious issue for all of us. In the Indian subcontinent, cancer problem is increasing due to inadequate medical facilities and poor living standards (Ali et al. 2011).

References

Ali I, Wani WA, Saleem K (2011) Cancer scenario in India with future perspectives. Cancer Ther 8:56–70

Bayli SB, Ohm JE (2006) Epigenetic gene silencing in cancer – a mechanism for early oncogenic pathway addiction? Nat Rev Cancer 6:107–117

Bhatt AN, Mathur R, Farooque A et al (2010) Cancer biomarkers – current perspectives. Indian J Med Res 132:129–149

Cho WC (2007) Contribution of oncoproteomics to cancer biomarker discovery. Mol Cancer 6:25

Ediriwickrema A, Saltzman WM (2015) Nanotherapy for cancer: targeting and multifunctionality in the future of cancer therapies. ACS Biomater Sci Eng 1:64–78

Weiss L (2000) Early concepts of cancer. Cancer Metastasis Rev 19:205–217

World Health Organization (2009) Global health risks: mortality and burden of disease attributable to selected major risks. WHO Library Cataloguing-in-Publication Data

Hallmarks of Cancer Cell

2

The fascinating complexity of a cancer cell is derived collectively from six basic alterations of cell physiology that result in sustained malignant proliferation (Hanahan and Weinberg 2000). However in in vitro cancer cells, four of the variations are easily detectable in yielding a phenotype of persistent proliferation and aversion from apoptosis. Cell migration/metastasis and angiogenesis are the other two which may be only observed in vivo. Genetic mutations in proto-oncogenes, onco-suppressors, and environmental conditions like hypoxia or inflammation contribute to malignant growth. The loss of tumor suppressor genes (TSG) by mutation may contribute to uncontrolled cell growth leading to cancer. Similarly proto-oncogenes are active in the signaling pathway for cell growth, and on mutation they transform into oncogenes triggering nonstop cell divisions leading to hyper-proliferation. Data implies that tumor cells are different from normal cells in at least six ways relating to growth control: sustaining proliferative signaling, evading growth suppressors, resisting cell death, inducing angiogenesis, invading replicative immortality, and activating invasion and metastasis (Hanahan and Weinberg 2011) (Fig. 2.1).

Sustaining Proliferative Signaling

Enhanced and sustainable cell proliferation is the most fundamental trait of cancer cells and one of the most important hallmarks of cancer, which may be identified using a number of histological, biochemical, and flow cytometric analysis (Bhatt et al. 2010). In normal tissues, there is control on production and release of growth-promoting signals that regulate cell growth and division. In this manner upholding of cell number and thus normal tissue architecture and function, thereby maintaining homeostasis. Deregulating of these signals in cancer cells makes them sufficient for their own growth. Growth factors typically containing intracellular tyrosine kinase domains bind cell surface receptors to enable signals largely via branched intracellular signaling pathways that control progression through cell cycle and cell

S. Rashid, *Cancer and Chemoprevention: An Overview*,
DOI 10.1007/978-981-10-2579-2_2

Fig. 2.1 represents six hallmarks of cancer cell (Adapted and modified figure from Hanahan and Weinberg 2011). Six biological capabilities encompass the six hallmarks of cancer required for the progression of human tumors. They include sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis. The fundamental mechanism behind these hallmarks is genome instability through which genetic diversity originates which advances to their acquisition and inflammation and thereby promotes multiple hallmark functions

growth. There are also other cell biological properties like cell survival and energy metabolism which are influenced by these signals. However, still relatively little is known about the mechanisms controlling the release of these mitogenic signals (Witsch et al. 2010; Lemmon and Schlessinger 2010).

Moreover, the understanding of these mechanisms is intricate because the growth factor signals controlling cell number and position within tissues are thought to be transmitted in a temporally and spatially regulated fashion from one cell to its neighbors, and such paracrine signaling is hard to access experimentally. Moreover, growth factor bioavailability is regulated by sequestration in the pericellular space and extracellular matrix besides actions of complicated network of proteases, sulfatases, and other enzymes liberating and activating them in an extremely specific and localized manner (Lemmon and Schlessinger 2010). Cancer cells attain the competence to maintain proliferative signaling in a number of unconventional ways: they do so by producing growth factor ligands themselves and respond to them through the expression of cognate receptors leading to autocrine proliferative stimulation. On the other hand, they promote normal cells within the tumor-associated stroma which respond by providing the cancer cells with diverse growth factors (Cheng et al. 2008).

Deregulation of receptor signaling by growing the levels of receptor proteins present at the cancer cell surface, making such cells hyperresponsive to otherwise

limiting number of growth factor ligands; the similar conclusion can result from structural variations in the receptor molecules facilitating ligand-independent firing. Combined stimulation of constituents of signaling pathways operating downstream of these receptors may contribute to growth factor independence, preventing the necessity to activate these pathways by ligand-modulated receptor activation. Cancer cells can sustain this proliferative signaling by three main hypotheses (Hanahan and Weinberg 2011).

Evading Growth Suppressors

Cancer cells must also evade checkpoints which regulate cell proliferation by several ways either by inactivation of tumor suppressor genes or by passing cell cycle checkpoints. Cell growth and proliferation are limited by dozens of tumor suppressors operating in various ways through their characteristic inactivation in one or other form of animal or human cancers. The prototypical type tumor suppressors which function as fundamental regulatory points within two key corresponding cellular control circuits and manage the decisions of cells to proliferate or activate senescence and apoptotic programs are RB (retinoblastoma)-associated and TP53 proteins. The RB protein incorporates signals from varied extracellular and intracellular sources and later on chooses fate of a cell to go forward or not (Velez and Howard 2015).

RB pathway function serves as critical gatekeeper of cell cycle progression, while its dearth permits constant cell proliferation which is found in cancer cells. It transduces growth-inhibitory signals originating chiefly outside of the cell, while TP53 receives inputs from stress and abnormality sensors functioning within intracellular operating systems of the cell. TP53 can stop the progress of cell cycle progression when the degree of damage to the genome is extreme or levels of nucleotide pools, growth-promoting factors, glucose, and oxygenation are suboptimal. Until these conditions have been normalized, it can trigger apoptosis if there is devastating or irreparable damage to such cellular subsystems. Evidently, the various effects of activated TP53 are intricate and highly context reliant depending on cell type, rigorousness, and determination of conditions of cell stress and genomic damage. TP53 and RB are the two canonical suppressors of proliferation having paramount significance in regulating cell proliferation (Noa et al. 2011; Dick and Rubin 2013; Hanahan and Weinberg 2011).

Resisting Cell Death

Functional studies carried out over the last two decades have recognized the concept that programmed cell death by apoptosis serves as a natural barrier to cancer development. The mechanism of triggering apoptosis in response to various physiological stresses that cancer cells undergo during the course of tumorigenesis or as a result of anticancer therapy has been well elucidated in the form of signaling

circuitry program governing apoptosis (Lowe et al. 2004). Signaling imbalances resulting from elevated levels of oncogene signaling and DNA damage associated with hyper-proliferation are prominent among the apoptosis-inducing stresses. In contrast, research has revealed pathways of attenuation in tumors that succeed in resistance to therapy and progressing to states of high-grade malignancy (Adams and Cory 2007). Tumor cells develop various strategies to limit or avoid apoptosis; for instance, the loss of TP53 tumor suppressor, which is the most common, eradicates this critical damage sensor from the apoptosis-inducing circuitry (Junttila and Evan 2009).

Enabling Replicative Immortality

That cancer cells require uncontrolled replicative potential in order to generate macroscopic tumors was extensively accepted by 2000. This is in marked contrast with the behavior of regular cell lines in the body, which possess the capacity to pass through only an inadequate number of consecutive cell growth and division cycles owing this restraint to be related with two separate barriers to proliferation: senescence, which is classically irreversible entry into a nonproliferative but viable state, and crisis, which entails cell death (Kuilman et al. 2010). Consequently in culture when cells are propagated repeatedly into many cycles lead first to initiation of senescence and then for those cells that thrive in crossing this obstruction result to crisis phase resulting in death of immense bulk of cells in the population.

Alternatively some cells come out from a population in crisis and demonstrate unlimited replicative potential which rarely is a trait that most recognized cell lines acquire by virtue of their capability to propagate in culture lacking senescence or crisis. This transition is called immortalization which is conferred to telomeres shielding the ends of chromosomes that are centrally implicated to have potential for unlimited proliferation which has been known by multiple lines of its evidence from various studies (Blasco 2005; Dolcetti et al. 2014). The telomeres are made up of numerous tandem hexanucleotide repeats which condense gradually in non-immortalized cells disseminated in culture. By this, they ultimately lose their capability to protect the ends of chromosomal DNAs from end-to-end fusions, and by doing so, they generate unstable dicentric chromosomes whose resolution results in scrambling of karyotype, thereby, threatening cell viability. Telomeric DNA length demonstrates the number of consecutive cell generations its progeny can pass through before telomeres are largely eroded and lose their defensive functions, eliciting cell into crisis. Telomeric DNA at its ends has telomere repeat segments added to it by specialized DNA polymerase called telomerase which is articulated at functionally substantial levels in the enormous majority (90 %) of instinctively immortalized cells including human cancer cells but almost absent in non-immortalized cells (Passos et al. 2007; Landa et al. 2013). Hence, the two obstacles to propagation are senescence and crisis or apoptosis which act as critical anticancer defense strategies being mounted to hinder the outcomes of clones of preneoplastic and neoplastic cells. The ultimate immortalization of rare abnormal cells which progress to

form tumors is because of their capacity to sustain telomeric DNA at lengths adequate to shun senescence or apoptosis. This is attained either by upregulating the expression of telomerase commonly or via an unconventional recombination-based telomere preservation mechanism utilized less frequently. Thus, telomere shortening is an important marker demonstrating restricted replicative potential of normal cells and must be deregulated in cancer cells (Feldser and Greider 2007; Artandi and DePinho 2010; Gutschner and Diederichs 2012).

Inducing Angiogenesis

Tumors do need nutrients, oxygen, detoxification of metabolic wastes, and carbon dioxide like normal tissues. Angiogenesis is a process of generation of tumor-associated neovasculature which covers up these needs. There is generation of vasculature which involves development of new endothelial cells and their assembly into tubes called as vasculogenesis at embryonic stage, besides development of new vessels from existing ones called angiogenesis (Senger and Davis 2011). After progressing through this stage, the normal vasculature becomes largely quiescent. However in the adult, in case of wound healing and female reproductive cycling, angiogenesis is turned on briefly for a short while. On the contrary, angiogenic switch is almost always activated and remains on during tumor progression resulting in persistent sprouting of new vessels that help in sustenance of escalating neoplastic growths. Vascular endothelial growth factor A (VEGF-A) and thrombospondin 1 (TSP-1) are the well-known prototypes of angiogenesis inducers and inhibitors, respectively.

During embryonic and postnatal development, new blood vessel growth, homeostasis of endothelial cells, and physiological and pathological states are governed by VEGF. Conditions like hypoxia and oncogene signaling also upregulate VEGF gene expression (Ferrara 2009). Chronic angiogenesis within tumors and abnormal pro-angiogenic signals are classically anomalous: tumor neovasculature is discernible by convoluted and excessive vessel branching, precocious capillary sprouting, erratic blood flow, distorted and enlarged vessels, micro-hemorrhaging, leakiness and anomalous endothelial cell proliferation, and apoptosis (Nagy et al. 2010). During the multistage development of invasive cancers in animal models and humans, angiogenesis is induced surprisingly early which has been revealed by histological analysis of premalignant, noninvasive lesions, including dysplasias and in situ carcinomas arising in a variety of organs (Raica et al. 2009).

Activating Invasion and Metastasis

The mechanisms behind invasion and metastasis were enigmatic till 2000. It was revealed by local invasion and distant metastasis that carcinomas originating from epithelial tissues progressed to higher pathological grades of malignancy. Similarly the associated cancer cells alter their shape and attachment with other cells and with

the extracellular matrix (ECM) which is best characterized by loss of E-cadherin in carcinoma cells. E-cadherin is an important cell-to-cell adhesion molecule. It forms adherens junctions with adjacent epithelial cells. Upregulation of E-cadherin antagonizes invasion and metastasis, and its downregulation potentiates these phenotypes which is seen in human carcinomas providing strong evidence for its role as an excellent suppressor of this hallmark potential (Berx and van Roy 2009). Furthermore cell-to-cell and cell-to-ECM adhesion molecules like cytostasis are noticeably downregulated in some highly aggressive carcinomas and conversely often upregulated in embryogenesis and inflammation.

There is a sequence of discrete steps often termed the invasion-metastasis cascade (Talmadge and Fidler 2010) which involves a series of biological changes in cell, commencing with local invasion, then intravasation into nearby blood and lymphatic vessels by cancer cells, and transit through lymphatic and hematogenous systems by cancer cells, followed by escape of cancer cells from the lumina of such vessels into the parenchyma of distant tissues (extravasation), the formation of small nodules of cancer cells (micrometastases), and finally the growth of micrometastatic lesions into macroscopic tumors, this final step being termed colonization (Fidler 2003).

Enabling Characteristics and Emerging Hallmarks

During the course of multistep tumorigenesis, hallmarks permit cancer cells to continue to exist, multiply, and spread via distinct mechanisms at various times, and these functions are obtained in diverse tumor types which are made plausible by two enabling features. The growth of genomic instability in cancer cells is the most prominent, generating random mutations including chromosomal rearrangements. Inflammatory state of premalignant and malignant lesions determined by cells of the immune system that serve to advance tumor progression through different ways is found to be the second enabling characteristic (Sonnenschein and Soto 2013).

Two additional hallmarks of cancer are involved in the pathogenesis of cancers evidenced from research which includes the ability to amend or reprogram cellular metabolism in order to most successfully maintain neoplastic proliferation and second allows cancer cells to escape immunological devastation (Hanahan and Weinberg 2011; Negrini et al. 2010 and Colotta et al. 2009) (Fig. 2.2).

An Enabling Characteristic: Genome Instability and Mutation

Successions of alterations in the genomes of neoplastic cells are necessary for acquisition of the multiple hallmarks. It has been revealed that some mutant genotypes exhibit selective advantage on subclones of cells to enable their outgrowth and dominance in a local tissue environment. Although epigenetic mechanisms such as DNA methylation and histone modifications also result in inactivation of tumor suppressor genes in hereditary (Berdasco and Esteller 2010) along with some

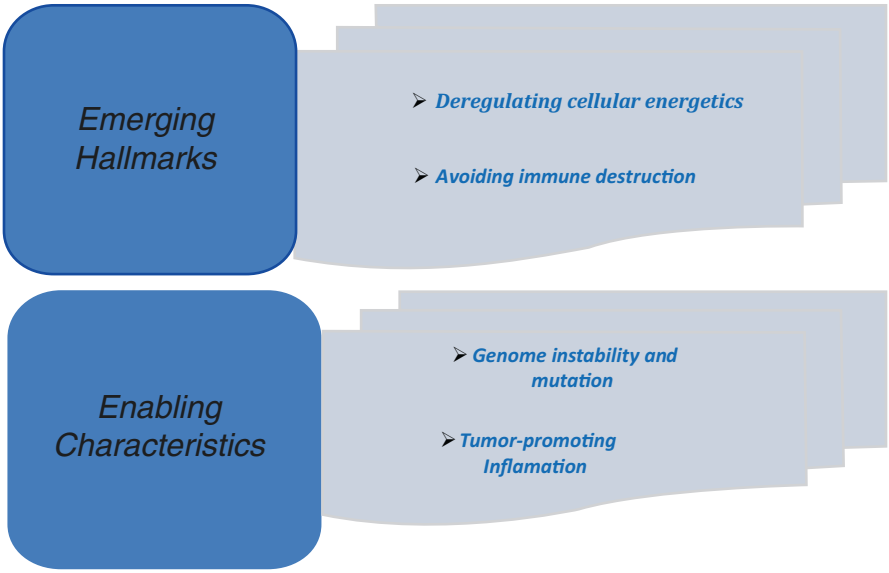


Fig. 2.2 represents the emerging hallmarks of cancer and enabling characteristics (Adapted and modified figure from Hanahan and Weinberg 2011). Additional hallmarks of cancer are involved in the pathogenesis of cancers. Two substantial features of neoplasia assist acquirement of both center and emerging hallmarks. Genomic instability and variability provide cancer cells with genetic variations that motivate tumor progression. Inflammation by innate immune cells intended to fight infections and heal wounds results in their involuntary support of several hallmark capabilities

nonmutational changes affecting the regulation of gene expression, rates of unplanned mutation are usually very low during each cell generation due to surprising ability of genome maintenance systems to discover and determine defects in the DNA. Additionally the extent of mutations can be accelerated by deregulating the surveillance systems that regulate genomic integrity and compel genetically damaged cells into either senescence or apoptosis (Jackson and Bartek 2009). TP53 plays a central role here to be entitled as guardian of the genome (Lane 1992). However defects in regulatory genes include those whose products are involved in:

- Detection of DNA damage and activation of repair machinery
- Direct repairing of damaged DNA
- Inactivation of mutagenic molecules before damaging DNA (Negrini et al. 2010; Ciccia and Elledge 2010)

These regulatory genes act greatly like tumor suppressor genes, whose functions are lost either through inactivating mutations or via epigenetic suppression during the course of tumor progression. Mutant copies of such regulatory genes have been commenced into the mouse germ line resulting in increase in cancer incidences favoring evidence about their possible association in human cancer progress (Barnes and Lindahl 2004).

An Enabling Characteristic: Tumor-Promoting Inflammation

It was known by 2000 that the tumor-related inflammatory response had the amazing contradictory effect in upgrading tumorigenesis and progression by facilitating early neoplasias to obtain hallmark potential. In the subsequent time, research connecting inflammation and cancer pathogenesis has flourished by producing copious evidences about tumor-promoting effects that immune cells have on neoplastic progression (Quail and Joyce 2013; Cantor and Sabatini 2012).

The multiple hallmark capabilities are contributed by inflammation via supplying bioactive molecules like growth factors to maintain proliferative signaling; survival factors to limit cell death; proangiogenic factors; extracellular matrix-modifying enzymes to assist in angiogenesis, invasion, and metastasis; and other hallmark-assisting programs to the tumor microenvironment (DeNardo et al. 2010; Grivennikov et al. 2010). In addition to this, reactive oxygen species are generated by inflammatory cells which are mutagenic for cancer cells in close proximity and accelerate their genetic evolution toward condition of uncontrollable malignancy (Grivennikov et al. 2010).

An Emerging Hallmark: Reprogramming Energy Metabolism

Neoplastic disease is often characterized by abnormal and chronic cell proliferation that involves not only unaltered cell proliferation but also subsequent demand of energy metabolism in order to energize cell growth and division. Normal cells convert glucose to pyruvate via glycolysis in the cytosol under aerobic conditions and later to carbon dioxide in the mitochondria. However under anaerobic conditions, glycolysis is preferred, and relatively little pyruvate is transmitted to oxygen-consuming mitochondria (Hsu and Sabatini 2008).

Otto Warburg was the first to observe an atypical characteristic of cancer cell energy metabolism by limiting their energy metabolism largely to glycolysis called as aerobic glycolysis as well as by reprogramming their glucose metabolism and energy production. Such reprogramming of energy metabolism is urgently needed wherein cancer cells must balance for 18-fold lower efficiency of ATP production afforded by glycolysis in comparison to mitochondrial oxidative phosphorylation. They achieve so by upregulating glucose transporters like GLUT1 which considerably enhances entry of glucose into the cytoplasm (Jones and Thompson 2009).

Tumors often are under hypoxic conditions. Here the hypoxia response system acts pleiotropically to upregulate glucose transporters and multiple enzymes of the glycolytic pathway, hence dependence on glycolysis (Semenza 2010). A practical underlying principle for the glycolytic switch in cancer cells has been obscure, because of the deprived efficiency of generating ATP by glycolysis compared to oxidative phosphorylation in mitochondria. According to a recent hypothesis (Vander Heiden et al. 2009), enhanced glycolysis confers the progression of

glycolytic intermediates into different biosynthetic pathways which includes generation of nucleosides and amino acids, thereby, helping in biosynthesis of the macromolecules and organelles required for assembling new cells (Ganapathy-Kanniappan and Geschwind 2013).

An Emerging Hallmark: Evading Immune Destruction

The function of the immune system in defending against or eliminating growth and development of early neoplasias, late-stage tumors, and micrometastases is still an unsolved issue surrounding tumor formation. It has been suggested by a theory that cells and tissues are continually monitored by an ever-alert immune system called immune surveillance which has the capacity to recognize and eliminate enormous majority of initiated cancer cells and hence emerging tumors (Teng et al. 2008; Bruttel and Wischhusen 2014).

However some solid tumors that grow manage somehow to evade detection by immune system or limit the amount of immunological killing resulting in escaping eradication which is well authenticated by the remarkable enlargement of certain cancers in immune-compromised individuals either because of defective immunological status (Vajdic and van Leeuwen 2009).

References

Adams JM, Cory S (2007) The Bcl-2 apoptotic switch in cancer development and therapy. Oncogene 26:1324–1337

Artandi SE, DePinho RA (2010) Telomeres and telomerase in cancer. Carcinogenesis 31:9–18

Barnes DE, Lindahl T (2004) Repair and genetic consequences of endogenous DNA base damage in mammalian cells. Annu Rev Genet 38:445–476

Berdasco M, Esteller M (2010) Aberrant epigenetic landscape in cancer: how cellular identity goes awry. Dev Cell 19:698–711

Berx G, van Roy F (2009) Involvement of members of the cadherin superfamily in cancer. Cold Spring Harb Perspect Biol 1:a003129

Bhatt AN, Mathur R, Farooque A et al (2010) Cancer biomarkers – current perspectives. Indian J Med Res 132:129–149

Blasco MA (2005) Telomeres and human disease: ageing, cancer and beyond. Nat Rev Genet 6:611–622

Bruttel VS, Wischhusen J (2014) Cancer stem cell immunology: key to understanding tumorigenesis and tumor immune escape? Front Immunol 5:360

Cantor JR, Sabatini DM (2012) Cancer cell metabolism: one hallmark, many faces. Cancer Discov 2:881–898

Cheng N, Chytil A, Shyr Y et al (2008) Transforming growth factor-beta signaling-deficient fibroblasts enhance hepatocyte growth factor signaling in mammary carcinoma cells to promote scattering and invasion. Mol Cancer Res 6:1521–1533

Ciccia A, Elledge SJ (2010) The DNA damage response: making it safe to play with knives. Mol Cell 40:179–204

Colotta F, Allavena P, Sica A et al (2009) Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. Carcinogenesis 30:1073–1081

DeNardo DG, Andreu P, Coussens LM (2010) Interactions between lymphocytes and myeloid cells regulate pro- versus anti-tumor immunity. Cancer Metastasis Rev 29:309–316
Dick FA, Rubin SM (2013) Molecular mechanisms underlying RB protein function. Nat Rev Mol Cell Biol 14:297–306
Dolcetti R, Giunco S, Jessica DC et al (2014) Epstein-Barr virus and telomerase: from cell immortalization to therapy. Infect Agent Cancer 9:8
Feldser DM, Greider CW (2007) Short telomeres limit tumor progression *in vivo* by inducing senescence. Cancer Cell 11:461–469
Ferrara N (2009) Vascular endothelial growth factor. Arterioscler Thromb Vasc Biol 29: 789–791
Fidler IJ (2003) The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. Nat Rev Cancer 3:453–458
Ganapathy-Kanniappan S, Geschwind JF (2013) Tumor glycolysis as a target for cancer therapy: progress and prospects. Mol Cancer 12:152
Grivennikov SI, Greten FR, Karin M (2010) Immunity, inflammation, and cancer. Cell 140:883–899
Gutschner T, Diederichs S (2012) The hallmarks of cancer A long non-coding RNA point of view. RNA Biol 9:703–719
Hanahan D, Weinberg RA (2000) The hallmarks of cancer. Cell 7:57–70
Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144:646–674
Hsu PP, Sabatini DM (2008) Cancer cell metabolism: Warburg and beyond. Cell 134:703–707
Jackson SP, Bartek J (2009) The DNA-damage response in human biology and disease. Nature 461:1071–1078
Jones RG, Thompson CB (2009) Tumor suppressors and cell metabolism: a recipe for cancer growth. Genes Dev 23:537–548
Junttila MR, Evan GI (2009) p53—a Jack of all trades but master of none. Nat Rev Cancer 9:821–829
Kuilman T, Michaloglou C, Mooi JW et al (2010) The essence of senescence. Genes Dev 24:2463–2479
Landa I, Ganly I, Timothy AC et al (2013) Frequent somatic TERT promoter mutations in thyroid cancer: higher prevalence in advanced forms of the disease. J Clin Endocrinol Metab 98:E1562–E1566
Lane DP (1992) Cancer. p53, guardian of the genome. Nature 358:15–16
Lemmon MA, Schlessinger J (2010) Cell signaling by receptor tyrosine kinases. Cell 141:1117–1134
Lowe SW, Cepero E, Evan G (2004) Intrinsic tumour suppression. Nature 432:307–315
Nagy JA, Chang SH, Shih SC et al (2010) Heterogeneity of the tumor vasculature. Semin Thromb Hemost 36:321–331
Negrini S, Gorgoulis VG, Halazonetis TD (2010) Genomic instability— an evolving hallmark of cancer. Nat Rev Mol Cell Biol 11:220–228
Noa R, Ran B, Moshe O et al (2011) Mutations in the p53 tumor suppressor gene. Genes Cancer 2:466–474
Passos JF, Saretzki G, von Zglinicki T (2007) DNA damage in telomeres and mitochondria during cellular senescence: is there a connection? Nucleic Acids Res 35:7505–7513
Quail DF, Joyce JA (2013) Micro environmental regulation of tumor progression and metastasis. Nat Med 19:1423–1437
Raica M, Cimpean AM, Ribatti D (2009) Angiogenesis in pre-malignant conditions. Eur J Cancer 45:1924–1934
Semenza GL (2010) HIF-1: upstream and downstream of cancer metabolism. Curr Opin Genet Dev 20:51–56
Senger DR, Davis GE (2011) Cold Spring Harb Perspect Biol 3:a005090

Sonnenschein C, Soto AM (2013) The aging of the 2000 and 2011 Hallmarks of Cancer reviews: a critique. J Biosci 38:651–663

Talmadge JE, Fidler IJ (2010) AACR centennial series: the biology of cancer metastasis: historical perspective. Cancer Res 70:5649–5669

Teng MWL, Swann JB, Koebel CM et al (2008) Immune-mediated dormancy: equilibrium with cancer. J Leukoc Biol 84:988–993

Vajdic CM, van Leeuwen MT (2009) Cancer incidence and risk factors after solid organ transplantation. Int J Cancer 125:1747–1754

Vander Heiden MG, Cantley LC, Thompson CB (2009) Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 324:1029–1033

Velez AMA, Howard M (2015) Tumor-suppressor genes, cell cycle regulatory checkpoints, and the skin. N Am J Med Sci 7:176–188

Witsch E, Sela M, Yarden Y (2010) Roles for growth factors in cancer progression. Physiology (Bethesda) 25:85–101

Classification of Cancer

3

Tumors can be either benign or malignant. However benign tumors are usually slow-growing extensive masses that compress rather than invade surrounding tissue. Benign tumors are not cancerous, i.e., cells do not extend to other parts of the body. But malignant tumors are cancerous. Cells in these tumors invade nearby tissues and extend to other parts of the body, and when it spreads from one part of the body to another, it is termed as metastasis. Cancers are categorized in two ways depending upon the type of tissue in which cancer originates known as primary site or the position in the body where the cancer first develops. Malignant tumors are generally fast growing which invade surrounding tissue and colonize distant organs extensively. The capacity of tumor cells to separate from the original mass (the primary tumor) and spread to other organs sets up metastasis. There are hundreds of different cancers from histological point of view which are grouped into six major categories:

Carcinoma is any malignant cancer that originates from epithelial tissues lining the inner or outer surfaces of the body, generally arising from endodermal or ectodermal germ layers during embryogenesis. Carcinomas invade surrounding tissues and organs and may metastasize or spread to lymph nodes and other sites (Witkiewicz et al. 2011). Common malignancies like breast, colon, and lung cancer are categorized as carcinomas.

Sarcoma is a malignancy that originates from altered cells of mesenchymal origin in bone, muscle, or connective tissue. Thus, malignant tumors found in cancellous bone, cartilage, fat, muscle, or connective tissues are termed as sarcomas. They occur rarely in humans.

Leukemia is a neoplastic disease that usually begins in the bone marrow and results in the abnormal development of white blood cells and is generally classified into acute and chronic forms. Additionally it is classified depending upon the type of white blood cells affected by the disease (Isaacs 2009).

S. Rashid, *Cancer and Chemoprevention: An Overview*,
DOI 10.1007/978-981-10-2579-2_3

Lymphoma is a kind of blood cell tumor of lymphocytes. It originates in the glands or nodes of the lymphatic system, a network of vessels, nodes, and organs that purify bodily fluids and develop infection fighting white blood cells or lymphocytes (Cupedo et al. 2011).

Myeloma arises in the plasma cells of bone marrow. Sometimes myeloma cells accumulate in one bone and form a single tumor called plasmacytoma. Yet in other cases, the myeloma cells accumulate in several bones developing many tumors termed as multiple myeloma. It is also known as plasma cell myeloma, myelomatosis, or Kahler's disease. In myeloma, unusual plasma cells accumulate collectively in the bone marrow and obstruct the production of normal blood cells (Fonseca and Valdez 2002).

Adenocarcinoma is a cancer of epithelial tissue that has glandular origin, glandular characteristics, or both. They form the part of larger grouping of carcinomas. Carcinoma is just not limited to epithelial or skin or glands but a diversity of other tissues that lines the cavities and organs of the body. Thus invasive ductal carcinoma, the most common form of breast cancer, is adenocarcinoma which does not use the term in its name, but esophageal adenocarcinoma does to distinguish it from the other common type of esophageal cancers, esophageal squamous cell carcinoma, which is not adenocarcinoma. Several of the most common forms of cancer are adenocarcinomas, and the various sorts of adenocarcinomas vary greatly in all their aspects, so that few useful generalizations can be made about them.

Blastoma arises in embryonic tissue of organs. It is a cancerous tumor that originates from the immature cells that form the basis for an organ's structure. It occurs in the cells which are undifferentiated, i.e., they have not developed a specific role within the body yet. It usually occurs in childhood and may rarely occur in early adulthood. Though osteoblastoma (blastoma of the bone) is a non-cancerous tumor, otherwise most blastomas are cancerous which include nephroblastoma, medulloblastoma, and retinoblastoma (Harada et al. 2006).

References

Cupedo T, Coles MC, Veiga-Fernandes H (2011) Development and structure of lymph nodes in humans and mice. Developmental Biology of Peripheral Lymphoid Organs, pp 59–74

Fonseca R, Valdez R (2002) Plasma cell neoplasms. Diagnostic Techniques in Hematological Malignancies 244:30–36

Harada T, Sukoh N, Hakuma N et al (2006) Pulmonary blastoma within bronchioloalveolar cell carcinoma. Respirology 11:339–342

Isaacs R (2009) Lymphosarcoma cell leukemia. Landmark Papers in Internal Medicine: the first 80 years of annals of internal medicine, vol 441

Witkiewicz AK, Wright TC, Ferenczy A et al (2011) Carcinoma and other tumors of the cervix. Blaustein's pathology of the female genital tract, vol 6, pp 253–303

Factors Affecting Cancer Development

4

There are a number of innate and extrinsic features coupled with the progression of cancer. The innate features consist of age and hormonal significance of the individual, ancestral history, and hereditary make. The external features comprise diet and lifestyle, smoking and alcohol use, contact to deadly chemicals and radiation, several infections, etc. A number of agents in external factors are chemical and environmental pollution, dyes, food additives, and exhaust from automobiles functioning as promoters in carcinogenesis. Intrinsic factors also termed as natural features include:

Age and Hormonal Status

Cancer has been suggested to occur at late stages of life. A number of cancers are age dependent like they occur in people above 50–55 years, e.g., prostate and kidney cancers. Similarly cervical cancer in women is more commonly detected at pre- or postmenopausal ages. Nevertheless, no age group is immune to this disease. Hormonal factors play an essential role in the development of gender-specific cancers, e.g., estrogens in cancers of the ovary and uterus in female (Henderson et al. 1998).

Family Record

A number of cancers are designated to have a relationship with ancestral incidences; e.g., a woman having close family members like grandmother, mother, maternal aunt, or sister who have undergone breast cancer is 3 times at advanced threat of developing breast cancer compared to not having such a familial account. Similar phenomena govern cervical cancer in females and prostate cancer in males (Kohno and Yokota 2002).

S. Rashid, *Cancer and Chemoprevention: An Overview*,
DOI 10.1007/978-981-10-2579-2_4

Genetic Predisposition

Certain genetic conditions predispose individuals to cancer like in the case of those with genetic conditions like xeroderma pigmentosum, ataxia telangiectasia, Bloom's syndrome, and Fanconi's anemia that are found to be extremely vulnerable to diverse kinds of malignancy (Elliot and Mignon 2013).

Extrinsic Factors

Diet, *alcohol*, *and tobacco use*: Personal habits like taking alcohol, tobacco chewing, smoking, and diet contribute more than 50 % of all cancers. Breast cancer has been associated with high-fat diet and obesity. A positive relationship linking age attuned breast cancer death rates, and the standard per capita fat utilization daily has been reported. Likewise deep-fried, overcooked food and high-salt food are coupled by way of escalating gastric cancer incidences. Usual eating of food having small fiber ingredients and loaded with animal fat increases the threat of stomach and esophageal malignancies. High incidence of gastric cancer in the USA has been associated largely with eating of red meat and diet having less fiber (Key et al. 2004). Tobacco smoke has nitrosamine, a chemical which is potent enough to develop neoplastic changes in the lungs. It has been reported that there is an increase in the cancers of upper alimentary canal and buccal mucosa due to nonsmoking tobacco habits like chewing. India leads in the prevalence of oral cancers worldwide which corresponds to the tobacco chewing habit (Jha 2009). There is also an increase in the risk of liver and bladder cancers due to alcoholism. Smoking and alcohol consumption further increase the risk of breast, esophageal, liver, stomach, and urinary bladder cancers. However, hepatitis B virus infection and alcoholism are sterner risk factors in liver cancer (Gary et al. 2008).

Radiation and Cancer

Ionizing radiation has both initiating and promoting properties which has been obtained from reports on the occupationally exposed workers like early radiologists, radium dial painters, and atom bomb sufferers of Japan. There has been an increase in infant cancers due to exposure to X-rays by mothers. It has been found that the radiation-induced tumors have comparatively long latencies varying as per species. However latency also varies with age at the time of exposure with the type of neoplasm initiated in a given species (Jemal at al. 2010).

Viruses and Cancer

Cancer-inducing viruses play a vital function in explicit human cancers like human papillomavirus in cervical cancer, human T-cell leukemia in leukemia, hepatitis B virus in hepatocellular carcinoma, and Epstein–Barr virus in Burkitt lymphoma and

nasopharyngeal carcinoma. There are two types of viruses: DNA and RNA viruses in which the former integrates into the cellular genome and the latter causes alteration of cellular genome resulting in malignancy in the infected cell (Stebbing et al. 2009; Ledford 2015).

Role of Free Radicals

During normal metabolic processes and by interacting with extrinsic contaminated agents like radiation and lethal chemicals, reactive oxygen species (ROS) and other free radicals in the body are produced. ROS includes superoxide anions, hydroxyl radicals, peroxy-radicals, and hydroperoxides. They lead to cell transformation via interacting with DNA and develop genetic aberrations and chromosomal abnormalities. They play a key task in the initiation of malignancy by producing DNA adducts and DNA mutations (Gulam and Haseeb 2006).

References

Elliot S, Mignon LL (2013) Genetic predispositions to childhood leukemia. Ther Adv Hematol 4:270–290

Gary LD, Jane DRN, James DM et al (2008) Hepatocellular carcinoma: management of an increasingly common problem. Proc (Baylor Univ Med Cent) 21:266–280

Gulam W, Haseeb A (2006) Reactive oxygen species: role in the development of cancer and various chronic conditions. J Carcinog 5:14

Henderson BE, Ross RK, Bernstein L (1988) Estrogens as a cause of human cancer. Cancer Res 48:246–253

Jemal A, Siegel R, Xu J et al (2010) Cancer statistics. CA: A Cancer J Clin 60:277–300

Jha P (2009) Avoidable global cancer deaths and total deaths from smoking. Nat Rev Cancer 9:655–664

Key TJ, Arthur S, Willett WC et al (2004) Diet, nutrition and the prevention of cancer. Public Health Nutr 7:187–200

Kohno T, Yokota J (2002) Genetic factors involved in cancer susceptibility. Gan to kagaku ryoho 29:1255–1262

Ledford H (2015) Cancer-fighting viruses near market. Nature News 526:622

Stebbing J, Duru O, Bower M et al (2009) Non-AIDS-defining cancers. Curr Opin Infect Dis 22:7

Carcinogenesis

5

Carcinogenesis is an extensive and multistep process involving initiation where initiated cell is formed and selected. Promotion is a step where initiated cell is selectively expanded. Progression occurs as a result of an imbalance between cell proliferation and apoptosis which further leads to invasion and metastasis. For the development from initiated cells to malignant tumors, many genetic and epigenetic events are necessary to exhibit one or another type of growth advantage leading to progressive alteration of normal human cells into cancer cells (Salvador 2012). Many remarkable preneoplastic mutations result in upregulation of oncogenes (e.g., myc, ras, abl, bcl-2) or downregulation of tumor suppressor genes (e.g., p53, Rb) which confers advantage of selective growth or survival to the cell.

Abnormal signal transduction, inappropriate expression of growth factor receptors (epidermal growth factor receptor), deregulation of cell cycle checkpoints, resistance to apoptosis, decreased need for metabolites, altered signal transducers and growth factors, and formation of neoangiogenesis are some phenotypic changes found in preneoplastic mutations. DNA damage must resist DNA repair processes and must be decipherable by DNA polymerase, which creates and locks mutations. However, this damage to the DNA may result in selective growth or survival advantage to the cell resulting in precancerous lesions. Normal cells can turn on and off genes that help them survive toxic signals transiently as induction of P450 enzymes for metabolism of toxic chemicals and drugs when under prolonged stress during chronic inflammation wherein a mutation may lock in the growth-advantaged phenotype (Federico et al. 2007).

Stages of Carcinogenesis

In vitro, in vivo, and epidemiologic studies have enabled researchers to affirm that neoplastic pathogenesis is a complex process from functional aspects which can be divided into three distinct stages involving changes in the genome's structure (Pitot 2001; Luch 2005). Human life is led under very different conditions from these

S. Rashid, *Cancer and Chemoprevention: An Overview*,
DOI 10.1007/978-981-10-2579-2_5

experimental procedures. Even though carcinogenesis is alike for man and experimental animals, humans are exposed to different chemical compounds throughout their lives which modify the speed and the frequency of mutation, the speed of cell growth, and hence the phenotypical expression of the mutated genes. Apart from individual's susceptibility and defense mechanisms, they have their own interaction which takes care of each of the neoplastic stages (Barcellos-Hoff et al. 2013).

Initiation

It forms the first stage of carcinogenesis characterized by irreversible genetic changes which predispose vulnerable normal cells to malign evolution, and immortality has been concluded from various studies (Shacter and Weitzman 2002). It is a speedy, permanent phenomenon which is transmitted to daughter cells. After successive genotypical and phenotypical changes are initiated, the cell is converted into a neoplastic one (Trosko 2003; Lagasse 2008). The initiated cell is similar to the remaining cells from a phenotypical perspective and undergoes mutations which induce proliferation but not differentiation (Trosko 2001; Oliveira et al. 2007). It has been well validated that DNA damage is the prime event to initiate chemical carcinogenesis (Santella and Gammon 2005) which can be repaired by enzymatic mechanisms. Proliferating cells are unable to fix the damaged DNA because of less time and remove covalent bonds that chemicals make with the DNA known as adducts (Frowein 2000).

It has been found that initiated cells can remain dormant for weeks, months, or years or they can grow in an autonomous and clonal fashion at this stage (Player et al. 2004). There is a mitogenic process caused by an increase in the number of new cells and apoptosis inhibition preventing initiated cells from dying off resulting into clonal expansion (Trosko 2001; Greaves and Maley 2012). DNA damage to stem cells is worst because of their long survival and exists in several tissues (Williams 2001). In 1978, Potter explained that all neoplastic cells had monoclonal origin from a stem cell and could display a phenotype established between the embryonic aspect and the terminal differentiation. As defined, stem cells are immortal cells until they differentiate or death is induced, and their delayed differentiation results in initiation and collects these cells in tissues as clones of abnormal cells (Trosko 2003). Reports reveal that stem cells are present in every tissue although they are not traceable in most tissues (Player et al. 2004).

Fundamentally initiation requires cell proliferation and is an additive process. The injury becomes permanent and irreversible if DNA repair systems remain inactivated either at or before cell division or when cellular division occurs. Carcinogenic dose is directly proportional to neoplastic development, i.e., escalating the dose increases the incidences and multiplicity of resultant neoplasias and reduces the latency period of its manifestation. It is essential that genes regulating terminal differentiation must be mutated or else all cells of a living organism exposed to an initiator agent will not be initiated even after mutations (Klaunig et al. 2000). Spontaneous mutations can commence initiation which can further undergo normal processes such as DNA

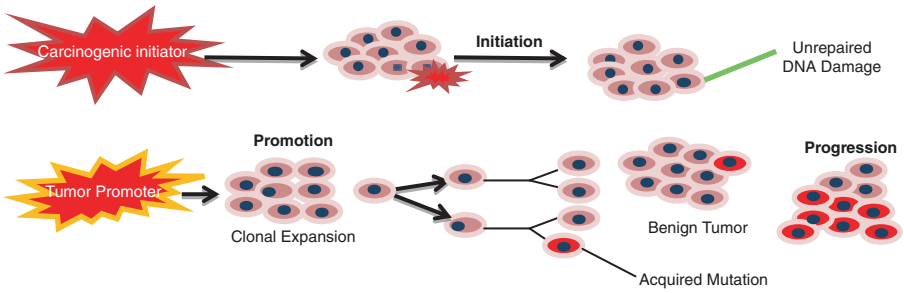


Fig. 5.1 represents the stages of cancer (Adapted and modified from Caligur 2008). The classical model of carcinogenesis consists of initiation, promotion, and progression. Changes in the genome's structure occur across the three stages of neoplastic development. Changes in gene expression take place during the promotion stage, with selective proliferation of initiated cells with unrepaired DNA damage leading to the development of preneoplastic cells and benign tumors. During initiation and promotion, apoptosis and cell proliferation can occur at different rates while remaining balanced. During progression, this balance is modified and from there malignancy

depurination and deamination. Errors in DNA replication are also associated with initiation. All living organisms have spontaneously initiated cells, and occurrence of spontaneous neoplasias in laboratory animals has confirmed its role, although it is less common than induced initiation (Trosko 2001; Spitsbergen et al. 2012) (Fig. 5.1).

Promotion

Metabolic activation is necessary for promoters to cause its effect and does not interact directly with DNA (Williams 2001). They contribute to fasten mutations, enhance cell proliferation in susceptible tissues, augment alterations in genetic expression, and change cellular growth control. They can also indirectly damage DNA via oxidation (Gutiérrez and Salsamendi 2001). It was first believed that epigenetic mechanisms were coupled with these occurrences, but now it is broadly accepted that genetic changes are also involved in promotion (Hanahan 2000). Promoters impede the natural inhibition of the quiescent cells or in G0 by gap junctions (Bertram 2001). Its most important activity is mitogenesis, wherein genotoxicity and mutations are not obligatory at this stage. In order to be efficient, the exposure with promoter must be for weeks, months, and years, and its efficacy depends on its concentration in the target tissue. If promoter is removed or somehow made unavailable, a regression in cell proliferation occurs via induction of apoptosis. Hence, promotion is a reversible stage at early stages and can be modified by physiological factors limiting the extent of experimental carcinogenesis. It has been found that some promoters are tissue specific, while others act concurrently upon numerous tissues. Prolonged exposure of promoters and high doses nearly of all the promoters in chemical carcinogenesis induce neoplasias without initiation (Gutiérrez and Salsamendi 2001) with examples of phenobarbital, benzene, asbestos, and arsenic without using initiator agents forming neoplasias (Trosko 2001).

This contradiction may be either due to mutagenicity and genotoxicity assays not detecting genotoxic effect or initiated cells emerging spontaneously. Promoter acts by increasing the frequency of cell division incorporating errors in DNA replication and mutations thereby acting indirectly. Not all cells exposed to promoters take part in the promotion stage; only cells which are undifferentiated, are stimulated to divide, and resist apoptosis can contribute to instability between growth and cell death leading to the appearance of a malign neoplasia; hence all cells exposed to promoters don't participate in the process of promotion (Trosko 2001).

Progression

Histopathology identifies the sequence of lesions between initiation and promotion which is called as preneoplastic lesions or benign neoplasias. The last stage of carcinogenesis is the most extended wherein transformation into malign lesions occurs undergoing labeled progression. Genetic and epigenetic mechanisms lead to the formation of a neoplastic phenotype, and cell proliferation is independent of stimulus in progression (Shacter and Weitzman 2002; Lutz 2002; Gutiérrez and Salsamendi 2001).

Alterations in the biochemical, morphological, and metabolic characteristics of cells occur in progression. Moreover, progression is characterized by faster growth, irreversibility, genetic instability, invasion, and metastasis (Klaunig et al. 2000; Gutiérrez and Salsamendi 2001; Dixon and Kopras 2004). Angiogenesis that occurs by epigenetic mechanisms is indispensable to neoplastic progression, and acquisition of such phenotype heads the development of features that contribute to malignancy, whereas its inhibition delays neoplastic development (Hawighorst et al. 2001).

Tumor metastasis A typical feature of tumor progression is that cells lose their adherence character, get detached from the primary tumor, and invade the surrounding tissues. The detached cells also enter blood and lymph circulation and transport to other tissues and organs away from the site of the primary growth and develop into secondary tumors at new sites. These form widespread metastasis, resulting in extensively spread cancers. Cancer metastasis consists of many steps in which main steps are common in all tumors. The progress of neoplastic disease depends on invasion of local normal tissues, entry and transfer of neoplastic cells in the blood and lymphatic systems, and consequent development of secondary tumors at distant sites (Wan et al. 2013).

Cadherins are one of the most important cell adhesion molecules which influence the behavior of tumors. Downregulation of E-cadherin results in low expressions which are concurrent with metastatic behavior in vivo, signifying that cadherins act as invasion suppressor gene. It is the metastatic process and tumor spreading that is mainly responsible for the lethal effects of many common human tumors. The driving force behind tumor metastasis is gene mutations along with the formation of tumor vasculature playing an imperative role in the disease progression (Stacker et al. 2002; Oliveria et al. 2010).

References

Barcellos-Hoff MH, David L, Timothy CW (2013) The evolution of the cancer niche during multistage carcinogenesis. Nat Rev Cancer 13:511–518
Bertram JS (2001) The molecular biology of cancer. Mol Asp Med 21:167–223
Caligur V (2008) Carcinogenesis and Epigenetics. BioFiles 3:18–23
Dixon K, Kopras E (2004) Genetic alterations and DNA repair in human carcinogenesis. Semin Cancer Biol 14:441–448
Federico A, Morgillo F, Tuccillo C et al (2007) Chronic inflammation and oxidative stress in human carcinogenesis. Int J Cancer 121:2381–2386
Frowein J (2000) Hypothesis: chemical carcinogenesis mediated by a transiently active carcinogen receptor. Cytogenet Cell Genet 91:102–104
Greaves M, Maley CC (2012) Clonal evolution in cancer. Nature 481:306–313
Gutiérrez JB, Salsamendi AL (2001) Fundamientos de ciencia toxicológica. Diaz de Santos, Madrid, pp 155–177
Hanahan D (2000) The hallmarks of cancer. Cell 100:57–70
Hawighorst T, Velasco P, Streit M et al (2001) Thrombospondin-2 plays a protective role in multistep carcinogenesis: a novel host anti-tumor defense mechanism. EMBO J 20:2631–2640
Klaunig JE, Kamendulis LM, Xu Y (2000) Epigenetic mechanisms of chemical carcinogenesis. Hum Exp Toxicol 19:543–555
Lagasse E (2008) Cancer stem cells with genetic instability: the best vehicle with the best engine for cancer. Gene Ther 15:136–142
Luch A (2005) Nature and nurture – lessons from chemical carcinogenesis. Nat Rev Cancer 5:113–125
Lutz WK (2002) Differences in individual susceptibility to toxic effects of chemicals determine the dose-response relationship and consequences of setting exposure standards. Toxicol Lett 126:155–158
Oliveira PA, Colaço A, Chaves R et al (2007) Chemical carcinogenesis. Ann Brazilian Acad Sci 79:593–616
Pitot HC (2001) Animal models of neoplastic development. Dev Biol (Basel) 106:53–57
Player A, Barrett JC, Kawasaki ES (2004) Laser capture microdissection, microarrays and the precise definition of a cancer cell. Expert Rev Mol Diagn 4:831–840
Salvador JDC (2012) Tumor heterogeneity: mechanisms and bases for a reliable application of molecular marker design. Int J Mol Sci 13:1951–2011
Santella RM, Gammon M (2005) DNA adducts DNA repair genotype/phenotype and cancer risk. Mutat Res 592:29–35
Shacter E, Weitzman SA (2002) Chronic inflammation and cancer. Oncology 6:217–226
Spitsbergen JM, Buhler DR, Peterson TS (2012) Neoplasia and neoplasm associated lesions in laboratory colonies of Zebrafish emphasizing key influences of diet and aquaculture system design. ILAR J 53:114–125
Stacker SA, Baldwin ME, Achen MG et al (2002) The role of tumor lymphangiogenesis in metastatic spread. FASEB J 16:922
Trosko JE (2001) Commentary: is the concept of "tumor promotion" a useful paradigm? Mol Carcinog 30:131–137
Trosko JE (2003) The role of stem cells and gap junctional intercellular communication in carcinogenesis. J Biochem Mol Biol 36:43–48
Wan L, Pantel K, Kang Y (2013) Tumor metastasis: moving new biological insights into the clinic. Nat Med 19:1450–1464
Williams GM (2001) Mechanisms of chemical carcinogenesis and application to human cancer risk assessment. Toxicology 166:3–10

Molecular Mechanism of Carcinogenesis

6

Most of the genetic changes in cancer consist of two categories: gain of function mutations in proto-oncogenes stimulating cell growth, division, and survival and loss of function mutations in tumor suppressor genes which normally check unrepressed cellular growth and promote DNA repair and commencement of cell cycle checkpoints (Lee and Muller 2010). The genes involved in cell cycle control, apoptosis, DNA repair, aging and immortalization, angiogenesis, and metastasis play a significant role in cancer. The most important genes affecting cell growth and mutation in cancers are oncogenes and tumor suppressor genes. The modifications are generally somatic events, while germ line mutations predispose a person to heritable or familial cancer passing on to future generations. A single genetic mutation may not be sufficient for the development of a malignancy but a multistep process of sequential alterations in a number of tumor suppressor genes, oncogenes, and DNA repair mechanisms (Croce 2008).

So the main focus of most of the human cancer studies involves tumor suppressor genes, oncogenes, and DNA repair mechanisms (Zingde 2001). The detailed molecular mechanism of RCC remains poorly understood in spite of its progress in management (Aravalli et al. 2008). RCC is a complex and multistep process involving the activation of both epigenetic and genetic events. Together these genetic and epigenetic alterations trigger positive regulators of cellular proliferation along with cellular proto-oncogenes and their mitogenic signaling pathway and inactivate negative regulators of cellular proliferation including tumor suppressor genes resulting in cells with self-sufficient growth potential (Levy and Sherman 2002; Feitelson et al. 2002). The understanding of the sequence of molecular events leading to progression from the constantly diseased kidney to the occurrence of hyperplastic and dysplastic nodules and ultimately to initiation and promotion of RCC is still poorly understood (Kojiro and Roskams 2005). Extensive research is being focused on the detection of key oncogenes and tumor suppressor genes regulating cell cycle and apoptosis associated with the development of RCC during recent years.

S. Rashid, *Cancer and Chemoprevention: An Overview*,
DOI 10.1007/978-981-10-2579-2_6

Oncogenes

Cells have normal genes that regulate cell proliferation, and when these genes are mutated, they lead to uncontrolled cell proliferation. Proto-oncogenes are the normal forms of these regulatory genes, while the mutated cancer-causing forms are called oncogenes (Alberts et al. 2008) which are transformed by alterations in their structures due to mutation or chromosomal rearrangement or by amplification. Common cytogenetic abnormalities in cancer cells are chromosomal inversions and translocations. When mutation occurs in a proto-oncogene, it is activated, and the structure of an encoded protein is distorted which enhances its transforming activity. Examples are the RAS oncogenes (KRAS, HRAS, and NRAS) which encode proteins with guanosine nucleotide-binding activity and intrinsic guanosine triphosphatase activity (Kufe et al. 2003).

Initiating events include mutations and translocations which occur during tumor promotion, whereas amplification particularly occurs during progression stage. Mutations in proto-oncogenes are typically dominant in nature, and the mutated proto-oncogenes become oncogenic in nature. Proto-oncogenes encode proteins that function to stimulate cell division, inhibit cell differentiation, and arrest cell death. All of these processes are essential for the maintenance of tissues and organs in normal human development. Oncogenes, on the other hand, usually exhibit increased production of these proteins resulting into increased cell division, decreased cell differentiation, and inhibition of cell death leading to extensive proliferation beyond limits (Chial 2008). Oncogenic proteins are currently modified and targeted in cancer treatment.

Tumor Suppressor Genes

Tumor suppressor genes are the genes which regulate cell growth and division and also stimulate cell death to keep the cells in balance. Some of these genes are also involved in DNA repair mechanisms which help in preventing the accumulation of mutations. Thus, tumor suppressor genes act as brakes not to allow cells to become malignant (Chial 2008). The first tumor suppressor gene, the Rb gene, was isolated in 1986. Unlike oncogenes, tumor suppressor genes in general follow the "two-hit hypothesis," which demonstrates that both alleles coding for a particular gene must be affected totally to achieve a significant effect. Because if only one allele for the gene is mutated, the other copy of allele can still generate the correct protein. In other words, mutant tumor suppressor alleles are usually recessive, whereas mutant oncogene alleles are dominant.

The two-hit hypothesis was first proposed by A.G. Knudson for cases of retinoblastoma. The methods which either eliminate or diminish the functions of the tumor suppressor genes are loss of heterozygosity, methylation, cytogenetic aberrations, genetic mutations, gain of auto inhibitory function, and polymorphism (Zingde 2001). Persons affected with von Hippel–Lindau syndrome (VHL) take over one standard copy and one malformed copy of the VHL gene, similar to

various diseases caused by deficiency of tumor suppressor gene potential. As a result of somatic mutation or deficit of the normal VHL gene function, people are prone to an extensive range of tumors which include renal cell carcinomas, retinal angiomas, cerebellar hemangioblastomas, pheochromocytomas, and so on. Additionally, some people with VHL maintain somatic modifications to both wild-type genes. This latter event is apparent in the bulk of sporadic clear cell renal carcinomas cases. p53 is one of the most important tumor suppressor genes which can be targeted for diagnosis, prognosis, and therapeutic intervention. Induction of p53 by DNA damage may act to cause cell cycle arrest or cell death by altering the transcription program of damaged cells (Chial 2008).

References

Alberts B, Johnson A et al (2008) Cancer. Molecular biology of the cell. New York: Garland Science; vol 5, pp 1231–1240

Aravalli RN, Steer CJ, Cressman EN (2008) Molecular mechanisms of hepatocellular carcinoma. Hepatology 48:2047–2063

Chial H (2008) Proto-oncogenes to oncogenes to cancer. Nature Educ 1(1):33

Croce CM (2008) Oncogenes and cancer. N Engl J Med 358:502–511

Feitelson MA, Sun B, Satiroglu Tufan NL (2002) Genetic mechanisms of hepatocarcinogenesis. Oncogene 21:2593–2604

Kojiro M, Roskams T (2005) Early hepatocellular carcinoma and dysplastic nodules. Semin Liver Dis 25:133–142

Kufe DW, Pollock RE, Weichselbaum RR et al (2003) Holland-Frei cancer medicine, 6 edn. BC Decker, Hamilton

Lee EY, Muller WJ (2010) Oncogenes and tumor suppressor genes. Cold Spring Harb Perspect Biol 2:32–36

Levy I, Sherman M (2002) Staging of hepatocellular carcinoma: assessment of the CLIP, Okuda, and Child-Pugh staging systems in a cohort of 257 patients in Toronto. Gut 50:881–885

Zingde SM (2001) Cancer genes. Curr Sci 81:508–514

Oxidative Stress-Induced Carcinogenesis 7

Oxidative Stress

Oxygen is a blessing for the existence of all the aerobic organisms on earth. Nevertheless, it plays an adverse role in biological systems by involving the phenomenon of oxidative stress. In biological systems, oxygen constantly undergoes metabolic reactions to generate oxygen-derived free radicals in the form of superoxide (O^{2-}), hydroxyl (OH), alkoxyl (RO), and peroxyl (RO2) plus non-radicals in the form of hydrogen peroxide (H_2O_2), peroxynitrite (ONOO–), hypochlorous acid (HOCl), and hypobromous acid (HOBr). Reactive species are divided into four groups depending on the main atom involved: ROS, reactive nitrogen species (RNS), reactive sulfur species (RSS), and reactive chloride species (RClS); their half-lives vary from a few nanoseconds to hours depending on the stability of the molecule. During intracellular metabolic processes like electron transport chain, ROS and RNS are also produced. Normal physiological state in a living system is achieved by a proper equilibrium between the generation and neutralization of ROS and does not lead to any oxidative damage (Roberts et al. 2009). A group of researchers illustrated that an imbalance between the generation of ROS (prooxidant) and antioxidant defense system leads to oxidative stress.

This imbalance occurs due to two reasons: either by the attenuation in the elimination of ROS by oxidant defense mechanisms or by the overproduction of ROS such as the superoxide radical or hydroxyl radical (OH) which exhausts the endogenous antioxidant reservoirs in a cell. This excessive production of ROS alters and damages various intracellular molecules like DNA, RNA, lipids, and proteins by creating nicks in DNA, malfunctions in the DNA repair system, and DNA oxidation generating 8-hydroxy-2-deoxyguanosine, which has been reported to enhance aging and carcinogenesis by developing mutations in DNA (Roberts et al. 2010; Rashid et al. 2013a). Moreover, reactive species cause oxidation of cell membrane because it is vulnerable and rich in polyunsaturated lipids, thereby inducing lipid peroxidation and consequently enhancing the permeability of cell membrane leading to cell death. The most affected by reactive species are the proteins since they undergo

S. Rashid, *Cancer and Chemoprevention: An Overview*,
DOI 10.1007/978-981-10-2579-2_7

accumulation of thiol groups (−SH) and carbonyl groups (aldehydes and ketones) which get transformed into sulfur reactive radicals resulting in the modification of the protein structure and function. Furthermore, various enzymes such as cyclooxygenases, xanthine oxidase, uncoupled NOS, and NADPH oxidases enhance the production of ROS. Additionally various anticancer drugs, e.g., doxorubicin, 5-FU (Rashid et al. 2013b), and cisplatin (Khan et al. 2012), analgesics like acetaminophen (Ahmad et al. 2011); toxicants such as acrolein; heavy metals like As, Pb, Cd, and Hg; xenobiotics; ultraviolet (UV) irradiation; environmental pollutants like oxides of nitrogen, SO_2, CO_2, etc.; and other factors contribute to ROS production. Dismutation of O_2 generates H_2O_2 via manganese superoxide dismutase which is further converted to HO radical through Fenton or Haber–Weiss reaction and produces substantial cellular lesions.

Oxidative stress is associated with an extensive variety of human diseases such as neurodegenerative diseases, inflammatory diseases, cardiovascular diseases, allergies, immune system dysfunctions, diabetes, aging, and cancer. It does so by the release of chemical mediators of inflammation from inflammatory cells, predominantly ROS. In case of chronic exposure, tremendous ROS is produced which saturates the cell defense mechanisms, i.e., antioxidants with the result of serious damage to intracellular molecules, thereby affecting surrounding neighboring cells as well. H_2O_2 drastically changes and damages structure and function of mitochondrial membrane lipids. Oxidative stress in mitochondria may result in rigorous genomic DNA lesions which may promote severe repercussions such as apoptosis. All the pathways and mechanisms involved in oxidative stress are conserved in mammalian cells. ROS upregulates several characteristics of tumor development and progression like cellular proliferation (from EGFR to mTOR), evasion of apoptosis (via PI3K/Akt activation), tissue invasion and metastasis (MMP secretion into extracellular matrix), and angiogenesis (VEGF, angiopoietin) (Rashid et al. 2013b; Poljsak et al. 2013).

Antioxidants are the cell's defense mechanisms by which scavenging of reactive species occurs and can be classified into different groups according to their properties like endogenous antioxidants including various enzymes and molecules and exogenous antioxidants which include natural and synthetic antioxidants. Endogenous antioxidants include glutathione, alpha-lipoic acid, coenzyme Q, ferritin, uric acid, bilirubin, metallothionein, L-carnitine, melatonin, enzymatic superoxide dismutase (SOD), catalase (CAT), glutathione peroxidases (GPXs), thioredoxins (TRX), and peroxiredoxins (PRXs). Natural antioxidants coexist in a delicate balance with oxidative inputs. Natural antioxidants from the diet include carotene (vitamin A), ascorbic acid (vitamin C), tocopherol (vitamin E), lipoic acid, reduced glutathione, and polyphenol metabolites, while synthetic antioxidants comprise of N-acetyl cysteine (NAC), iron, pyruvate, selenium, and desferrioxamine. SOD is said to catalyze the conversion of O_2 to H_2O_2; CAT converts H_2O_2 into H_2O and O_2. GPx catalyzes the reduction of two molecules of peroxide to produce oxidized glutathione (GSSG) using reduced glutathione (GSH) and water (Savaskan et al. 2007). Besides glutathione (GSH) along with glutathione S-transferase (GST) and glutathione reductase (GR) in combination plays a variety of vital roles in an array of antioxidant defense mechanisms (Esra et al. 2012; Kumar 2011).

Oxidative Stress-Induced Carcinogenesis

Oxidative stress is coupled with a superfluous pathological phenomenon which includes infection, inflammation, ultraviolet and γ-irradiation, overload of transition metals, exposure to certain chemical agents, etc. Association between chronic oxidative conditions and carcinogenesis has been authenticated from various human epidemiological studies revealing that multiple modifications in the original genome are chief molecular mechanisms responsible for carcinogenesis. DNA single- and double-strand breaks and cross-links are caused by excessive generation of reactive oxygen and nitrogen species and a variety of other alterations leading to transformed genome despite vigorous countermeasures endorsed by repair mechanisms and apoptotic pathways. Such changes in genetic information are called mutations which include point mutations, deletions, insertions, and chromosomal translocations and may cause constant activation of oncogenes or inactivation of tumor suppressor genes (Toyokuni 2008; Dayem et al. 2010).

References

Ahmad ST, Arjumand W, Seth A et al (2011) Preclinical renal cancer chemopreventive efficacy of geraniol by modulation of multiple molecular pathways. Toxicology 290:69–81

Dayem AA, Choi HY, Kim JH et al (2010) Role of oxidative stress in stem, cancer, and cancer stem cells. Cancers (Basel) 2:859–884

Esra B, Umit MS, Cansin S et al (2012) Oxidative stress and antioxidant defense. World Allergy Organ J 5:9–19

Khan R, Khan AQ, Qamar W et al (2012) Chrysin abrogates cisplatin induced oxidative stress, p53 expression, goblet cell disintegration and apoptotic responses in the jejunum of Wistar rats. Br J Nutr 108:1574–1585

Kumar S (2011) Free radicals and antioxidants: human and food system. Adv Appl Sci Res 2:129–135

Poljsak B, Šuput D, Milisav I (2013) Achieving the balance between ROS and antioxidants: when to use the synthetic antioxidants. Oxid Med Cell Longev 2013;Article ID 956792, 11 pages

Rashid S, Ali N, Nafees S et al (2013a) Abrogation of 5-flourouracil induced renal toxicity by bee propolis via targeting oxidative stress and inflammation in Wistar rats. J Pharm Res 7:189–194

Rashid K, Sinha K, Sil PC (2013b) An update on oxidative stress-mediated organ pathophysiology. Food Chem Toxicol 62:584–600

Roberts RA, Laskin DL, Smith CV (2009) Nitrative and oxidative stress in toxicology and disease. Toxicol Sci 112:4–16

Roberts RA, Smith RA, Safe S (2010) Toxicological and pathophysiological roles of reactive oxygen and nitrogen species. Toxicology 276:85–94

Toyokuni S (2008) Molecular mechanisms of oxidative stress-induced carcinogenesis: from epidemiology to oxygenomics. IUBMB Life 60:441–447

Major Mediators Linking Inflammation and Cancer

8

Inflammation has been considered and included as the seventh hallmark of cancer (Hanahan and Weinberg 2011). The corelation between inflammation and cancers was noticed some 150 years ago as Virchow denoted that cancers have a tendency to occur at chronic inflammatory sites, and this was known as early as 1863. However, evidence from epidemiological studies suggests that persistent inflammatory diseases are often connected with increased risk of cancers. The investigation aiming at the association between inflammation and cancers initially led to the determination whether the reactive nitrogen and oxygen species produced by inflammatory cells like leukocytes recruited to the inflammatory foci to kill infectious agents may also cause mutagenic injury, thereby resulting in the initiation of tumor.

Inflammation is a physiological process which occurs in response to tissue damage (Del Prete et al. 2011). Neutrophils are the first cells to migrate to the inflammatory sites under the regulation of molecules produced by rapidly responding macrophages and mast cells pre-stationed in tissues at the initiation stage of inflammation. Later on, various types of leukocytes, lymphocytes, and other inflammatory cells are activated and fascinated to the inflammation site by a signaling network which involves an immense number of growth factors, cytokines, and chemokines (Karin et al. 2006). A swift programmed dissolution of inflammatory cells including neighboring macrophages, dendritic cells, and backup phagocytes is needed to induce apoptosis and perform phagocytosis for the regulation of inflammation. An anti-inflammatory response is elicited by phagocytosis of apoptotic cells enhancing the production of anti-inflammatory mediator transforming growth factors. On the other hand, if inflammation is deregulated, cellular response gets converted into chronic inflammation in which inflammatory foci are dominated by lymphocytes, plasma cells, and macrophages with varying morphology. DNA damage occurs because macrophages and other inflammatory cells produce an enormous amount of growth factors, cytokines, and reactive oxygen and nitrogen species. Macrophages that remain in the activated state constantly result in continuous tissue damage. Hence a microenvironment comprised of all the above elements inhabits the

S. Rashid, *Cancer and Chemoprevention: An Overview*,
DOI 10.1007/978-981-10-2579-2_8

sustained cell proliferation induced by persistent tissue damage, thus predisposing chronic inflammation to neoplasia. Various epidemiological and clinical studies have illustrated the association between inflammation and cancer (Hussain et al. 2007; Lu et al. 2006).

Chronic inflammation is involved in all three stages of cancer, i.e., initiation, promotion, and progression. When a tissue is under constant inflammation, there is tremendous production of ROS that can cause genomic instability leading to initiation of cancer. On unregulated proliferation, a single initiated cell produces clones of mutated cells forming premalignant mass known as tumor promotion. Among them, some preneoplastic cells exhibit additional mutations and turn out to be malignant. This process is known as tumor progression. Tumor cells in proliferation with their neighboring host stromal cells and tumor-infiltrating inflammatory cells produce a tumor microenvironment that reveals a constant inflammatory condition. A variety of pro-inflammatory mediators participate in an intricate inflammatory signaling which aids in extravasation of tumor cells from the stroma, thereby encouraging tumor progression within tumor microenvironment (Simone et al. 2010; Mantovani 2010).

Tumor promotion and progression are regulated chiefly by inflammation via several mechanisms which include enhancing cell cycle progression and cell proliferation, escaping apoptosis, and activation of tumor neovascularization. Nowadays, it has been recognized that the process of development of cancers from inflammation might be driven by inflammatory cells in addition to a variety of mediators which include cytokines, chemokines, and enzymes setting up an inflammatory microenvironment (Miki et al. 2007). The most remarkable and main members implicated in the process from inflammation to cancer axis are inflammatory markers like cytokines, COX-2, prostaglandins, i-NOS, NO, chemokines, and NFkB. These inflammatory cells release diverse pro-inflammatory mediators which function in an autocrine or paracrine manner to further trigger inflammatory signaling, tumor cell to host stroma communication, and chemoattraction of furthermore inflammatory immune cells in the microenvironment.

Tumor cells interrupt the homeostasis in the surrounding normal tissue by varied mechanisms like direct cell-to-cell contact, contact between cell and extracellular matrix, and secretion of various potent factors accelerating the inflammation within premalignant tissues in the early phase of tumorigenesis. They repeatedly secrete cytokines which cause infiltration of certain inflammatory cells in the tumor microenvironment. Tumor growth is accelerated by many of these pro-inflammatory mediators by promoting angiogenesis. Mutations in proto-oncogenes or tumor suppressor genes or other genetic alterations and DNA may occur due to reactive species derived from inflammatory stress leading to initiation of carcinogenesis. Promotion and progression stages by stimulating the proliferation of initiated cells, inducing angiogenesis and metastasis, and evasion of apoptosis to neoplastic cells through genetic or epigenetic mechanisms may be contributed by inflammation (Jung et al. 2002; Kundu et al. 2008a; Colotta et al. 2009).

Cytokines

Cytokines like tumor necrosis factor-α (TNF-α), growth factors, interleukins (ILs), and differentiation factors are either secreted or membrane-bound small protein molecules which regulate varied processes involved in physiology including growth, development, differentiation, wound healing, and immune response (Miki et al. 2007). Cytokine signaling instigates binding of explicit cytokines to cell-specific cognate receptors which further activate intracellular kinases like phosphatidylionositol-3-kinase (PI3/K)/Akt, Janus-activated kinase (JAK), IKK, and MAP kinases along with consequent activation of transcription factors, primarily NFkB, STAT, and AP-1.

Cytokines are generally divided into two groups, namely, as inflammatory (IL-1, IL-6, IL-17) and anti-inflammatory (IL-10). It has been reported that some cytokines are involved in inflammation-associated carcinogenesis. Optimal conditions for cell growth within tumor microenvironment are provided by cytokines produced by cancer cells, and those secreted by stromal cells influence the behavior of malignant cells (Rigby et al. 2007; Lin and Karin 2007). Cytokine signaling can stimulate cell growth and differentiation as well as inhibition of apoptosis of transformed cells at the inflammatory site, thereby contributing to the progression of tumors (Reuter et al. 2010).

Tumor Necrotic Factor-α (TNF-α)

TNF-α plays dual role in carcinogenesis and acts as a representative inflammatory cytokine with pleiotropic functional nature, although elevated levels of TNF-α are harmful to tumor vasculature and cause necrosis. It may on the other hand activate growth of certain tumor cells and fibroblasts like epidermal growth factor (EGF) or serum-depleted cervical cancer cells inhibiting proliferation of normal cervical keratinocytes. It has been detected in various human cancers such as lymphoma, leukemia, breast, prostate, colorectum, bladder, and RCC. It has an endogenous tumor-promoting ability which has been revealed from various preclinical studies such as mice in which TNF-α or TNF-α receptor is absent and shows resistance to carcinogenesis.

A large number of studies suggest that TNF-α and chemokines are the most important molecules linking between inflammation and cancer which have been suggested from several studies. It may instigate an inflammatory cascade of other inflammatory cytokines, chemokines, growth factors, and endothelial adhesion factors thereby employing a diversity of activated cells at the site of tissue damage (Glauben et al. 2014). It has been reported that high-dose administration of TNF-α might destroy tumor vasculature and necrotizing effects on tumors. On the contrary, TNF-α has been found to be a requisite in chemically induced carcinogenesis and a major inducer of nuclear factor-kB (NFkB) activation also, which is anti-apoptotic. The paradoxical roles of TNF-α in regulating cell death might be accredited to the

varied modifications of TNF-α receptor complexes activating opposite pathways. Additionally, TNF-α acts as a growth factor for tumor cells and induces DNA damage and inhibits DNA repair. TNF-α has been found to mediate epithelial–mesenchymal transition of RCC via GSK3β and be competent of promoting proliferation and metastasis of RCC cells. TNF-α induces a variety of angiogenic factors, thymidine phosphorylase, and MMPs promoting angiogenesis and tumor growth (Balkwill and Mantovani 2001; Aung et al. 2006).

Interleukin-6 (IL-6)

IL-6 is another major pro-inflammatory cytokine in nature which participates in inflammation-associated carcinogenesis. It transforms the expression of genes involved in inhibition of apoptosis and cell cycle progression principally through JAK-STAT signaling pathway. Elevation in IL-6 levels has been implicated in the pathogenesis of diverse cancers. On the contrary, mice deficient in IL-6 are less vulnerable to development of plasmacytoma. It has been reported that IL-6 levels in serum have been found to be considerably increased and have positive correlation with tumor burden in cancer patients. In multiple myeloma, non-Hodgkin's lymphoma, bladder cancer, colorectal cancer, and renal cell carcinoma (RCC), IL-6 acts as a paracrine growth factor. Autocrine IL-6 production in RCC has been linked with the involvement of p53. RCC cell lines containing mutant p53 produced higher levels of IL-6 than those containing wild-type p53 signifying its probable role in tumorigenesis. Reports reveal that both IL-6 and IL-10 are strongly expressed in RCC stroma and cells (Fig. 8.1).

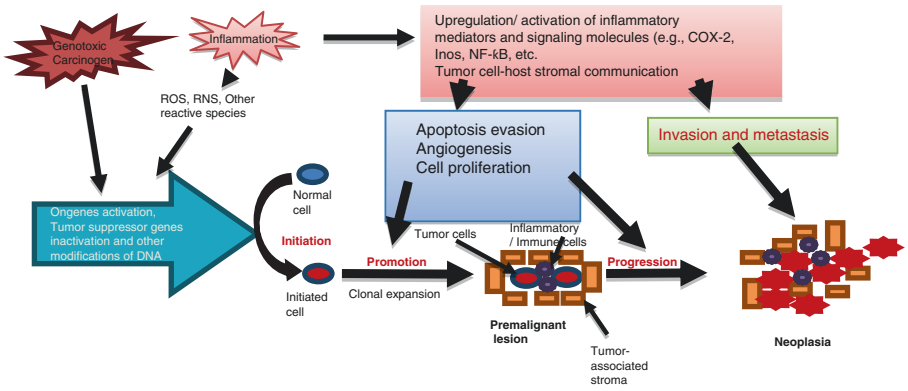


Fig. 8.1 Represents the development of cancer: implication of inflammation in multistage carcinogenesis (Adapted and modified from Kundu and Surh 2008a, b). ROS or RNS or other reactive species can attack DNA and cause mutations in oncogenes or tumor suppressor genes or other genetic alterations which lead to initiation of carcinogenesis. Inflammation also contributes to promotion and progression stages by stimulating the proliferation of initiated or premalignant cells, augmenting angiogenesis and metastasis through epigenetic mechanisms

Additionally more advanced tumors had higher IL-10 levels suggesting that IL-6 and IL-10 may be valuable markers linked with the development and progression of RCC (John Rose and Schooltink 2006; Aggarwal et al. 2006; Cozen et al. 2004). Besides, in vivo ras-induced release of IL-6 is required for the growth of implanted ras-transformed human kidney cells. It can be concluded therefore that IL-6 is fundamental for ras-driven tumorigenesis (Ancrile et al. 2007).

Nuclear Factor Kappa-B (NFkB)

NFkB is a protein bound to the kappa immunoglobulin gene enhancer found in the nuclei of B cells. In brief, mammalian NFkB transcription factors consist of five homologous subunits, i.e., RelA/p65, c-Rel, RelB, p50/NFkB1, and p52/NFkB2, which are held in the cytoplasm by specific proteins, the inhibitors of NFkBs (IkBs), and occur as dimmers. IkB kinase (IKK) complex consists of two catalytic (IKKa and IKKb) and one regulatory (IKKg/NEMO) subunits and lies immediately upstream from the IkB-bound NFkB dimmers. Numerous pathways of cell stimulation assemble to activate IKK complex and that phosphorylates IkBs, aiming them for ubiquitination and degradation by 26S proteosome which liberates NFkBs that later move to the nucleus and engages in transcriptional activities. IkB undergoes phosphorylation, ubiquitination, and proteolytic degradation via canonical IkB kinase pathway or noncanonical NFkB-inducing kinase pathway in response to extracellular stimuli including cytokines or stress (Fig. 8.2).

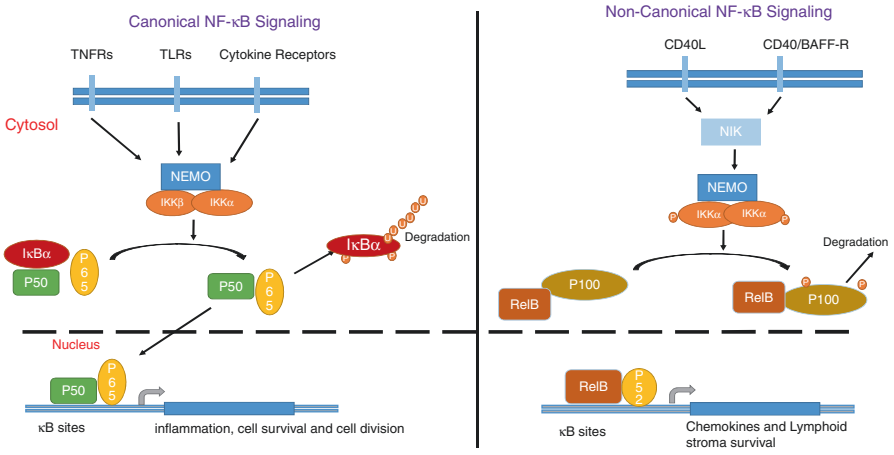


Fig. 8.2 Represents the canonical and noncanonical pathway of NFK-B (Adapted and modified from Kundu and Surh 2008a, b). The canonical NF-κB pathway (*left*) induced by signals including antigens, TLR ligands, and cytokines activate IKKβ subunit of IKK complex. IKKβ phosphorylation of classical IκB proteins bound to NF-κB dimers such as p50-p65 results in ubiquitination (Ub) of IκB and proteasome-induced degradation. This allows NF-κB to enter the nucleus where it binds specific DNA sequences (κB sites) involved in controlling the transcription of genes encoding functions as diverse as inflammation, cell survival, and division. The noncanonical pathway (*right*) requires NIK to activate IKKα, which then phosphorylates p100 (NFκB2), triggering its proteosomal processing needed for the activation of p52-RelB dimmers to control gene expression for organogenesis

Phosphorylation by casein kinase 2 also may lead to IkB degradation due to which NFkB is released and translocated to the nucleus, where it binds to the promoter regions of its target genes (Hoesel and Schmid 2013). Two most recognized pathways are canonical and noncanonical, even though there is a broadening complexity to NFkB signaling.

Canonical depends on NEMO, IKKb activation, and nuclear localization of RelA/p50 dimers and is associated with inflammation, while noncanonical depends on IKKa activation possibly via the upstream kinase NIK and nuclear localization of p52/RelB dimers, and both pathways have now been involved in carcinogenesis. NFkB activation has been associated with many cancers which include breast cancer, melanoma, lung cancer, colon cancer, multiple myeloma, pancreatic cancer, esophageal adenocarcinoma, and various types of leukemia and lymphoma (Kundu et al. 2008a). Investigators are trying to find correlation between exact functions of NFkB activation to the carcinogenesis process, tumor progression, and metastatogenesis in experimental mouse models of cancer with the beginning of recent advances. It regulates the expression of a wide array of inflammatory molecules including cytokines and adhesion factors which act as a significant mediator in progression of inflammation. It has also been reported that NFkB regulates inflammatory cell apoptosis and phagocytosis. It inhibits apoptosis by a variety of mechanisms in response to cancer. Inhibition of p53-mediated apoptosis was established through the activation of NFkB pathway in mucosa-associated lymphoid tissue lymphoma (Philpott and Ferguson 2004; O'Byrne and Dalgleish 2001; Herszenyi et al. 2007).

Besides this, it contributes to tumor development by activating cell proliferation via stimulating expression of growth factor genes, proto-oncogene c-Myc, and cell cycle regulator cyclin D1. It is stimulated by inflammatory stimuli and its constitutive activation is found in cancers. Therefore, it has long been assumed to be a vital promoter assisting the development from inflammation into cancer. NFkB may also contribute to genomic instability in two aspects. It is found to promote the generation of ROS, which are potential enough to cause mutations. Also its anti-apoptotic property averts mutated precancerous cells from being eliminated. The association between NFkBs and the induction of pro-inflammatory cytokines like IL-6 and TNF-α, chemokines like IL-8, adhesion molecules, MMPs, COX-2, and i-NOS may decipher NFkB to be implicated in linking inflammation to cancer. Direct instances for the role played by NFkB at the tumor promotion stage in the development of cancers from chronic inflammation have been proved from recent studies using different animal models. Reduction in both the tumor incidences and the sizes of tumors occurred on NFkB pathway inactivation due to decrease of several pro-inflammatory factors facilitating tumor growth which has been found in cancer (Seril et al. 2003; Cooke et al. 2003; Wang et al. 2004; Pikarsky et al. 2004).

In response to inflammatory stimuli, an extensive array of DNA-binding proteins are atypically triggered leading to improper initiation of various pro-inflammatory genes in tumor cells, in tumor-associated stromal cells, and in surrounding host tissues. There is an abnormal turn on or switch off of different transcription factors in various human malignancies, one of which is NFkB, the most broadly studied since it is present ubiquitously and serves with many several functions. It is well evident from reports that inappropriate activation of NFkB contributes to tumorigenesis either by trans-activating some target genes having inflammatory functions like COX-2, i-NOS,

and TNF-α; anti-apoptotic proteins like IAP, Bcl-2, Bcl-3, and Bcl-XL; cell cycle regulatory proteins like cyclin D1; and proangiogenic like VEGF and angiopoietin functions or by downregulating apoptosis-inducing genes like p53, Bax, and Bad. Hence, NFkB has been recognized as a promising molecular link between inflammation and cancer. Transcriptional activation of NFkB triggers induction of pro-inflammatory cytokines like IL-6 and TNF-α; chemokines like IL-8, COX-2, i-NOS, and MMP; and various adhesion molecules (Wojdasiewicz et al. 2014).

NFkB-dependent activation of cell adhesion molecules like vascular cell adhesion molecule (VCAM) and intercellular adhesion molecule (ICAM) is implicated in leukocyte adhesion, and movement in tumor microenvironment is usually found to be increased in various cancers. It regulates cytokine expression primarily, while the cytokine expression is regulated primarily by NFkB; however tumor cell-derived cytokines further promote stimulation of NFkB-mediated transcription of pro-inflammatory genes in tumor cells, tumor-associated stromal cells, and host tissues. In this manner, a constant chronic inflammatory state in tumor microenvironment is generated. NFkB inhibition has also been shown to inhibit cancer growth in human neoplasias including breast, lung, melanoma, colon, and B-cell lymphoma. Different experimental models have deciphered the role of NFkB in chronic inflammation-driven tumor promotion (Naugler and Karin 2008; Elsharkawy and Mann 2007). Oxidative stress can activate a variety of transcription factors including NFκB, AP-1, p53, HIF-1α, PPAR-γ, β-catenin/Wnt, and Nrf2. It was revealed that apoptosis in 786 0 RCC cell lines was induced via siRNA-mediated inhibition of p65. In ACHN and VMRC-RCW RCC cell lines SN50, specific NFkB inhibitory peptide reduced proliferation and nuclear translocation of NFkB. In case of RCC, the genetic approaches are restricted to in vitro and so far to be translated in vivo successfully. RCC samples revealed a considerable increase in the phosphorylated p65 and IkB as compared to normal tissues indicating that NFkB is constitutively triggered in human RCC tumors with particularly clear cell RCC which showed an elevated expression of phosphorylated p65 as compared to other subtypes.

Nevertheless no significant correlation is found between tumor grade and NFkB activation. However there is a corelation between p50 expression and tumor grade, VEGF, EGFR, Bcl-2 and p53. Nearly half of the long-term dialysis patients develop cystic changes of the kidneys, out of which approximately 6 % develop RCC. Reports suggest that upregulation of NFkB may be involved in the development of renal cysts and their consequent conversion into tumors (Morais et al. 2011). Transcriptional pathway of NFkB is implicated in many basic biological processes like immune response, inflammation, cell proliferation, apoptosis, cell migration, and angiogenesis (Sourbier et al. 2007 and Xia et al. 2014).

Cyclooxygenase-2 (COX-2)

It is an inducible form of cyclooxygenase which serves as a connecting link between inflammation and cancer. In response to various external stimuli, it is rapidly upregulated in certain tissues via pro-inflammatory cytokines, bacterial LPS, UV, ROS, and chemicals. Its oncogenic function can be endorsed to its facilitation in cellular proliferation, inhibition of apoptosis, augmentation of angiogenesis, and invasiveness.

It has been found in the pathogenesis of various types of malignancies that there is an anomalous induction of COX-2 (Cyril et al. 2010). Genetically engineered mice were made to overexpress COX-2 in the mammary glands, skin, or stomach which resulted in developing malignancies in these organs, whereas COX-2 knockout mice are found to be less susceptible to intestinal tumorigenesis, skin papillomagenesis, and mammary carcinogenesis. Either functional inactivation of COX-2 in adenomatous polyposis coli (APC) D716 knockout mice, a murine model of human adenomatous polyposis demonstrating both reduction in the number and size of intestinal polyps, or the administration of rofecoxib, a selective COX-2 inhibitor, provides evidence about association between anomalous upregulation of COX-2 and tumorigenesis. In case of chronic UV-induced skin carcinogenesis model, there was 50–65 % decrease in tumor multiplicity and a discernible reduction in tumor size in SKH-1 hairless mice due to the lack of one allele of COX-2, whereas transgenic mice overexpressing COX-2 developed 70 % more tumors than wild-type mice.

It has been reported that inhibition of COX-2 pharmacologically by celecoxib in rats impeded the development of esophageal inflammation to metaplasia and adenocarcinoma. But it has been reported to enhance the development and progression of cancer in a state of chronic inflammation. COX-2 is upregulated in many cancers including RCC. Furthermore, COX-2 and IGF-IR may generate a synergistic effect in the oncogenesis and progression of RCC. Currently, sunitinib used in clinics is a first-line drug targeting VEGF pathways for the treatment of advanced RCC. It is documented that COX-2 inhibition enhanced the action of sunitinib in human RCC. COX-2 is strong biomarker than p53 for the development of metastases in RCC (Zhang et al. 2014; Muller-Decker et al. 2002; Lu et al. 2006). Overexpression of COX-2 triggers the upregulation of anti-apoptotic proteins like Mcl-1 and Bcl-2, induces sustained cellular proliferation of angiogenic factors like VEGF-A, and escalates metastasis (Fig. 8.3).

Inducible Nitric Oxide Synthase (i-NOS)

i-NOS is an enzyme whose expression is induced in macrophages and epithelial cells which leads to production of NO endogenously during arginine metabolism and during inflammation via different isoforms of NOS, another important inflammatory mediator linking chronic inflammation and cancer. It is found to be upregulated in chronic inflammation and associated diseases as well as various types of cancers. It gets transactivated by some transcription factors like NFkB, besides response to inflammatory cytokines (TNF-α and IL-1b) and other inflammatory stimuli (chemicals, environmental pollutants, UVB, LPS, and DSS). Various carcinomas have been detected with overexpression of i-NOS (David et al. 2011). From a clinical study, analysis of an isolated prostate cancer has revealed strong i-NOS expression to be positively correlated with rapid cancer cell proliferation, dedifferentiation, and progression to advance stage cancer.

Reider et al. confirmed elevated expression of i-NOS to be associated with the development of intestinal metaplasia in the biopsy specimens from patients with

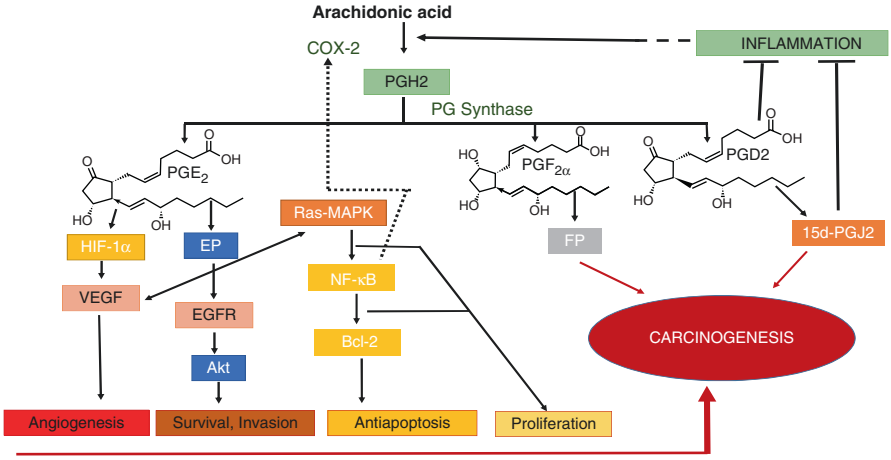


Fig. 8.3 Represents the role of COX-2 and PGs in inflammation-induced carcinogenesis (Adapted and modified from Sonawane et al. 2011). PGs are derived mainly from arachidonic acid released from membrane by phospholipases. Inflammatory signaling activates initiation of COX-2 expression and consequently production of group of prostaglandins. Cox-2 catalyzes the conversion of arachidonic acid to PGG2 and then to PGH2 which is subsequently converted to various physiologically active prostanoids, prostacyclin and thromboxane, by the relevant enzymes in a variety of cell types. COX-2 derived PGE2 can activate EGFR signaling and thereby stimulate cell proliferation. PGE2 stimulates angiogenesis via the transcription factor hypoxia-inducible factor-1 (HIF-1 alpha) leading to the induction of VEGF. COX-2-derived PGs regulate programmed cell death and reduce the apoptotic rate via inhibition of the mitochondrial apoptotic pathway. COX-2 derived PGs play a key role in the tumorigenesis. The tumor-promoting effect of PGs is due to their ability to stimulate cell proliferation and migration, inhibit the apoptosis, and increase angiogenesis. PGE2 is associated with carcinogenesis; others (e.g., PGI2) have cytoprotective effects. Another group of prostaglandins comprising PGD2 and 15d-PGJ2 PGDH inactivate PGE2 and act as a tumor suppressor

stomach carcinoma as well as *H. pylori*-induced gastritis. Ulcerative colitis patients had overexpression of i-NOS in colon tissues suggesting that i-NOS may contribute to the pathogenesis of colitis-related neoplasia. It was revealed that ablation of i-NOS genetically resulted in 80 % decrease in mouse lung tumorigenesis. In an animal model study, selective NOS inhibitor was found to prevent the progression of rat esophageal tumorigenesis induced by N-nitrosomethylbenzylamine. In chronic inflammation, the continuous generation of NO may lead to DNA damage, disruption of DNA repair, and cancer-prone posttranslational modification. NO is an imperative regulatory signaling molecule in inflammation response as well as cancer development. Trans-repression of i-NOS expression and NO production and increased expression of i-NOS in p53 knockout mice imply that the loss of wild-type p53 by oxidative or nitrosative stress during chronic inflammation may obstruct p53-mediated negative regulation of i-NOS, as a result enhancing NO production with successive stimulation of NO-dependent angiogenic process (Hussain and Harris 2007; Rieder et al. 2003; Kundu and Surh 2008a, b; Landskron et al. 2014).

References

Aggarwal BB, Shishodia S, Sandur SK et al (2006) Inflammation and cancer: how hot is the link. Biochem Pharmacol 72:1605–1621

Ancrile B, Lim KH, Counter CM (2007) Oncogenic Ras-induced secretion of IL-6 is required for tumorigenesis. Genes Dev 21:1714–1719

Aung HT, Schroder K, Himes SR et al (2006) LPS regulates proinflammatory gene expression in macrophages by altering histone deacetylase expression. FASEB J 20:1315–1327

Balkwill F, Mantovani A (2001) Inflammation and cancer: back to Virchow? Lancet 357:539–545

Colotta F, Allavena P, Sica A et al (2009) Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. Carcinogenesis 30:1073–1081

Cooke MS, Evans MD, Dizdaroglu M et al (2003) Oxidative DNA damage: mechanisms, mutation, and disease. FASEB J 17:1195–1214

Cozen W, Gill PS, Ingles SA et al (2004) IL-6 levels and genotype are associated with risk of young adult Hodgkin lymphoma. Blood 103:3216–3221

Cyril S, Claudia C, Mario D et al (2010) The role of cyclooxygenase-2 in cell proliferation and cell death in human malignancies. Int J Cell Biol 2010;Article ID 215158, 21 pages

David AW, Harry BH, Robert YSC et al (2011) Nitric oxide and redox mechanisms in the immune response. J Leukoc Biol 89:873–891

Del Prete A, Allavena P, Santoro G et al (2011) Molecular pathways in cancer-related inflammation. Biochem Med (Zagreb) 21:264–275

Elsharkawy AM, Mann DA (2007) Nuclear factor-kB and the hepatic inflammation fibrosis- cancer axis. Hepatology 46:590–597

Glauben L, De La Fuente M, Peti T et al (2014) Chronic inflammation and cytokines in the tumor microenvironment. J Immunol Res 19:149–163

Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144:646–674

Herszenyi L, Miheller P, Tulassay Z (2007) Carcinogenesis in inflammatory bowel disease. Dig Dis 25:267–269

Hoesel B, Schmid JA (2013) The complexity of NF-κB signaling in inflammation and cancer. Mol Cancer 2:12–86

Hussain SP, Harris CC (2007) Inflammation and cancer: An ancient link with novel potentials. Int J Cancer 121:2373–2380

John Rose S, Schooltink H (2006) Cytokines are a therapeutic target for the prevention of inflammation-induced cancers. Recent Results Cancer Res 174:57–66

Jung YD, Fan F, McConkey DJ et al (2002) Role of P38 MAPK, AP-1, and NFKB in interleukin-1b-induced IL-8 expression in human vascular smooth muscle cells. Cytokine 18:206–213

Karin M, Lawrence T, Nizet V (2006) Innate immunity gone awry: linking microbial infections to chronic inflammation and cancer. Cell 124:823–835

Kundu JK, Surh YJ (2008a) Cancer chemopreventive and therapeutic potential of resveratrol: Mechanistic perspectives. Cancer Lett 269:243–261

Kundu JK, Surh YJ (2008b) Inflammation: gearing the journey to cancer. Mutat Res 659:15–30

Landskron G, Fuente MDL, Thuwajit P (2014) Chronic inflammation and cytokines in the tumor microenvironment. J Immunol Res 2014;Article ID 149185, 1–19

Lin WW, Karin M (2007) A cytokine-mediated link between innate immunity, inflammation, and cancer. J Clin Invest 117:1175–1183

Lu H, Ouyang W, Huang C (2006) Inflammation, a key event in cancer development. Mol Cancer Res 4:221–233

Mantovani A (2010) Molecular pathways linking inflammation and cancer. Curr Mol Med 10:369–373

Miki C, Inoue Y, Araki T et al (2007) Cytokines and cancer development. J Surg Oncol 95:10–11

Morais C, Gobe G, Johnson DW et al (2011) The emerging role of nuclear factor kappa B in renal cell carcinoma. Int J Biochem Cell Biol 43:1537–1549

Muller-Decker K, Neufang G, Berger I et al (2002) Transgenic cyclooxygenase-2 overexpression sensitizes mouse skin for carcinogenesis. Proc Natl Acad Sci 99:12483–12488

Naugler WE, Karin M (2008) NF-kappaB and cancer-identifying targets and mechanisms. Curr Opin Genet Dev 18:19–26

O'Byrne KJ, Dalgleish AG (2001) Chronic immune activation and inflammation as the cause of malignancy. Br J Cancer 85:473–483

Philpott M, Ferguson LR (2004) Immunonutrition and cancer. Mutat Res 551:29–42

Pikarsky E, Porat RM, Stein I et al (2004) NFKB functions as a tumour promoter in inflammation-associated cancer. Nature 431:461–466

Reuter S, Gupta SC, Chaturvedi MM et al (2010) Oxidative stress, inflammation, and cancer: how are they linked? Free Radic Biol Med 49:1603–1616

Rieder G, Hofmann JA, Hatz RA et al (2003) Up-regulation of inducible nitric oxide synthase in Helicobacter pylori-associated gastritis may represent an increased risk factor to develop gastric carcinoma of the intestinal type. Int J Med Microbiol 293:403–412

Rigby RJ, Simmons JG, Greenhalgh CJ et al (2007) Suppressor of cytokine signaling 3 (SOCS3) limits damage-induced crypt hyper-proliferation and inflammation-associated tumorigenesis in the colon. Oncogene 26:4833–4841

Seril DN, Liao J, Yang GY et al (2003) Oxidative stress and ulcerative colitis associated carcinogenesis: studies in humans and animal models. Carcinogenesis 24:353–362

Simone R, Subash C, Gupta MMC et al (2010) Oxidative stress, inflammation, and cancer: how are they linked? Free Radic Biol Med 49:1603–1616

Sonawane CS, Jagdale DM, Kadam VJ (2011) Role of cyclooxygenase – 2 in cancer. IJRPC 1:2231–2781

Sourbier C, Danilin S, Lindner V et al (2007) Targeting the nuclear factor-KB rescue pathway has promising future in human renal cell carcinoma therapy. Cancer Res 67:11668–11676

Wang D, Wang H, Shi Q et al (2004) Prostaglandin E2 promotes colorectal adenoma growth via transactivation of the nuclear peroxisome proliferator-activated receptor delta. Cancer Cell 6:285–295

Wojdasiewicz P, Poniatowski LA, Szukiewicz D (2014) The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of osteoarthritis. Mediators Inflammation 2014;Article ID 561459, 19 pages

Xia Y, Shen S, Verma IM (2014) NF-κB, an active player in human cancers. Cancer Immunol Res 2:823

Zhang GM, Zhu Y, Ye DW (2014) Metabolic syndrome and renal cell carcinoma. World J Surg Oncol 12:236

Apoptosis

9

It is a widespread phenomenon of planned cell loss that has a crucial function in a multitude of physiological and pathological courses. The phrase apoptosis is originated from a Greek word implicating falling off or dropping off. One of the essential features for the progress and persistence of the biological system of a living being is the steady-state equilibrium between propagation of cells and their loss. It was studied first in a nematode *Caenorhabditis elegans* at some point in its development phase. It has since then been documented and established as a characteristic and an essential manner of planned/programmed cell death (Sankari et al. 2012). Cell death during apoptosis can be divided into two phases. Initially biochemical intermediaries try to repair an injured cell, and if they fall short, subsequently, the cell proceeds to the next stage also called as execution stage. In this stage, changes in the cell structure/morphology involving nuclear changes and alterations in the cell membrane and in the intracytoplasmic organelles occur, therefore leading to cell death. Changes in both chromatin and nuclear membrane occur in the nucleus. Chromatin becomes dense clumps shifting toward the nuclear membrane. Redistribution of nuclear pores occurs, although the nuclear membranes remain intact, along with changes in nuclear protein.

There is degradation of DNA in mitochondria as well as loss of mitochondrial transmembrane potential. There is deformation of cytoplasmic membrane of apoptotic cell which leads to blebbing. There is loss of endoplasmic reticulum via widening and fusion of cisternae and change in the direction of phospholipid cell membrane, and coming in contact with the outside environment, the portion of the cell membrane forms apoptotic bodies. Phagocytes engulf apoptotic bodies when released in the outer location resulting in no inflammatory reaction (Chaabane et al. 2013). However there is instigation of proteolytic enzymes at molecular level which propagate the breaking of DNA into oligonucleosomal portions and a huge number of explicit protein substrates. Apoptosis is a firmly regulated and regimented cell death mechanism involving several features. Every cell has inherent mechanisms indicating death or survival, and discrepancy in such indications leads to apoptosis (Cheung et al. 2012; Elmore 2007; Gewies 2003).

S. Rashid, *Cancer and Chemoprevention: An Overview*,
DOI 10.1007/978-981-10-2579-2_9

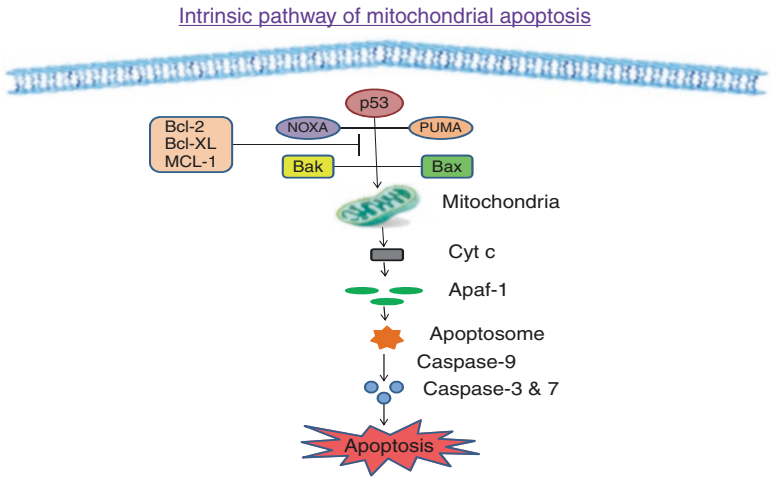


Fig. 9.1 represents the intrinsic signaling pathway for apoptosis that implicates non-receptor-mediated intracellular signals, persuading activities in the mitochondria that initiate apoptosis (Adapted and modified from Rastogi et al. 2009). Stimuli for the intrinsic pathway cause damage to the cellular DNA which induces the activation of the intrinsic pathway for apoptosis that results in the loss of transmembrane potential, causing the release of pro-apoptotic proteins into the cytosol. Pro-apoptotic proteins activate caspases that mediate the destruction of the cell through many pathways. These proteins also translocate into the cellular nucleus. The regulation of pro-apoptotic events in the mitochondria occurs through activity of members of the Bcl-2 family of proteins and the tumor suppressor protein p53

Caspases play a foremost and an essential task in apoptosis. Caspases is the word obtained from cysteine-dependent aspartate-specific proteases which are activated by three signal transduction pathways, namely, intrinsic (mitochondrial) pathway, extrinsic pathway or death receptor-dependent pathway, and lately identified intrinsic endoplasmic reticulum pathway which is less understood. Intrinsic and extrinsic pathways lead eventually to regular pathway or the execution pathway of apoptosis (Sankari et al. 2015) (Fig. 9.1).

Intrinsic Pathway

Various anomalous factors such as both extra- and intracellular stresses like oxidative stress, hypoxia, cytotoxic drugs, genetic aberrations, irradiation, and high quantity of calcium ions in cytosol elicit the activation of intrinsic apoptotic pathway elevating permeability of mitochondria. There is activation of apoptogenic factors like cytochrome c to be released from the mitochondrial inter-membrane space to the cytosol on apoptotic stimuli. This pathway is regulated by a group of proteins belonging to Bcl-2 family and is initiated within the cell. Bcl-2 family proteins consist of two groups, i.e., pro-apoptotic and anti-apoptotic proteins. Pro-apoptotic family proteins include Bax, Bak, Bad, Bcl-Xs, Bid, Bik, Bim, and Hrk, and

anti-apoptotic family proteins include Bcl-2, Bcl-XL, Bcl-W, Bfl-1, and Mcl-1. Pro-apoptotic proteins proceed to leak cytochrome c from mitochondria, while anti-apoptotic proteins cause their obstruction. However, balance between pro- and anti-apoptotic proteins is responsible for the initiation of apoptosis (Hassen et al. 2012; Rahman et al. 2012).

Consequently cytochrome c protein is released in cytoplasm forming apoptosome. Apoptosome consists of cytochrome c, apoptotic protease-activating factor-1(APAF-1), and caspase-9, formed on release of cytochrome protein in cytoplasm-activating caspase-3. Additionally, there are other apoptotic factors like apoptosis-inducing factor (AIF), second mitochondria-derived activator of caspase (Smac), and direct inhibitor of apoptosis protein (IAP)-binding proteins which are released from the mitochondrial inter-membrane space into the cytoplasm. Smac binds to IAPs triggering caspase activation which subsequently leads to interference in the interaction of IAPs with caspase-3 or caspase-9. The major plan of curative approaches for malignancy is to repair the equilibrium between deterioration and propagation of cells. Macrophages and neighboring cells effectively remove the products of cell death in case of apoptosis occurring as a normal physiological process, while in diseased environment, organization/system is damaged and noticeable measure of cell death products can get collected in the circulation.

The tumor suppressor genes have fundamental function in the protection and resistance against cancer progression. Some genes are reported to be deficient in their function in 50 % of all human cancers. Bcl-2 gene expression modification in tumor cells adds considerably to cancer growth and survival via direct suppression of apoptosis. A steady state is retained in the human body between cells formed by mitosis and cell death by apoptosis. The apoptotic factors like AIF, Smac, direct IAP-binding protein with low pI (DIABLO), and Omi/high-temperature requirement protein A (HtrA2) (Rebecca 2011; Rahman et al. 2012) are released from the mitochondrial inter-membrane space into the cytoplasm. Smac/DIABLO or Omi/HtrA2 binds to IAPs and cause caspase activation which subsequently leads to disruption in the interaction of IAPs with caspase-3 or caspase-9. Apoptotic signaling mechanism understanding is highly appreciable as its deregulation is involved in an extensive array of diseases. Thus understanding apoptotic mechanisms has provided insights to develop competent and explicit curative/beneficial strategies such as targeted stimulation of pro-apoptotic tumor suppressor genes or the obstruction of anti-apoptotic oncogenes in malignant states and management of untimely cell death in neurodegenerative diseases (Schroder and Kaufman 2005 and Sankari et al. 2012).

Extrinsic Pathway

This pathway is paramount by the commencement of cell surface receptors, namely, death receptors that pass on apoptotic signals after ligation with particular ligands. TNFR1 (tumor necrosis factor receptor 1)-related protein called Fas and their ligands, TNF and Fas ligand (FasL), are the known death receptors. These

receptors have an intracellular death domain which makes way for adaptor proteins. These are TNF receptor-associated death domain (TRADD), Fas-associated death domain (FADD), and cysteine proteases like caspase-8. When death ligand binds to its death receptor which in turn creates a binding site, an adaptor protein is formed. The absolute ligand receptor adaptor protein assembly is known as the death-inducing signaling complex (DISC). The DISC instigates the complex and stimulates pro-caspase-8 which on activation further activates caspase-8 and proceeds toward downstream effector caspases which afterward cut particular substrates leading to apoptosis (Rebecca 2011; Plati et al. 2011; Belizário et al. 2015; Ghavami et al. 2009).

References

Belizário J, Vieira-Cordeiro L, Enns S (2015) Necroptotic cell death signaling and execution pathway: lessons from Knockout mice. Mediators Inflamm Article ID 128076

Chaabane W, User SD, El-Gazzah M (2013) Autophagy, apoptosis, mitoptosis and necrosis: interdependence between those pathways and effects on cancer. Arch Immunol Ther Exp (Warsz) 61:43–58

Cheung H-H, Liu X, Rennert OM (2012) Apoptosis: reprogramming and the fate of mature cells. ISRN Cell Biol 2012:1–8

Elmore S (2007) Apoptosis: a review of programmed cell death. Toxicol Pathol 35:495–516

Gewies A (2003) Apo review – introduction to apoptosis. 1–26.

Ghavami S, Hashemi M, Ande SR et al (2009) Apoptosis and cancer: mutations within caspase genes. J Med Genet 46:497–510

Hassen S, Ali N, Chowdhury P (2012) Molecular signaling mechanisms of apoptosis in hereditary non-polyposis colorectal cancer. World J Gastrointest Pathophysiol 3:7–79

Plati J, Bucur O, Khosravi-Fara R (2011) Apoptotic cell signaling in cancer progression and therapy. Integr Biol (Camb) 3:279–296

Rahman A, Sultan T, Islam R (2012) Apoptosis and cancer: insights molecular mechanisms and treatments. Int J Biomol Biomed 2:1–16

Rastogi RP, Sinha R, Sinha RP (2009) Apoptosis: molecular mechanisms and pathogenicity. EXCLI J 8:155–181

Rebecca SYW (2011) Apoptosis in cancer: from pathogenesis to treatment. J Exp Clin Cancer Res 30:1–14

Sankari SL, Masthan KM, Babu NA et al (2012) Apoptosis in cancer–an update. Asian Pac J Cancer Prev 13:4873–4878

Sankari SL, Babu NA, Rajesh E et al (2015) Apoptosis in immune-mediated diseases. J Pharm Bioallied Sci 7:S200–S202

Schroder M, Kaufman RJ (2005) The mammalian unfolded protein response. Annu Rev Biochem 74:739–789

Chemoprevention

10

Cancer is a worldwide health burden with diagnosis in 1.6 million new cases and resulted in more than 585,000 deaths in the USA as per prediction reports of 2014 (Siegel et al. 2014). There is an improvement in cancer treatment with more efficient drugs being better safety options and target oriented over the last two decades. Unnecessary side effects constitute a major problem in spite of the progresses made. Incidences of cancer are increasing worldwide and so are the costs of new treatment, i.e., newer cancer treatments are costly. Ponatinib, a tyrosine kinase inhibitor which is used for the treatment of chronic myeloid leukemia, costs approximately $140000 (USD) per patient per year (Ferlay et al. 2015). There was 34 % per GDP capita in 1995–1999, 53 % in 2000–2004, and 67 % in 2005–2009 was the nonhormonal drug cost for the average course of cancer treatment (Savage 2012) which signifies the trend is increasing and no. of treatment options are limiting accepted by the National Institute for Health and Care Excellence (NICE) in the UK.

Therefore the best intervention would be prevention. Some of these costly treatments could be evaded if the development of cancer could be prevented or reduced benefitting health-care providers globally by preventing or lessening the onsets of cancer. Currently, cancer therapy regimens used are antiproliferative but are less effective in terms of anti-invasive and anti-metastases. Even though more target-selective drugs have been developed lately with improved results for patients by ameliorating unwanted side effects, benefits are being achieved by the combination of these with the concept of stratified medicine.

Patients selected for stratified medication aim that those who benefit from drug A will be given drug A and similarly drug B will be prescribed to those who benefit from drug B comprising the five rights: the right drug for the right disease to the right patient at the right time with the right dose. Although these approaches are beneficial, the option for management of treatment of cancer after development can be dealt by chemoprevention. It provides a sigh of relief for the health-care providers internationally under economic pressure to provide therapy besides being highly advantageous for the patients. Tamoxifen was the first chemopreventive agent

S. Rashid, *Cancer and Chemoprevention: An Overview*,
DOI 10.1007/978-981-10-2579-2_10

approved in 1998 by the FDA (Lippman and Brown 1999). Conversely, the concept of chemoprevention has not been accepted extensively (Pennya and Wallace 2015).

The term cancer chemoprevention was given by Dr. Michael B. Sporn in 1976 to describe the use of natural or synthetic compounds to repeal, repress, or thwart the cancer growth to malignancy (Singh 2000). Later Lee W. Wattenberg defined it as administration of one or several chemical compounds to prevent cancer and called it chemoprophylaxis (Laine 2001). It is obligatory to understand that if one is diagnosed with cancer, the patient is kept under the management of a medical team comprising of an oncologist, surgical oncologist, and radiation oncologist or as needed. Chemoprevention has the efficacy to suppress disease progression hence to avoid bewilderment; if cancer has to occur, it will occur. Nevertheless intervention by chemoprevention during cancer is promising but when not at malignant state. Mukhtar et al. (2016) depicts chemoprevention as slowing the process of carcinogenesis which he has best explained while giving an example of prostate cancer chemoprevention. If prostate cancer development process is slowed down, then disease could occur around 80–100 years. Here, if one could aim to slow the process of cancer development, then one could envision the disease to occur around 80–100 years of age instead at customary age of 40–50 years. Therefore slowing of disease progression concept may be applied to most solid malignancies like breast, colon, lung, and bladder (Rouzer and Marnett 2009; Mukhtar 2012).

Only mild improvement has been made in diminishing the morbidity and mortality of this lethal disease since it comprises the massive cause of deaths worldwide. Tumorigenesis is a multistep process which has been deciphered from various widespread preclinical and clinical researches which has led to the insights that most human malignancies need to be fought on manifold faces. Accordingly, cancer prevention is an essential approach to manage cancer besides cancer therapy (Hail 2005; Sun et al. 2004). Keeping away from known cancer-causing agents, bettering lifestyle, improvement of host defense mechanisms against cancer, and chemoprevention are a few common prevention strategies.

As the matter of fact, a potent chemopreventive agent mediates prematurely in carcinogenesis process to eradicate premalignant cells so that there is no chance of malignancy or shield normal cells from undergoing conversion which is hard to execute. Since otherwise healthy individuals may possibly require exposure of a particular chemopreventive agent lifelong to attain effectiveness, it could be rationally disagreed that by evading exposure to known cancer-causing agents and consuming a balanced diet, the equivalent benefit could be derived. This methodology is currently favored for the design of most human cancer chemoprevention trials so far.

Soon after introduction of chemoprevention, the synthetic analogs of vitamin A were evaluated for their chemopreventive potential. Conclusions drawn from then were that retinol (vitamin A) was not as efficient as all-*trans*-retinoic acid, since it exhibited teratogenicity coupled with toxicity leading to the production of analogs of retinoic acid with the aim of generating a less toxic and more effective chemopreventive compound. Wattenberg gave the concept of selective inhibition of carcinogenesis during initiation, promotion, or progression stages. Since then the field of cancer chemoprevention has explored.

It has been acknowledged that prevention of every disease is better than its treatment. It has been reported that the healthy diets along with exercise prevent the onset of various diseases including cancer. Ayurveda is an ancient system of medicine that originated

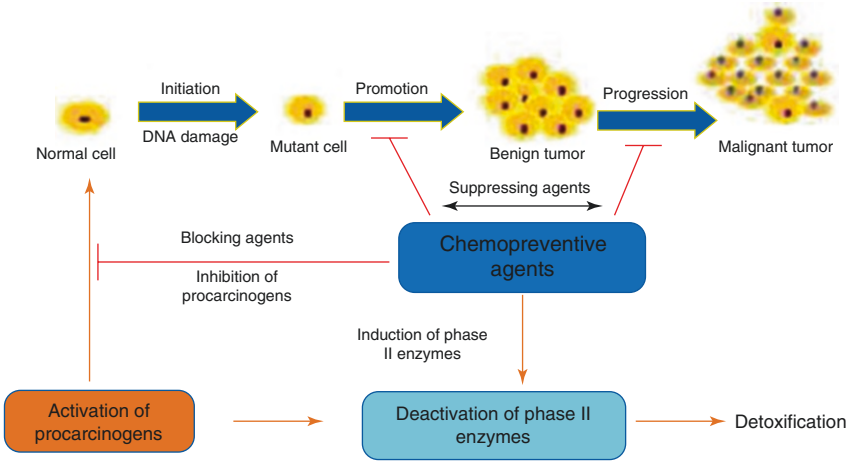


Fig. 10.1 represents diagrammatic representation of chemopreventive mechanism (Adapted and modified from Surh 1999). Carcinogenesis is initiated with the transformation of the normal cell into a cancer cell (initiated cell). These cells undergo tumor promotion into preneoplastic cells, which progress to neoplastic cells. Some chemopreventive phytochemicals (blocking agents) inhibit metabolic activation of procarcinogens to their ultimate electrophilic species or their subsequent interaction with DNA and therefore block tumor initiation or detoxify carcinogens. Other phytochemicals (suppressing agents) suppress promotion and progression of multistage carcinogenesis

in India some centuries ago and is still being practiced efficiently in various parts of the world based on the idea of using natural products to prevent and treat diseases (Balachandran and Govindrajan 2005). Likewise, traditional Chinese medicine (TCM) also symbolizes an ancient culture which uses natural products to maintain health and cure diseases (Xutian et al. 2009). Recently cytotoxic activity of many plants used in traditional medicine has been established against different cancer cell lines (Mena-Rajon et al. 2009). Hence, the use of natural products for the prevention of disease is not a new concept. The literature shows that ancient civilizations used herbs or natural products to circumvent unnecessary toxic effects of the therapeutic agents.

Currently, the question of toxicity persists to be a subject of big concern. For instance, the use of chemotherapeutic agents in cancer patients is often associated with varied toxicities owing to the noxious nature of the drugs. These side effects are acceptable provided that benefits surpass the hazard. Similarly, chemopreventive agents recommended for the healthy population must not have toxicity accompanied with their use in order to be acceptable. There has been an enormous emphasis on studying diet-derived compounds as possible chemopreventive agents. Various phytochemicals and their synthetic analogs have been explored for their chemopreventive efficiency. Experimental carcinogenesis models of the mammary gland, colon, prostate, lung, skin, pancreas, and esophagus are in clinical studies (Naithani et al. 2008). The main objective of the current study in cancer prevention is to find out the mechanisms of action of the phytochemicals and plant chemopreventive agents known to inhibit one or more stages of carcinogenesis, i.e., initiation, promotion, and progression. The basic hypothesis underlying thesis studies is that simultaneous treatment with various natural agents or synthetic compounds to inhibit and suppress different stages of carcinogenesis leads to prevention of cancer (Fig. 10.1).

Characteristics of an Ideal Chemopreventive Agent

An ideal chemopreventive compound is expected to mediate at the beginning stage of carcinogenesis to remove premalignant cells so that malignancy is not acquired. They do so by impeding or interfering in the promotion or progression of premalignant or malignant cells by modulating cell proliferation or differentiation (Hail 2005; Sun et al. 2004). Hence individuals with elevated risk of cancer development should be chronically administered with a chemopreventive agent.

- It should have excellent bioavailability at the targeted site with more than one mechanism of action so that it can fight the disease on multiple fronts.
- Moreover, it should be potent and efficient, easily administrable, affordable, least toxic, and available.
- Epidemiological studies and long-term exposure of dietary compounds to humans displaying lack of toxicity make them ideal compounds for use.
- These dietary agents can retard or prevent the process of carcinogenesis by multiple mechanisms, namely:
  - Enhanced detoxification of the carcinogenic intermediates through induction of phase II drug metabolism
  - Suppression of cytochrome P450-dependent monooxygenases function resulting in reduced carcinogenic activation
  - Perturbations in cell cycle events
  - Promotion of apoptosis selectively in cancerous or precancerous cells
  - Suppression of angiogenesis and metastasis (Stan et al. 2010)

Dietary compounds mediating apoptosis can have a vital effect on carcinogenesis as apoptosis offers a physiologic method for eradicating abnormal cells. Dietary interventions may stimulate apoptosis in precancerous cells proposing that it may be a plausible cancer defensive mechanism. Apoptosis may also damage initiated cells prior to their conversion into malignant state and further progression. Most human cancers should be managed and treated in numerous ways that have been conceived by the deep insights in the pathogenesis of cancer. Therefore cancer prevention is an important strategy of regulating cancer besides improvised traditional and conventional cancer therapies. In addition to cancer therapy, it has become an essential means of controlling cancer (Sun et al. 2004). Common prevention approaches include evading contact with procarcinogens and carcinogens, improvement in efficiency of host defense mechanisms against cancer, lifestyle adaptations, and chemoprevention (Sun et al. 2004) (Fig. 10.2).

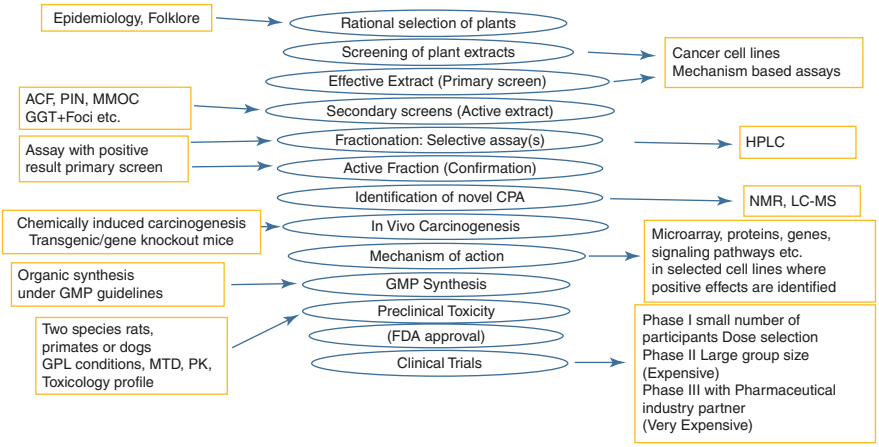


Fig. 10.2 represents various screening strategies of selection of a new phytocompound as a chemopreventive agent (Adapted and modified fig. from Mehta 2014). Plants are selected depending on their prior medicinal values and in the form of extracts are evaluated in vitro to investigate various cellular mechanisms and properties which are then taken to in vivo systems if found efficient enough. The compounds are then fractionated to find the efficacy at individual level purposely to develop more effective and less toxic chemopreventive agents

References

Balachandran P, Govindrajan R (2005) Cancer an Ayurvedic perspective. Pharmacol Res 51:19–30

Ferlay J, Soerjomataram I, Dikshit R et al (2015) Int J Cancer 136:359–386

Hail N Jr (2005) Mitochondria, a novel target for the chemoprevention of cancer. Apoptosis 10:687–705

Laine L (2001) Approaches to nonsteroidal anti-inflammatory drug use in the high-risk patient. Gastroenterology 120:594–606

Lippman SM, Brown PH (1999) Tamoxifen prevention of breast cancer, an instance of the fingerpost. J Natl Cancer Inst 91:1809–1819

Mehta GR (2014) Current paradigms of cancer chemoprevention. Turk J Biol 38:839–847

Mena-Rajon G, Caamal-Fuentes E, Cantillo-Ciau Z et al (2009) *In vitro* cytotoxic activity of nine plants used in Mayan Traditional medicine. Ethnopharmacol 121:462–465

Mukhtar H (2012) Chemoprevention, making it a success story for controlling human cancer. Cancer Lett 326:123–127

Mukhtar E, Adhami VM, Siddiqui IA, Verma AK, Mukhtar H (2016) Fisetin Enhances Chemotherapeutic Effect of Cabazitaxel against Human Prostate Cancer Cells. Mol Cancer Ther 15(12):2863–2874. Epub 2016

Naithani R, Huma LC et al (2008) Comprehensive review of cancer chemopreventive agents evaluated in experimental carcinogenesis models and clinical trials. Curr Med Chem 15: 1044–1071

Pennya LK, Wallace HM (2015) The challenges for cancer chemoprevention. Chem Soc Rev 44:8836–8847

Rouzer CA, Marnett LJ (2009) Cyclooxygenases, structural and functional insights. Lipid Res 50:29–34

Savage PJ (2012) Development and economic trends in cancer therapeutic drugs in the UK from 1955 to 2009. Oncol Pharm Pract 18:52–56

Siegel R, Ma J, Zou Z et al (2014) Cancer statistics. CA Cancer J Clin 64:9–29
Singh G (2000) Gastrointestinal complications of prescription and over-the-counter nonsteroidal anti-inflammatory drugs, a view from the ARAMIS database. Arthritis, Rheumatism, and Aging Medical Information System. Am J Ther 7:115–121
Stan SD, Singh SV, Brand RE (2010) Chemoprevention strategies for pancreatic cancer. Nature reviews Gastroenterology & hepatology. 7(6):347–356. doi:10.1038/nrgastro.2010.61
Sun SY, Hail JN, Lotan R (2004) Apoptosis as a novel target for cancer chemoprevention. J Natl Cancer Inst 96:662–672
Surh Y (1999) Molecular mechanisms of chemopreventive effects of selected dietary and medicinal phenolic substances. Mutat Res 428:305–327
Xutian S, Zhang J, Louise W (2009) New exploration and understanding of traditional Chinese medicine. Am J Chin Med 37:411–426

Classification of Chemopreventive Agents

11

Chemopreventive Agents

Chemopreventive agents are the chemicals or substances which have anticancer properties to hamper carcinogenesis either by blocking DNA damage at initiation stage or by arresting or reversing the processes at stages of promotion and progression (Kelloff et al. 1999). An efficient chemopreventive agent can impede the early stages of carcinogenesis and eradicate premalignant cells ahead of their malignancy. Most of the compounds used in cancer chemoprevention studies are natural phytochemicals which are present in food (Surh 2003). Wattenberg classified chemopreventive agents into two categories on the basis of inhibition stages as blocking agents and suppressing agents.

Blocking agents prevent metabolic activation or interaction with DNA, RNA, and proteins at initiation stages and hence obstruct carcinogens from reaching the target sites. Suppressing agents restrain the conversion of initiated cells into malignant cells at promotion or the progression stage. Some more agents work on all three stages of carcinogenesis and hence are classified into both categories (Hu et al. 2010). Cancer is a multistep process that occurs over an extensive period of time; as a result, there are a number of plausible stages at which it could be inhibited, slowed down, or even reversed. Wattenberg classified the chemopreventive agents into two major types (Wattenberg 1985).

Blocking Agents

Blocking agents may be defined as the agents that thwart carcinogens from reaching or reacting with critical target sites, i.e., they hinder initiation either by restraining the formation of carcinogens from precursor molecules or thwarting the ultimate electrophilic and carcinogenic species to interact with critical cellular target molecules like DNA, RNA, and proteins (Wattenberg 1985). Initiation of carcinogenesis which involves DNA damage can be prevented or reduced by blocking agents that

S. Rashid, *Cancer and Chemoprevention: An Overview*,
DOI 10.1007/978-981-10-2579-2_11

are primarily efficient if taken before the carcinogen exposure. These agents alter both phase I and II drug-metabolizing enzymes, rate of DNA repair, and scavenging of reactive oxygen and other free radical species (Boone et al. 1990). They limit additional adduct formation even if DNA has been damaged. They avert carcinogens from modifying DNA, hence preventing mutations. This is classically accomplished by growing the expression of detoxification and antioxidant enzymes in target tissues; besides modifications in the pharmacokinetics of xenobiotics may also provide protection against tumorigenesis.

They also thwart the activation of carcinogens, augment the detoxification of carcinogens, and trap cancer-producing compounds before they reach or react with target sites in tissues (Greenwald 2002). Examples of blocking agents are caffeic acid, ellagic acid, ferulic acid, p-hydroxycinnamic acid, coumarin, indole-3-acetonitrile, indole-3-carbinol, benzyl isothiocyanates, phenyl isothiocyanates, and quercetin. CYP-450 plays a critical role in the metabolic activation of carcinogens that result in the development of highly electrophilic intermediates. Blocking agents can restrain the conversion of carcinogen by inhibiting the CYP. Allium vegetable which has diallyl sulfide as a constituent inhibits cytochrome (CYP) enzymes (Yang et al. 2001) and tumorigenesis in different animal models. In vivo and in vitro reports show that curcumin inhibits the metabolic activation of different carcinogens by inhibiting CYP (Duvoix and Blasius 2005).

There is another natural chemopreventive agent, namely, resveratrol, which inhibits carcinogenesis by inhibiting the CYP (Delmas and Lançon 2006). This stimulation of detoxification enzymes by naturally occurring or synthetic agents represents a promising approach for chemoprevention of cancer and shielding cells from an abundant range of carcinogens and endogenous toxins (Wilkinson and Clapper 1997). Stimulation of phase II detoxification system or antioxidant genes demonstrates considerable cellular defense in response to electrophilic and oxidative injury.

Phytochemicals are promising cancer-blocking agents that can thwart the incidence of DNA mutation caused by carcinogens. While some of them explicitly react with carcinogens, several of them show their chemopreventive effects indirectly during the modulation of phase II metabolizing enzymes in the tissues where carcinogens or procarcinogens are metabolized (Johnson 2007). Diminished mutagenic risk could result from the induction of numerous phase II detoxifying or antioxidant enzymes resulting in the excretion or inactivation of the carcinogen (Gopalakrishnan and Tony 2008). It has been reported that numerous natural products lessen or reduce the cancer risk by stimulation of detoxifying enzymes like GST, QR, GPx, and GR (Zhao et al. 2010a, b; Surh 2003).

In a nutshell we can say that blocking agents are those natural compounds or bio-molecules that restrain the initiation stage of carcinogenesis. Therefore they preserve DNA in its original form thereby preventing mutagenic interactions with DNA to react with dangerous target sites. They metabolize or impede carcinogen activation or activate free radical scavenging system or boost antioxidant armory or stimulate DNA repair mechanisms or mediate epigenetic mechanisms if there is any stable or irreparable DNA damage.

Suppressing Agents

Suppressing agents are those which impede the evolution of the preneoplastic process. Given that the initiation and progression phases are relatively transitory and permanent events, it seems reasonable that chemopreventive agents should intervene at the prodromal promotion phase. Three decades of research suggest that chemoprevention is a promising strategy to reduce the incidence of cancer, both in well-defined high-risk groups and in the general population (Manach et al. 2005; Kelloff et al. 1999; Kakizoe 2003; Kuno et al. 2012).

They act in the process of carcinogenesis particularly in either promotion or progression stage. Therefore there is an enormous prospective to suppress its development. Suppressing agents inhibit polyamine metabolism and oncogene activity, induce terminal cell differentiation, modulate signal transduction and hormonal or growth factor activity, promote intercellular communication, restore immune response, induce apoptosis, correct DNA methylation imbalances, and inhibit basement membrane degradation and arachidonic acid metabolism (Hail et al. 2008). They reduce the consequences of altered gene expression by diminishing the proliferation of initiated cells or maintain the process of apoptosis to normal levels, thereby preventing the accumulation of damaged or initiated cells (Surh 2003; Manson et al. 2000).

They also thwart the development of the neoplastic process in cells that are previously deformed by carcinogens (Wattenberg 1996). Some of them act by differentiation; others predominantly counteract the consequences of genotoxic events, in particular, oncogene activation, while some others inhibit the proliferation of neoplastic cells (Reddy et al. 1993). They inhibit cancer cell proliferation by downregulating STAT3 (signal transducer and activator of transcription 3), NFkB, and mTOR pathways. They also enhance apoptosis and suppress angiogenesis, epithelial–mesenchymal transition (EMT), and invasion. Chemoprevention is a potential approach to decrease the incidence of cancer targeting high-risk populations especially obtained from the reports of three decades of research (Manach et al. 2005; Kelloff et al. 1999; Kakizoe 2003; Kuno et al. 2012).

Initiation phase is more understandable as compared to post-initiation events, and for this reason, the classification of suppressing agents is more intricate. Signaling pathways that control apoptosis or cell proliferation are altered by suppressing agents. They also protect during the initiation phase of heterocyclic amine-induced carcinogenesis. Generally, the chemopreventive activity of these agents is accredited to their influential effect on cell proliferation, differentiation, senescence, and apoptosis, and they act by scavenging reactive oxygen species, altering gene expression, decreasing inflammation, suppressing proliferation, inducing differentiation, supporting apoptosis, augmenting immunity, and dampening angiogenesis. Examples of suppressing agents are fumaric acid, caffeine, soya bean protease inhibitor, benzyl isothiocyanate, B-sitosterol, and selenium. Indole-3-carbinol, a breakdown product of glucobrassicin vegetables, and curcumin, a major component of the spice turmeric, exhibit both blocking and suppressing mechanisms of action (Hudson et al. 2003).

Chemoprevention Encompasses Three Types

Primary Chemoprevention Prevention in developing precancerous lesions and cancer via targeting high-risk groups of the population. Another strategy could be lessening or completely avoiding contact with carcinogens and lifestyle modifications like quitting smoking, etc.

Secondary Chemoprevention Development of precancerous lesions into cancer is prevented either by blocking or reversing or suppressing of cancer to malignancy.

Tertiary Chemoprevention Reoccurrence of cancer is prevented (Meyskens et al. 2011; Smith et al. 2015; McCullough et al. 2011).

Limitless efforts have been put on basic and clinical cancer research which has resulted in cancer treatment management options. Benjamin Franklin quoted that an ounce of prevention is far far significant than a pound of cure. On the other hand, the attention toward prevention of cancer is also evolving with substantial progress being made in the field of chemoprevention in the past 30 years.

Tertiary Chemoprevention by Suppressing Agents

NFκB It is an inflammatory mediator and an important transcription factor. There is a definitive role of inflammation in initiation and progression of cancer. NFkB is constantly activated in the tumor microenvironment and hence oncogenic in action. NFkB is known to regulate more than 150 genes which are involved in the mechanisms of cell survival like evasion of apoptosis, increased proliferation, and development of cancer progression. Therefore anti-inflammatory agents are used as suppressing chemopreventive agents (DiDonato et al. 2012). Various phytochemicals have been demonstrated as inhibitors of NFκB signaling or having anti-inflammatory in diet like epigallocatechin-3-gallate (EGCG) in green tea polyphenols and curcumin. For example, through proteasomal inhibition, both the green tea polyphenol (−)-epigallocatechin-3-gallate (EGCG) and curcumin alleviate IκBα reported in cell lines as well as animal models via proteasomal inhibition resulting in nonnuclear translocation of NFκB and transcription of its target genes. No synthetic IKK inhibitors or NFκB inhibitors have yet been clinically approved in spite of antitumor effects obtained from chemopreventive agents in various cancer models (De Amicis et al. 2013).

Cytochrome P450s Cytochrome P450s (CYPs) are a superfamily of proteins representing a distinctive example for chemoprevention and carcinogenesis because of their role in instigation of procarcinogenic molecules like hormones to their carcinogenic forms and metabolism of dietary and environmental chemicals for removal from the body.

CYP is increased in human tumors. Therefore relationship of the CYPs in developing malignancy is necessary to be known. Blocking of estrogen binding to its receptor is the standard pharmacological treatment for hormone-dependent breast cancer which has been achieved by the drug tamoxifen. The first chemopreventive agent for individuals at high risk for developing breast cancer approved by the FDA was tamoxifen. There are some considerable caveats for its use.

Metastasis Blocked by Phytochemicals

Dietary phytochemicals are quite efficient in alleviating cancer cell progression to metastasis besides being synthetic aromatase inhibitors. Metastasis is an intricate phenomenon where there is cancer cell migration, invasion, and spread of cancer cell via lymph or blood vessels leading to colonization to different organs which is the reason behind the vast majority of cancer-related deaths despite availability of various antimetastatic drugs in the market because of their low efficiency and non-targeted action besides drug resistance being the biggest factor for such negative results (Weber 2013). If in total metastatic process is prevented, superior patient results will be achieved. It has been reported that the molecular pathways leading to metastatic events are being blocked by dietary phytochemicals. Silibinin, EGCG, curcumin, gingerol, and resveratrol (Deep et al. 2011; De Amicis et al. 2013; Chen et al. 2013; Shen et al. 2014; Kim and Kim 2013; Li et al. 2013) induce increased expression of E-cadherin which is otherwise suppressed in metastatic state and therefore decreases the mesenchymal specific proteins like N-cadherin. They also inhibit MMP expression and act as suppressing agents since MMPs degrade the extracellular matrix and basement membrane in tumor microenvironment thereby enhancing invasion. Curcumin, gingerol, and luteolin decreased the expression of MMPs in thyroid, colorectal, pancreatic, and ovarian in vitro and in vivo models, and it is well evident that chemoprevention not only blocks initiation but also mitigates in cancer growth and malignancy (Vanden 2012).

As we know carcinogenesis begins with cellular transformation and sustained proliferation leading to invasiveness, angiogenesis, and metastasis (Donaldson 2004) which can be triggered by either environmental carcinogens like cigarette smoke, industrial emissions, gasoline vapors, etc., or inflammatory agents like TNF-α or the use of chemical carcinogens like phorbol esters and okadaic acid. It has been found that people in Southeast Asian countries are at lower risk of acquiring colon, gastrointestinal, prostate, breast, and other cancers as compared to the people living in the West from a population-based study report. The reason behind it is prevention by their dietary ingredients like garlic, ginger, soy, curcumin, onion, tomatoes, cruciferous vegetables, chillies, and green tea by inhibiting the transformation, hyper-proliferation, and inflammatory processes that instigate carcinogenesis thereby resulting in inhibition of angiogenesis and metastasis (Deep et al. 2011; Stan et al. 2008; Toshiya et al. 2012).

References

Boone CW, Kelloff GJ, Malone WE (1990) Identification of candidate cancer chemopreventive agents and their evaluation in animal models and human clinical trials. Cancer Res 50:2–9

Chen CC, Sureshbabul M, Chen HW et al (2013) Curcumin suppresses metastasis via Sp-1, FAK inhibition, and E-cadherin upregulation in colorectal cancer. Evid Based Complement Alternat Med 2013:541695

De Amicis F, Perri A, Vizza D et al (2013) Epigallocatechin gallate inhibits growth and epithelial-to-mesenchymal transition in human thyroid carcinoma cell lines. J Cell Physiol 228:2054–2062

Deep G, Gangar SC, Agarwal C et al (2011) Role of E-cadherin in antimigratory and antiinvasive efficacy of silibinin in prostate cancer cells. Cancer Prev Res 4:1222–1232

Delmas D, Lançon A (2006) Resveratrol as a chemopreventive agent: a promising molecule for fighting cancer. Curr Drug Targets 7:423–442

DiDonato JA, Mercurio F, Karin M (2012) NF-κB and the link between inflammation and cancer. Immunol Rev 246:379–400

Donaldson MS (2004) Nutrition and cancer, a review of the evidence for an anti-cancer diet. Nutr J 3:19

Duvoix A, Blasius R (2005) Chemopreventive and therapeutic effects of curcumin. Cancer Lett 223:181–190

Gopalakrishnan A, Tony KA (2008) Anticarcinogenesis by dietary phytochemicals: cytoprotection by Nrf2 in normal cells and cytotoxicity by modulation of transcription factors NFkB and AP-1 in abnormal cancer cells. Food Chem Toxicol 46:1257–1270

Greenwald P (2002) Science, medicine, and the future: cancer chemoprevention. BMJ 324:714–718

Hail N Jr, Cortes M, Drake EN et al (2008) Cancer chemoprevention, a radical perspective. Free Radic Biol Med 45:97–110

Hudson EA, Howells LM, Gallacher-Horley B et al (2003) Growth-inhibitory effects of the chemopreventive agent indole-3-carbinol are increased in combination with the polyamine putrescine in the SW480 colon tumour cell line. BMC Cancer 3:2

Hu R, Saw CL, Yu R (2010) Regulation of NF-E2-Related Factor 2 Signaling for Cancer Chemoprevention: Antioxidant Coupled with Antiinflammatory. Antioxidants & Redox Signalling. 13:32–76

Johnson IT (2007) Phytochemicals and cancer. Proc Nutr Soc 66:207–215

Kakizoe T (2003) Chemoprevention of cancer—Focusing on clinical trials. Jpn J Clin Oncol 33:421–442

Kelloff GJ, Sigman CC, Greenwald P (1999) Cancer chemoprevention, progress and promise. Eur J Cancer 35:2031–2038

Kim SO, Kim MR (2013) Gingerol prevents disassembly of cell junctions and activities of MMPs in invasive human pancreas cancer cells through ERK/NF-κB/snail signal transduction pathway. Evid Based Complement Alternat Med 2013:761852

Li W, Ma J, Ma Q et al (2013) Resveratrol inhibits the epithelial–mesenchymal transition of pancreatic cancer cells via suppression of the PI-3K/Akt/NF-κB pathway. Curr Med Chem 2033:4185–4194

Manach C, Williamson G, Morand C et al (2005) Bioavailability and bioefficacy of poly- phenols in humans I. Review of 97 bioavailability studies. Am J Clin Nutr 81:230S–242S

Manson M, Gescher A et al (2000) Blocking and suppressing mechanisms of chemoprevention by dietary constituents. ToxicolLett 112:499–505

McCullough ML, Patel AV, Kushi LH et al (2011) Following cancer prevention guidelines reduces risk of cancer, cardiovascular disease, and all-cause mortality. Cancer Epidemiol Biomarkers Prev 206:1089–1097

Meyskens FL Jr, Curt GA, Benner DE et al (2011) Regulatory approval of cancer risk-reducing chemopreventive. drugs, moving what we have learned into the clinic. Cancer Prev Res (Phila) 43:311–323

Reddy BS, Rao CV et al (1993) Chemoprevention of colon carcinogenesis by organosulfur compounds. Cancer Res 53:3493–3498

Shen F, Cai WS, Li JL et al (2014) Synergism from the combination of ulinastatin and curcumin offers greater inhibition against colorectal cancer liver metastases via modulating matrix metalloproteinase-9 and E-cadherin expression. Onco Targets Ther 7:305–314

Smith RA, Mannassaram-Baptiste D, Brooks D et al (2015) Cancer screening in the United States, a review of Current American Cancer Society guidelines and current issues in cancer screening. CA Cancer J Clin 651:30–54

Stan SD, Kar S, Stoner GD, Singh SV (2008) Bioactive food components and cancer risk reduction. J Cell Biochem 104:339–356

Surh YJ (2003) Cancer chemoprevention with dietary phytochemicals. Nat Rev Cancer 3:768–780

Toshiya K, Testuya T, Akira H et al (2012) Cancer chemoprevention through the induction of apoptosis by natural compounds. JBPC 3:156–173

Vanden BW (2012) Epigenetic impact of dietary polyphenols in cancer chemoprevention, lifelong remodeling of our epigenomes. Pharmacol Res 65:565–576

Wattenberg LW (1985) Chemoprevention of cancer. Cancer Res 45:1–8

Wattenberg LW (1996) An overview of chemoprevention: current status and future prospects. Exp Biol Med (Maywood) 216:133

Weber GF (2013) Why does cancer therapy lack effective anti-metastasis drugs? Cancer Lett 3282:207–211

Wilkinson J, Clapper ML (1997) Detoxication enzymes and chemoprevention. Exp Biol Med (Maywood) 216:192

Yang CS, Chhabra SK, Hong JY et al (2001) Mechanisms of inhibition of chemical toxicity and carcinogenesis by Diallyl Sulfide (DAS) and related compounds from Garlic. J Nutr 131:1041S–1045S

Zhao CR, Gao ZH, Qu XJ (2010a) Nrf2-ARE signaling pathway and natural products for cancer chemoprevention. Cancer Epidemiol 34:523–533

Zhao Q, Wang J, Zou MJ et al (2010b) Wogonin potentiates the antitumor effects of low dose 5-fluorouracil against gastric cancer through induction of apoptosis by down-regulation of NF-kappaB and regulation of its metabolism. Toxicol Lett 197:201–210

Preclinical Development of Chemopreventive Agents for Chemoprevention

12

Chemopreventive agents regulate cellular and molecular events by modulation of phase I and phase II metabolizing enzymes inducing DNA repair and inhibiting growth and cell cycle progression and differentiation and apoptosis, growth hormonal activity modulation, ligands for nuclear receptors, and alteration of chromatin structure. These mechanisms are majorly interconnected or partially overlap each other. Modulation of a given end point may possibly be the result of a specific mechanism or the result of other upstream mechanisms (Flora and Ferguson 2005). Different studies have been done on evaluating the molecular mechanisms of chemopreventive agents. Some of the mechanisms of action are described below Fig. 12.1.

Modulation of Phase I and II Enzymes by Chemopreventive Agents

Exogenous chemical substances and xenobiotics are metabolized in the body generally via phase I and phase II metabolisms. They do so by activating phase I metabolism reactions involving activation of procarcinogens to highly reactive carcinogens or detoxification by phase II metabolism which involves conjugation process wherein polarity of the compounds increases, thus facilitating the process of elimination. The physiological balance of these drug-metabolizing enzymes between competing, activating, and detoxifying reactions illustrates the sensitivity of an individual toward carcinogens. Thus, phytochemicals that modify phase I and phase II enzymes provide protection against cellular damage induced by carcinogen (Issaa et al. 2006). A promoter region, namely, antioxidant response element (ARE) activation found in several genes encoding for detoxifying enzymes such as NAD(P)H:quinone oxidoreductase-1 (NQO1), heme oxygenase-1 (HO-1), glutathione S-transferase (GST), and superoxide dismutase (SOD), has been used to monitor for potential enzyme inducers. A member of the basic leucine zipper NF-E2, namely, transcription factor Nrf2 belonging to the family of transcription factors, binds and

S. Rashid, *Cancer and Chemoprevention: An Overview*,
DOI 10.1007/978-981-10-2579-2_12

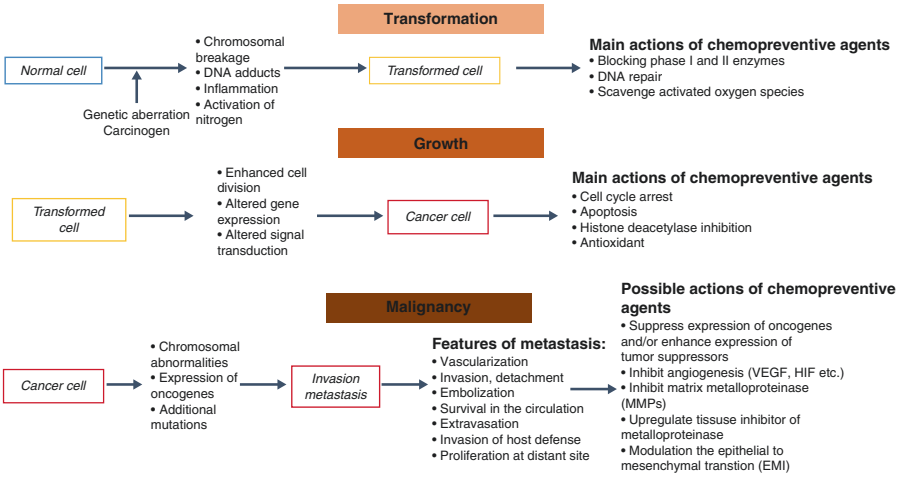


Fig. 12.1 Represents possible mechanism of action of various chemopreventive agents by modulation and regulation of various signaling pathways (Adapted and modified figure from Mehta 2014)

activates ARE. It is bound to Keap1 in cytoplasm; following dissociation, it migrates into the nucleus and increases gene transcription during binding to ARE. Destabilizing the Nrf2–Keap1 complex is a possible mechanism to activate cytoprotective enzyme expression targeted by chemopreventive agents (Yu and Kensler 2005).

Antioxidant Activity of Chemopreventive Agents

Oxidative stress produced as a result of ROS may result in dysfunctional cell growth, differentiation, and death which often occurs in collaboration with DNA mutations and finally results in the development of cancer. It has been reported that phytochemicals with antioxidant potential exert their effects by absorbing free electrons and radicals. It has been observed that compounds with hydroxyl groups attached to aromatic rings create an electron-rich environment trapping ROS thereby preventing them from reacting with nucleophilic centers of cellular proteins and DNA (Issaa et al. 2006). Antioxidants that target free radicals produced from normal oxygen metabolism or during inflammatory responses are being targeted by antioxidants.

ROS may either contain odd numbers of electrons, e.g., superoxide (O^{2-}), hydroxyl (OH^{-}), hydroperoxyl (HOO^{-}), peroxyl (ROO^{-}), and alkoxyl free radicals (RO), or even numbers of electrons such as hydrogen peroxide (H_2O_2) and lipid hydroperoxide (ROOH). NFκB, a redox sensitive transcription factor may be targeted by antioxidants because its activation promotes transcription of genes that are involved in cell cycle progression and cell proliferation (Loo 2003).

Anti-inflammatory Action of Chemopreventive Agents

Chemopreventive agents having anti-inflammatory properties can target arachidonic acid-dependent pathway or arachidonic acid-independent pathway. Arachidonic acid-dependent pathway includes COX, lipoxygenase (LOX), and phospholipase A2, while arachidonic acid-independent pathway includes NOS, LOX, peroxisome proliferator-activated receptor (PPAR), NSAID-activated gene 1 (NAG-1), and NFκB (Hyde and Missailidis 2009). There is an association between arachidonic acid metabolism and inflammation. Arachidonic acid is chiefly catalyzed by COX, LOX, and CYP P450 into eicosanoid metabolites which are lipid signaling mediators that play a vital role in various pathophysiological conditions. They have been identified as active carcinogens or tumor promoters (Hyde and Missailidis 2009). The most important mode of action of anti-inflammatory synthetic drugs or natural compounds depends on their capability to obstruct the COX activity of the COX enzymes. COX-2 is the inducible form of COX which contributes in various inflammatory and proliferative reactions (Kundu and Surh 2008a, b). Anti-inflammatory agents also target pro-inflammatory mediators like growth factors (EGF, TGFβ, and VEGF), cytokines (TNF-α and IL-6), oncogenes, and other factors which induce COX expression, and the products of COX and LOX pathways like prostaglandins, thromboxanes, and leukotrienes are also targeted by anti-inflammatory agents (Murakami and Ohigashi 2007).

Modulation of Cell Signaling Pathways by Chemopreventive Agents

Cellular signaling is an intricate signal communication network in cells controlling indispensable biological activities and organizes cellular events. Cancer cell growth depends on various pathways. There is alteration in the structure of proteins due to mutations or defects of genes which influence the way cells communicate with each other. It has been reported that chemopreventive agents like soy isoflavones including genistein and daidzein and indole-3-carbinol (I3C) and its dimeric product 3,3-diindolylmethane (DIM) from cruciferous vegetables target NFκB and phosphoinositide 3-kinase (PI3K)/Akt and MAPK pathways (Sarkar and Li 2004). Cellular targets like cell proliferation, apoptosis, inflammation, and stress response within the NFκB pathway are controlled by NFκB, IκB, and IKK; Akt pathway is activated by phosphoinositide-dependent kinase 1 (PDK1) and PDK2 which play a considerable role in survival of cells. MAPK pathways consist of a three-tiered kinase core in which a MAPK kinase (MAP3K) activates a MAPK kinase (MAP2K) which further activates a MAPK (ERK, JNK, p38) and thereby regulates cell growth and their survival. Notch receptors, p53 protein, and androgen receptors are other molecular targets which are involved in their respective pathways in regulation of cells, and these molecules are targeted by chemopreventive agents in chemoprevention of cancer (Doraia and Aggarwal 2004).

Inhibition of NFκB Signaling Pathway by Chemopreventive Agents

Carcinogens, inflammatory agents, and tumor promoters stimulate NFkB, and its nuclear translocation activates transcription of downstream target genes whose activation is lethal to the signaling pathways like cyclin D1 expression, apoptosis suppressor proteins like bcl-2 and bcl-XL, and metastasis and angiogenesis activators like MMP and VEGF. Natural compounds like curcumin, catechins, silymarin, caffeic acid phenethyl ester (CAPE), sanguinarine, anethole, emodin, piceatannol, resveratrol, capsaicin, ursolic acid, betulinic acid, flavopiridol, and oleandrin block the NFκB activation process (Gonzalez and Riboli 2006; Manach et al. 2005; Riboli and Norat 2001). Though regulated NFκB activity is vital for regular cellular functioning unlike constitutive NFκB activation leading enhanced growth as found in various cancers. Phytochemicals mentioned above might be incorporated in the diet of patients whose tumors are NFκB positive for beneficial effects in case of non-small cell lung carcinoma (NSCLC) and thyroid, colon, breast, stomach, and squamous head and neck carcinomas (Stan et al. 2008).

Inhibition of AP-1 Activation Pathway by Chemopreventive Agents

Activated protein 1 (AP-1) regulates the expression of several genes involved in cell differentiation and proliferation and is a transcription factor. Its functional activation is involved in cancer promotion and malignancy. AP-1 transcription complex is formed of members of the JUN and FOS family of proteins. Various external signals like growth factors, mitogen-activated protein kinases (MAPK), extracellular signal-regulated protein kinases (ERK), and JUN terminal kinases (JNK) can be mediated by AP-1 transcription of several target genes including cyclin D1, bcl-2, bcl-XL, VEGF, MMP, and urokinase plasminogen activator (uPA) which are quite similar to target genes activated by NFkB. AP-1 promotes one of the early steps in tumor metastasis, i.e., the conversion of tumor cells from an epithelial to mesenchymal morphology (Lepley et al. 1996).

Phytochemicals which suppress AP-1 activation process include curcumin, capsaicin, resveratrol, and green tea catechins (Riboli and Norat 2001). Proliferative signals stimulated by peptide growth and steroid growth factors are interfered by AP-1 obstruction (Lepley et al. 1996). Therefore phytochemicals specifically targeting AP-1 or its activating kinases may prove to be capable agents for chemoprevention of various cancers.

Induction of Apoptosis and Cell Cycle Arrest

Uncontrolled cell proliferation may be due to the insensitivity in induction of apoptosis and absence of normal cell cycle control leading to uncontrolled cell proliferation. A number of chemopreventive agents have been reported in literature to induce apoptosis through mitochondria-mediated pathway (Toshiya et al. 2012). Stress signals produced

by chemopreventive agents help in the regulation of pro-apoptotic proteins (Bax and Bak) or anti-apoptotic proteins (e.g., bcl-2 and bcl-XL), resulting in release of cytochrome c from the mitochondrial inner membrane, followed by formation of "apoptosome" (formed by cytochrome c, apoptotic protease-activating factor 1(APAF-1), and caspase 9). Caspase 9 further activates downstream effector caspases, such as caspase-3, caspase-6, and caspase-7, and degrades main intracellular proteins, which cause morphological changes showing the phenotype of apoptotic cells.

Disruption of the balance among cyclins, cyclin-dependent kinases (CDKs), and CDK inhibitors (CDKIs), which governs the progression of the cell cycle by chemopreventive compounds, potentially inhibits proliferation of neoplastic cells. Some chemopreventive agents activate upstream kinases such as JNK or inhibit PI3K/Akt pathway to induce apoptosis, inhibiting NFκB and AP-1 activation, therefore downregulating anti-apoptotic and cell cycle-regulating proteins, inducing caspase activation resulting in death of a cell and increasing p53 expression, and eliciting cell cycle arrest through the induction of CDKIs (p21 and p27) and the inhibition of CDK4, CDK2, cyclin D1, and cyclin E (Chen and Kong 2005).

Inhibition of Sustained Cell Proliferation and Initiation of Apoptosis by Chemopreventive Agents

Curcumin, green tea, 6-gingerol, and resveratrol have been found to inhibit NFkB or the AP-1 activation process leading to suppression of cell proliferation and sensitization of cells toward apoptosis, and activation leads to upregulation of these mechanisms (Skaper et al. 1997). NFkB has been reported to maintain cell survival and proliferation and downregulation of NFkB resulting in apoptosis. NFkB has been reported to upregulate bcl-2, bcl-XL, cIAP, survivin, cyclin D1, TRAF1, and TRAF2 (Riboli and Norat 2001) genes which function by blocking the apoptosis pathway.

Curcumin has been reported to decrease the expression of apoptosis suppressor proteins like bcl-2 and bcl-XL in various cancer cell lines either by intrinsic tyrosine kinase activity or by intracellular non-receptor tyrosine kinase recruitment. Constitutive activation of STAT3 and STAT5 has been involved in human cancers like multiple myeloma, lymphomas, leukemias, and solid tumors, and out of which, seven known STAT proteins make them rational targets for cancer therapy. They thwart apoptosis via upregulating anti-apoptotic proteins leading to cell survival, growth, and angiogenesis. JAK-STAT-mediated signaling has been shown to be inhibited by various chemopreventive phytochemicals in multiple myeloma (Hollman and Katan 1999).

Modulation of Multidrug Resistance by Chemopreventive Agents

Drug resistance, one of the serious side effects of chemotherapeutic drugs used for the treatment of cancer, is mediated mainly by classical ATP-driven drug efflux pumps such as P-glycoproteins and MRP family of proteins. Kidney, prostate, and

colon cancers have highly expressed multidrug resistance (MDR)-related P-glycoprotein. Curcumin and genistein have provided promising results in reversal of MDR. Curcumin downregulated P-glycoprotein and mRNA levels in the multidrug-resistant human cervical carcinoma cells (KB-V1) (Scalbert and Williamson 2000). Sensitivity to vinblastine was also increased as directly proportional to rhodamine (Rh123) dye. Recent reports from several laboratories suggest that agents such as curcumin may interfere with the drug resistance processes modulated by topoisomerase II (TOPO-II) poisons which intervene via suppression of heat shock proteins or by intracellular function of proteasome. Therefore these phytochemicals intervene at multiple sites and levels to mitigate with the traditional and other approaches of generation of resistance developed in microenvironment (Nijveldt et al. 2001).

Modulation of Angiogenesis by Chemopreventive Agents

The development of new blood vessels from existing ones is called angiogenesis where endothelial cells secrete MMP and heparanase to dissolve the extracellular matrix (ECM). Endothelial cells arrange themselves into new capillary tubes facilitating newly formed vessels to develop into fresh blood supply because of the alteration in tight junctions between the endothelial cells (Chou et al. 2010).

Tumor blood vessels have partial basement membranes with disorganized microvasculature having uneven endothelial cell-to-pericyte ratio. There is a disproportion of pro- and anti-angiogenic factors in the newly formed blood vessels that thus are hyperpermeable (Chou et al. 2010). Endothelial cell properties are mimicked by tumor cells forming vasculogenic meshwork. Various highly developed cancers can be intervened at angiogenic switch, vessel cooption, and vasculogenic mimicry (Nguyen et al. 2004).

Chemopreventive phytochemicals like curcumin, resveratrol, and catechins are known inhibitors of angiogenesis, while some more are in clinical trials (Senthilkumar et al. 2010; Van et al. 2005). Curcumin has been found to decrease the dissolution of ECM that forms the basis of angiogenic switch by mitigating MMP-2 and MMP-9 (Ramos et al. 2005) along with regulation of angiogenic and other growth factors resulting in non-budding of new blood vessels in the tumor via interfering in the mechanisms of angiogenic switch and vessel cooption (Galati et al. 2000). Src and FAK, non-receptor tyrosine kinases, are being acted upon by curcumin, genistein, and green tea components which impede the downstream PIK3 signaling giving rise to induction of the angiogenic target genes like COX-2, VEGF, IL-8, and MMPs (Giovannini et al. 2007; Sarkar and Li 2006). Curcumin is a negative regulator of MMP-2-mediated degradation of the lamin-5 isoform forming loose and primordial looking meshwork as found in melanoma and prostate cancers (Chan et al. 2003). Curcumin and genistein also hamper VEGF expression by transforming growth factor (TGF)-β release, COX-2 overexpression, hydrogen peroxide release from bone cells, constitutive and anomalous EGFR, Src signaling, as well as

NFkB signaling in traditional cancers. Inhibition of specific integrin engagement and usage to obstruct with the endothelial cell function is done by curcumin, genistein, and green tea.

Regulation of Cell Cycle Regulatory Proteins by Chemopreventive Agents

Mutations of p53 and retinoblastoma (Rb) are found in diverse cancers (Khafif et al. 1998), and curcumin, resveratrol, and catechins have been reported to regulate these cell cycle regulatory pathways making them promising therapeutic agents (Sharma et al. 2005; Jakubowicz-Gil et al. 2005). In G0/G1 and G2/M phase cell cycle, CDK inhibitors were upregulated p21Cip1 and p27Kip1 and downregulated cyclin B1 and CDC2 on treatment of curcumin which resulted in downregulation of apoptosis suppressor proteins such as bcl-2 and bcl-XL. Alleviation of cell survival mechanisms is specifically modulated by transcription factors like AP-1, STATs, and NFkB upon curcumin treatment. Promotion of apoptosis and inhibition of cyclin CDK complexes leading to cell cycle arrest were shown by ECGC treatment. Resveratrol inactivates p34 (CDC2) and CDK7 protein kinase activity thereby causing G2 phase arrest signifying its pro-apoptotic and antiproliferative effects (Hwang et al. 2005).

Chemopreventive Agents as Chemosensitizers and Radiosensitizers

Curcumin has been reported to inhibit ATP binding site of the MDR or MRP drug efflux pumps increasing the intracellular concentrations of vinblastine or vincristine. Genistein and green tea components (EGCG) that function by acting as efflux substrates for MDR or MRP pumps augment the concentration of the chemotherapeutic drug within the cell thereby sensitizing the cancer cell to be targeted well by chemotherapeutic agents. Curcumin has been reported to hinder MRP pump functioning as it inhibits GSH synthetase which is required for a stable supply of reduced glutathione (GSH). Third, curcumin can hinder with the functioning of pumps such as MRP which require a steady supply of antioxidant reduced glutathione (GSH) as it is known to be an inhibitor of GSH synthetase. This type of blockade might augment the sensitivity of these cancer cells overexpressing MRP to chemotherapeutic agents like vincristine, arsenicals, and platinum-based compounds by damaging their efflux (Siraki et al. 2004).

c-JUN expression is targeted to decrease intracellular GSH levels in clinical strategy since c-JUN increase is related to increased GSH synthetase levels via AP-1 (Depeint et al. 2002). Curcumin is reported to be a potent inhibitor and reduces GSH at the transcriptional level (Gomes et al. 2003). Glutathione S-transferase pi (GST-Pi) has been associated with the resistance of cancer cells to chemotherapeutic agents. Recently curcumin suppressed phorbol ester, and TNF-α induced NFκB

and AP-1 binding to the sites located on the GST-Pi gene promoter in K562 leukemia cells (Gomes et al. 2003) decreasing GST-Pi levels effecting in an obstruction with drug resistance and leading to apoptosis (Kurosaka et al. 2003). In prostate cancer cells, curcumin was reported to induce radiosensitization through the repression of NFκB. Similarly, curcumin was found to induce radiosensitization of prostate cancer cells through the suppression of NFκB activation (Elmore 2007; Doraia and Aggarwal 2004).

Phytochemicals can delay or thwart the process of carcinogenesis by numerous mechanisms, like induction of phase II drug-metabolizing enzymes, increased detoxification of the carcinogenic intermediates, repression of CYP P450-dependent monooxygenases which would in a way reduce carcinogenic activation, mitigation in cell cycle progression, induction of apoptosis in cancerous or precancerous cells selectively, and inhibition of angiogenesis and metastasis formation (Stan et al. 2008).

Possible Targets for Chemoprevention: MicroRNA

MicroRNAs are small noncoding RNAs consisting of 19–24 nucleotides in length. It activates translational repression, and RNA degradation thereby regulates gene expression. About 35 % of the genome's translational function is regulated by approximately 2000 miRNA (Ye and Cao 2014) because one miRNA regulates the expression of various mRNAs. miRNAs can act as important molecular targets for cancer chemoprevention since they are selectively expressed in normal and abnormal cancer cells. miRNAs may be classified as oncogenes or as tumor suppressors like let-7, which is a tumor suppressor that inhibits HMGA2 oncogene and regulates RAS oncogene via translational suppression. miRNA-82 is reported to be a potential oncogene (Peng et al. 2008; Ouyang et al. 2014). Chemopreventive agents like curcumin and folates have been found to amend miRNA expression; therefore miRNAs could be possible targets for chemoprevention (Sarkar et al. 2013). Let-7 miRNA expression was altered by stimulating oxidative stress in MCF7 cells, but vitamin D supplementation reversed the effect (Peng et al. 2010). Furthermore, miR-181 regulated vitamin D-activated cell differentiation via mediating p21 expression (Giangreco et al. 2013). MicroRNA as a regulatory molecule is a budding concept and may probably prove to be an important molecular target for carcinogenesis and chemoprevention.

Nano-chemoprevention: Another Evolving Strategy

Preventive agents used in chemoprevention must be safe to use and nontoxic which makes up the primary requisite for chemoprevention and usually restricts the utilization of many potential chemopreventive agents. Bioavailability is an additional chief concern because numerous potent chemopreventive agents are not readily bioavailable. Hence nanotechnology provides useful ways to transport test compounds

to the amiable sites which have been possible by these advances. It has been reported that EGCG release using nanoparticles sustain its anticancer properties (Khan et al. 2014). Resveratrol and vitamin D have also been found to be delivered through nanotechnology recently (Almouazen et al. 2013). There was decrease in the prevalence of chemically induced pancreatic cancer in Syrian golden hamsters as compared to the original combination of drugs upon combinatorial treatment with curcumin, aspirin, and sulforaphane in a solid lipid nano-encapsulated form. Thus providing an evidence for the nanotechnological approach to chemoprevention and further confirmation for other chemoprevention models provide a potent basis for using nanotechnology for chemoprevention in which bioavailability of the drugs is often a restraining factor. Interactive molecular signal transduction pathways through different drug combinational approaches may be identified for their action in chemoprevention.

Translational Application of Chemoprevention

From the past three decades, the researchers in the field of chemoprevention have resulted in the appliance of chemoprevention strategies under clinical setups. Chemoprevention trials also require recognizing safe dose and bioavailability (phase I and II) in humans just like clinical trials for chemotherapy. Thousands of contributors or volunteers are needed for the randomized clinical trials (phase III) for chemoprevention and need to be passed out over a long period of time which turns out to be cost ineffective, i.e., some studies employ phase 0 clinical trials (Steward and Brown 2013). New approaches to study pharmacokinetics and toxicity and very low doses of the chemopreventive agent are required. The discovery of urine- or blood-based biomarkers ideally which can predict malignancy or reaction to chemopreventive agents would be tremendously helpful. Presently only a small number of markers are normally utilized in clinical practice like ACF of the colon, colon polyps and adenomas, breast density and mammograms, DCIS in women, and prostatic intraepithelial neoplasia in men for prostate cancer. But there is an urgent and alarming requirement for generating new clinical noninvasive markers that can forecast the disease more precisely without much suffering. More than 50 cancer prevention trials have been reported, although majorly the trials are not having adequate numbers of participants or appropriate statistics are not used and thus in a way are incomplete (Naithani et al. 2008).

Breast Cancer Prevention Trial (BCPT) for high-risk women is the most popular chemoprevention trial in which more than 13,000 women participated and tamoxifen treatment indicated a positive outcome for tamoxifen as a chemopreventive agent by reducing invasive and noninvasive breast cancer. Nevertheless thromboembolic events and augmented incidence of endometrial cancers are indicative of tamoxifen toxicity (Cuzick et al. 2013). Another trial was carried out with an antiestrogen, raloxifene, which is similar to tamoxifen but is not associated with the incidence of endometrial cancers, though it was less effective than tamoxifen. Besides, few more clinical trials have been done which include an inhibitor,

5α-reductase enzyme, indispensable for the generation of dihydrotestosterone, namely, finasteride. Finasteride management reduced prostate cancer prevalence by 25 % for 7 years (Ankerst et al. 2013).

Aspirin, a nonsteroidal anti-inflammatory drug (NSAID), on which another chemoprevention trial was completed for colon cancers. A high dose of aspirin has been reported to decrease colon cancer-related deaths upon 3 year treatment, but a lower dose of aspirin is required to achieve similar effectiveness (<300 mg daily) for 5 year long treatment. Conversely a large clinical trial on 35,500 men for four arms upon selenium and α-tocopherol was carried out in which the men received a placebo, α-tocopherol, selenium, or both α-tocopherol and selenium. But unfortunately no positive results could be predicted, so this trial had to be wind up (Dunn et al. 2010), and instead possibility of developing prostate cancer for people consuming α-tocopherol was increased. So it could be inferred from the data available that there is a long way to go for chemoprevention trials, since they are very costly and need a large number of participants and the trials have to be conducted for a long period which is unlikely.

Outline

In brief chemoprevention has progressed from determining the efficiency of dietary treatment of synthetic analogs of vitamin A to estimating efficacy of hundreds of chemopreventive agents. Lately emphasis has been made toward evaluating the efficacy of chemopreventive agents against various molecular and signal transduction pathways and utilizing new strategies of safe targeted delivery entailing lower concentrations of the drugs and having reduced toxicity. Identification of early end point markers that could be evaluated with fewer invasive measures is another major focus which could be achieved by testing some molecular markers present in blood, urine, sputum, or biological fluids and would be ideal. Clinical trials for chemoprevention are important and desirable as well as are cost prohibitive and require large number of volunteers who may be at high risk of developing cancer. At the same time, recent failures of chemoprevention trials also raise concerns for such long-term expensive clinical trials (Jordan 2014).

References

Almouazen E, Bourgeois S, Jordheim LP et al (2013) Nano-encapsulation of vitamin D3 active metabolites for application in chemotherapy, formulation study and *in vitro* evaluation. Pharm Res 30:1137–1146

Ankerst DP, Till C, Boeck A et al (2013) Predicting risk of prostate cancer in men receiving finasteride, effect of prostate volume, number of biopsy cores, and American Urological Association symptom score. Urology 82:1076–1081

Chan MM, Fong D, Soprano KJ et al (2003) Inhibition of growth and sensitization to cisplatin-mediated killing of ovarian cancer cells by polyphenolic chemopreventive agents. J Cell Physiol 194:63–70

Chen C, Kong ANT (2005) Dietary cancer-chemopreventive compounds: from signaling and gene expression to pharmacological effects. Trends Pharmacol Sci 26:318–326
Chou CC, Yang JS, Lu HF et al (2010) Quercetin-mediated cell cycle arrest and apoptosis involving activation of a caspase cascade through the mitochondrial pathway in human breast cancer MCF-7 cells. Arch Pharm Res 33:1181–1191
Cuzick J, Sestak I, Bonanni B et al (2013) Selective oestrogen receptor modulators in prevention of breast cancer, an updated meta-analysis of individual participant data. Lancet 3819880:1827–1834
Cuzick J, Sestak I, Cawthorn S et al (2015) Tamoxifen for prevention of breast cancer, extended long-term follow-up of the IBIS-I breast cancer prevention trial. Lancet Oncol 161:67–61
Depeint F, Gee JM, Williamson G et al (2002) Evidence for consistent patterns between flavonoid structures and cellular activities. Proc Nutr Soc 61:97–103
Doraia T, Aggarwal BB (2004) Role of chemopreventive agents in cancer therapy. Cancer Lett 215:129–140
Dunn BK, Richmond ES, Minasian LM et al (2010) A nutrient approach to prostate cancer prevention, The Selenium and Vitamin E Cancer Prevention Trial SELECT. Nutr Cancer 62: 896–918
Elmore S (2007) Apoptosis, a review of programmed cell death. Toxicol Pathol 35:495–516
Flora SD, Ferguson LR (2005) Overview of mechanisms of cancer chemopreventive agents. Mutat Res 591:8–15
Galati G, Teng S, Moridani MY et al (2000) Cancer chemoprevention and apoptosis mechanisms induced by dietary polyphenolics. Drug Metabol Drug Interact 17:311–349
Giangreco AA, Vaishnav A, Wagner D et al (2013) Tumor suppressor microRNAs, miR-100 and -125b, are regulated by 1,25-dihydroxyvitamin D in primary prostate cells and in patient tissue. Cancer Prev Res (Phila) 6:483–494
Giovannini C, Scazzocchio B, Vari R et al (2007) Apoptosis in cancer and atherosclerosis, polyphenol activities. Ann Ist Super Sanita 43:406–416
Gomes CA, Cruz D, Andrade TG et al (2003) Anticancer activity of phenolic acids of natural or synthetic origin, a structure-activity study. J Med Chem 46:5395–5401
Gonzalez CA, Riboli E (2006) Diet and cancer prevention, where we are, where we are going. Nutr Cancer 56:225–231
Hyde CA, Missailidis S (2009) Inhibition of arachidonic acid metabolism and its implication on cell proliferation and tumour-angiogenesis. Int Immunopharmacol 9(6):701–15
Hollman PC, Katan MB (1999) Dietary flavonoids, intake, health effects and bioavailability. Food Chem Toxicol 37:937–942
Hwang JT, Ha J, Park OJ (2005) Combination of 5-fluorouracil and genistein induces apoptosis synergistically in chemo-resistant cancer cells through the modulation of AMPK and COX-2 signaling pathways. Biochem Biophys Res Commun 332:433–440
Issaa AY, Volatea SR, Wargovich MJ (2006) The role of phytochemicals in inhibition of cancer and inflammation: new directions and perspectives. J Food Compos Anal 19:405–419
Jakubowicz-Gil J, Paduch R, Piersiak T et al (2005) The effect of quercetin on pro-apoptotic activity of cisplatin in HeLa cells. Biochem Pharmacol 69:1343–1350
Jordan C (2014) Tamoxifen as the first targeted long term adjuvant therapy for breast cancer. Endocr Relat Cancer 21:R235–R246
Khafif A, Schantz SP, Chou TC et al (1998) Quantitation of chemopreventive synergism between epigallocatechin-3-gallate and curcumin in normal, premalignant and malignant human oral epithelial cells. Carcinogenesis 19:419–424
Khan N, Bharali DJ, Adhami VM et al (2014) Oral administration of naturally occurring chitosan-based nanoformulated green tea polyphenol EGCG effectively inhibits prostate cancer cell growth in a xenograft model. Carcinogenesis 35:415–423
Kundu JK, Surh YJ (2008a) Cancer chemopreventive and therapeutic potential of resveratrol: mechanistic perspectives. Cancer Lett 269:243–261
Kundu JK, Surh YJ (2008b) Inflammation: Gearing the journey to cancer. Mutat Res 659:15–30

Kurosaka K, Takahashi M, Watanabe N et al (2003) Silent cleanup of very early apoptotic cells by macrophages. J Immunol 171:4672–4679
Lepley DM, Li B, Birt DF et al (1996) The chemopreventive flavonoid apigenin induces G2/M arrest in keratinocytes. Carcinogenesis 17:2367–2375
Loo G (2003) Redox-sensitive mechanisms of phytochemical-mediated inhibition of cancer cell proliferation. Nutr Biochem 14:64–73
Manach C, Williamson G, Morand C et al (2005) Bioavailability and bioefficacy of polyphenols in humans I. Review of 97 bioavailability studies. Am J Clin Nutr 81:230S–242S
Mehta RG (2014) Current paradigms of cancer chemoprevention. Turk J Biol 38:839–847.
Murakami A, Ohigashi H (2007) Targeting NOX, INOS and COX-2 in inflammatory cells: chemoprevention using food phytochemicals. Int J Cancer 121:2357–2363
Naithani R, Huma LC, Moriarty RM et al (2008) Comprehensive review of cancer chemopreventive agents evaluated in experimental carcinogenesis models and clinical trials. Curr Med Chem 15:1044–1071
Nguyen TT, Tran E, Nguyen TH et al (2004) The role of activated MEK-ERK pathway in quercetin-induced growth inhibition and apoptosis in A549 lung cancer cells. Carcinogenesis 25:647–659
Nijveldt RJ, Van Nood E, Van Hoorn DE et al (2001) Flavonoids, a review of probable mechanisms of action and potential applications. Am J Clin Nutr 74:418–425
Niravath P, Rimawi MF, Osborne CK (2014) Aromatase inhibitor adverse effects, are we sweeping them under the rug? J Clin Oncol 3233:3779
Olin JL, St Pierre M (2014) Aromatase inhibitors in breast cancer prevention. Ann Pharmacother 4812:1605–1610
Ouyang M, Li Y, Ye S et al (2014) MicroRNA profiling implies new markers of chemoresistance of triple-negative breast cancer. PLoS One 9:e96228
Peng B, Cao J, Yi S, et al (2013) Inhibition of proliferation and induction of G1-phase cell-cycle arrest by dFMGEN., a novel genistein derivative., in lung carcinoma A549 cells. Drug Chem Toxicol 36:196–204
Peng X, Vaishnav A, Murillo G et al (2010) Protection against cellular stress by 25-hydroxyvitamin D3 in breast epithelial cells. J Cell Biochem 110:1324–1333
Potter JD (2014) The failure of cancer chemoprevention. Carcinogenesis 355:974–982
Ramos S, Alia M, Bravo L et al (2005) Comparative effects of food-derived polyphenols on the viability and apoptosis of a human hepatoma cell line HepG2. J Agric Food Chem 53:1271–1280
Riboli E, Norat T (2001) Cancer prevention and diet, opportunities in Europe. Public Health Nutr 4:475–484
Sarkar FH, Li Y (2004) Cell signaling pathways altered by natural chemopreventive agents. Mutat Res 555:53–64
Sarkar FH, Li Y (2006) Using chemopreventive agents to enhance the efficacy of cancer therapy. Cancer Res 66:3347–3350
Sarkar S, Azab BM, Das SK, Quinn BA, Shen X, Dash R, Emdad L et al (2013) Chemoprevention gene therapy (CGT): Novel combinatorial approach for preventing and treating pancreatic cancer. Curr Mol Med 13(7):1140–59
Scalbert A, Williamson G (2000) Dietary intake and bioavailability of polyphenols. J Nutr 130:2073S–2085S
Senthilkumar K, Elumalai P, Arunkumar R et al (2010) Quercetin regulates insulin like growth factor signaling and induces intrinsic and extrinsic pathway mediated apoptosis in androgen independent prostate cancer cells PC-3. Mol Cell Biochem 344:173–184
Sharma H, Sen S, Singh N (2005) Molecular pathways in the chemosensitization of cisplatin by quercetin in human head and neck cancer. Cancer Biol Ther 4:949–955
Siraki AG, Chan TS, O'Brien PJ (2004) Application of quantitative structure-toxicity relationships for the comparison of the cytotoxicity of 14 p-benzoquinone congeners in primary cultured rat hepatocytes versus PC12 cells. Toxicol Sci 81:148–159

Skaper SD, Fabris M, Ferrari. V, Dalle C et al (1997) Quercetin protects cutaneous tissue-associated cell types including sensory neurons from oxidative stress induced by glutathione depletion, Cooperative effects of ascorbic acid. Free Radic Biol Med 22:669–678

Stan SD, Kar S, Stoner GD et al (2008) Bioactive food components and cancer risk reduction. J Cell Biochem 104:339–356

Steward WP, Brown K (2013) Cancer chemoprevention, a rapidly evolving field. Br J Cancer 109:1–7

Toshiya K, Testuya T, Akira H, et al (2012) Cancer chemoprevention through the induction of apoptosis by natural compounds. JBPC 3:156–173

Van E, Roepman MJ, Van der Lende P et al (2005) Integrated assessment by multiple gene expression analysis of quercetin bioactivity on anticancer-related mechanisms in colon cancer cells *in vitro*. Eur J Nutr 44:143–156

Waters EA, McNeel TS, Stevens WM et al (2012) Use of tamoxifen and raloxifene for breast cancer chemoprevention in 2010. Breast Cancer Res Treat 1342:875–880

Ye JJ, Cao J (2014) MicroRNAs in colorectal cancer as markers and targets, recent advances. World J Gastroenterol 20:4288–4299

Yu X, Kensler T (2005) Nrf2 as a target for cancer chemoprevention. Mutat Res 591:93–102

Modulation of Wnt/β-Catenin Signaling by Chemoprevention

13

More than 100 years ago, this theory that natural compounds might impede cancer development was stated and the effect of small natural compounds obtained from plants and fungi was examined on tumor development and growth in various laboratories. Presently, many labs are working to check the efficacy of these small molecules in modulating various signaling pathways and regulations of basic cellular functions like cell proliferation, cell differentiation, and apoptosis. Hence upgrading the current knowledge and deciphering the molecular mechanisms that underlie the action of these small molecules, thereby, provide clear understanding of signaling pathways in healthy and pathological states (Lustig and Behrens 2003; Nathália et al. 2014; Phelps et al. 2009).

Wnt/β-catenin signaling pathway plays an imperative role in normal growth, stem cell preservation, and various malignancies including CRC, PC, melanomas, etc. Nuclear localization of β-catenin is the hallmark of Wnt signaling. Many of the downstream genes involved in proliferation and cellular transformation are acted upon by β-catenin, an important transcriptional manager which collaborates with members of the T-cell factor (TCF) family of transcription factors in the active state to stimulate their transcription (Weeraratna 2005; Elcheva et al. 2008).

The word Wnt came into existence by the combination of Int-1 which is mouse proto-oncogene and *Drosophila* segment polarity wingless gene (Chandra et al. 2012; Siegel et al. 2013). Certain food ingredients of fruits, vegetables, and spices reveal cancer chemopreventive properties as reports from various preclinical models (Beveridge et al. 2002; Saleem 2009) and therefore have gained huge consideration due to their inherent capacity to inhibit or delay or prevent cancer growth and progression (You et al. 2003). There are escalating evidences from various reports that dietary components are used alone as well as in combination with traditional chemotherapeutic agents to thwart the incidence of cancer, to increase its latency period, and to treat cancer also. Wnt plays an imperative role in the development and progression of cancer(s), which could be modulated by dietary factors.

A large number of secreted protein growth factors are encoded by Wnt genes which have been recognized in animals from hydra to humans (Lima et al. 2007).

S. Rashid, *Cancer and Chemoprevention: An Overview*,
DOI 10.1007/978-981-10-2579-2_13

Wnt genes and their chromosomal positions have been documented in humans (Lima et al. 2007). Wnt(s) have been found to have varied roles in maintaining cell fate, cell proliferation, cell movement, polarization, and apoptosis. In humans, β-catenin is encoded by CTNNB1 gene and is a 781-amino acid protein. β-Catenin levels in the cytoplasm are usually kept low through constant proteasome-mediated degradation by a destructive complex of adenomatous polyposis coli (APC)/glycogen synthase kinase-3b (GSK-3b)/Axin. The degradation of β-catenin is repressed when cells receive Wnt signals; thus levels of β-catenin accumulate in the cytoplasm and nucleus (Tarapore et al. 2010), wherein nuclear β-catenin interacts with transcription factors like T-cell factor/lymphoid enhancer-binding factor (Tcf/Lef) and acts as a transcription controller for various genes that in a way regulate tumor formation and progression (Tarapore et al. 2010). β-Catenin might alter the Tcf repressor complex into a transcriptional activator complex once it is in the nucleus by displacement of Groucho from Tcf/Lef and mobilization of the histone acetylase cyclic adenosine monophosphate response element-binding protein/p300.

Binding of cyclic adenosine monophosphate response element-binding protein to the β-catenin/Tcf complex may act as a coactivator (Hecht et al. 2000; Takemaru et al. 2000). Activating mutations in β-catenin detected in half of the colorectal cancers that demonstrate wild-type APC have led to the discovery of oncogenic role of β-catenin (Morin 1997, 1999; Rubinfeld et al. 1997). Wnt signaling is responded by one class of targets known as frizzled (Fz) along with induction of two cytoplasmic antagonists. Inhibition of Wnt signaling in *Drosophila* (Takemaru et al. 2000) and vertebrates occurs by (Wharton et al. 2001) the naked cuticle gene which binds openly to Dsh. Another negative regulator of Wnt signaling is the Axin2 gene that directly targets Wnt signaling.

Wnt/β-Catenin Signaling and Cancer

Mutations in Wnt/β-catenin signaling pathway which result in constitutive activation of this pathway lead to cancer. Axin2 which is Axin-like protein (Axil) or axis inhibition protein 2 (Axin2) or conductin which is encoded by the Axin2 gene in humans plays a vital role in the regulation of the constancy of β-catenin in the Wnt signaling pathway and is identified to exhibit a tendency to colon cancer (Lammi). Other tumors also decipher a variety of mutations in β-catenin, and APC has been observed (Chien et al. 2009; Giles et al. 2003; Clevers 2006) signifying that deregulation of Wnt/β-catenin signaling is an essential event in the derivation of many cancers as in the case of both primary hepatocellular carcinomas (HCC) and hepatoma cell lines (Miyoshi et al. 1998; Murata et al. 2001) where β-catenin mutations and interstitial deletions have been reported. Ser37 mutation was rarely observed in HCC, but alterations affecting the putative phosphorylation residues were common Fig. 13.1.

Studies in transgenic mice expressing c-Myc or H-ras in the liver contain β-catenin mutations demonstrating that β-catenin activation may interact with ras or Myc in progression of 50 % HCC (Murata et al. 2001; De et al. 1998). Exon 3 deletions and β-catenin mutations have been found to be exhibited in 48 % of sporadic

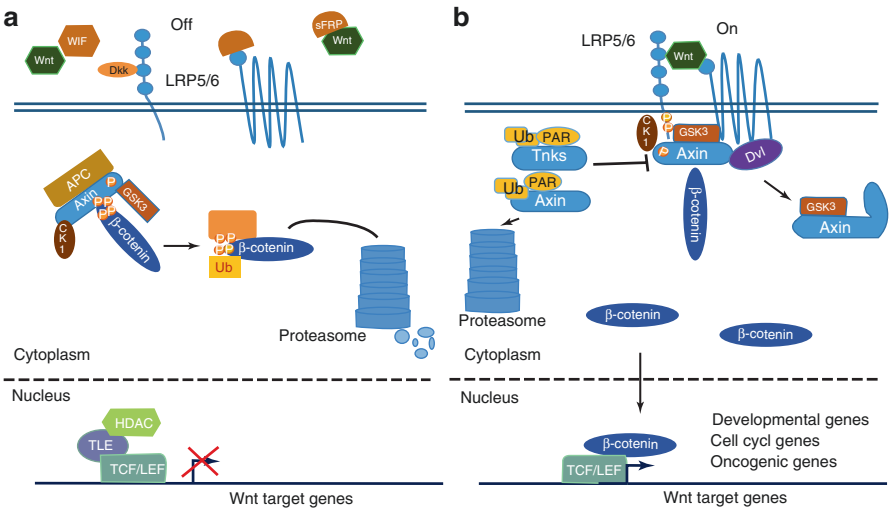


Fig. 13.1 depicts Wnt/β-catenin signaling pathway (Adapted and modified from Amado et al. (2014)). (**a**) When Wnt is not present. (**b**) In the presence of Wnt-ligand. *APC* adenomatous polyposis coli, *CK1* casein kinase 1, *Dkk* dickkopf, *Dvl* disheveled, *β-Trcp* beta-transducin repeat containing, *GSK3* glycogen synthase kinase 3, *LRP 5/6* LDL receptor-related protein 5/6, *TCF* T-cell factor. From APC: *FRP* frizzled-related protein, *WIF* Wnt inhibitor factor, *HDAC* histone deacetylase, *Tnks* tankyrases, *LEF* lymphoid enhancer-binding factor, *PAR* poly(ADP-ribose), *TLE* transducin-like enhancer proteins

hepatoblastomas, a type of childhood malignant liver tumor (Koch et al. 1999). β-Catenin mutations have been harbored in Wilms tumor which is a common childhood renal cancer. Interestingly 90 % of these tumors have β-catenin mutation at codon 45 in the abovementioned studies (Koesters et al. 1999; Kusafuka et al. 2002; Maiti et al. 2000).

Wnt/β-Catenin Signaling and Melanomas

The first study to suggest a mutation in β-catenin was a single amino acid substitution at the N-terminus of β-catenin identified as a melanoma-specific antigen which could result in cancer (Robbins et al. 1996). Mutations in CTNNB1 and unusually high levels of β-catenin have been reported in an array of melanomas (Rubinfeld et al. 1997). Here also like in HCC and RCC, mutations in Ser37 occur frequently in colon cancer (Rimm et al. 1999), though overexpression of APC decreased the levels of β-catenin in these lines similar to colon cancer (Rubinfeld et al. 1997).

Reports propose that deregulation of Wnt signaling may eventually lead to deregulation of microphthalmia-associated transcription factor-M (MITF-M) expression thereby resulting in inappropriate cellular functions in terms of differentiation, proliferation, and growth advantage (Larue et al. 2006, 2003). Morphological changes and loss of melanocytic and neural crest markers such as MITF and

tyrosinase have been associated with downregulation of Brn-2 in melanoma cells (Goodall et al. 2004) as well as loss of tumorigenicity of these cells in nude mice (Thomson et al. 1995). A Brn-2 promoter that has an active Lef/Tcf binding site (Goodall et al. 2004) entails that Wnt/β-catenin signaling can stimulate Brn-2 protein, which is implicated in cell proliferation and another protein (MITF) required for cell differentiation.

Wnt/β-Catenin Signaling and Colorectal Cancer

Colorectal cancer (CRC) is the third most common cause of cancer-related mortality in the USA (Phelps et al. 2009; Jemal et al. 2009). APC is one of the fundamental players in the sequence of molecule changes ensuing in CRC and has also been recognized as dependable germ line mutation in familial adenomatous polyposis patients (Kinzler et al. 1996; Fearon et al. 1990). APC forms a central component in the Wnt/β-catenin signaling pathway, and 80 % of non-inherited CRC cases have mutations in APC. At the early onset of colorectal carcinogenesis, mutations in CRC occur which corresponds that functional loss of APC is a necessity for additional progression toward malignant state (Munemitsu et al. 1995).

Reduction in the levels of β-catenin resulted in the reintroduction of wild-type APC. Familial adenomatous polyposis is an inherited disorder in which patients develop frequent polyps in the colon and rectum. Truncations in APC, which support anomalous activation of Wnt/β-catenin signaling pathway leading to adenomatous lesions, are the major cause behind familial adenomatous polyposis (Nishisho et al. 1991; Michils et al. 2005; Kinzler et al. 1996). In fact the loss of APC followed by anomalous Wnt/β-catenin signaling might instigate colon adenoma formation (Phelps et al. 2009) which is well evident from mouse models of APC truncation where nuclear β-catenin was found to be measurable soon after the loss of APC (Phelps et al. 2009). Nearly 7 % of sporadic human colon carcinomas had stabilizing mutations in β-catenin in the absence of mutations in APC. Hence these studies give genetic confirmation linking APC loss and β-catenin activation (Morin et al. 1997; Phelps et al. 2009; Iwao et al. 1998). Additionally, stabilized mutations in β-catenin in transgenic mice develop abundant intestinal adenomas (Phelps et al. 2009; Romagnolo et al. 1999).

Overall, these studies suggest that dysregulation of β-catenin is an important oncogenic incidence followed by loss of APC. However transfection of APC in colon cancer cells diminishes the unusually high Tcf reporter plasmid activity (Korinek et al. 1997). Amazingly some uncommon cell lines without APC mutations also show β-catenin/Tcf transcriptional activity which was calculated by luciferase reporter assay (Morin et al. 1997).

In 50 % of CRCs without APC mutations, displayed mutations in β-catenin gene represent 10 % of overall CRCs wherein mutations occur mostly in the microsatellite-instable tumors (Sparks et al. 1998; Kitaeva et al. 1997). A strong nuclear enrichment of β-catenin was seen at the invasion site as compared to large parts of the central tumor area where β-catenin was discovered in the cytoplasm and at the

membrane through immunolocalization of β-catenin in colon carcinomas indicating that high levels of nuclear β-catenin play a role in the conversion to the invasive state of tumor cells (Brabletz et al. 2000; Hlubek et al. 2007).

Wnt/β-Catenin Signaling and Prostate Cancer (PC)

Abnormal Wnt/β-catenin signaling appears to be a delayed event in PC approximately covering just a third of prostate tumors (Chesire et al. 2000). PC exhibits mutations in APC, β-catenin, and b-TrCP (Voeller et al. 1998; Gerstein et al. 2002). Single-strand conformation polymorphism in a panel of 104 PC recognized five mutations in the dogmatic site of β-catenin (Voeller et al. 1998) where four sites affected known phosphorylation sites of β-catenin and fifth affected a residue nearby to Ser33 similarly as seen in colon cancer (Sparks et al. 1998). Gleason scores, cellular level of β-catenin, and serum prostate-specific antigen levels were positively correlated with the expression levels of Wnt ligands (Wnt 1, Wnt 2, and Wnt 5a) (Yang et al. 2002; Iozzo et al. 1995; Verras et al. 2006). Truica et al. have reported that β-catenin is interrelated with androgen receptor and augments the transcriptional activity for androgen receptor in LNCaP cells suggestive of a promising mechanism of cross talk between Wnt and androgen signaling pathways which has been confirmed by various studies using yeast two-hybrid, in vitro, and in vivo models.

Wnt/β-Catenin Signaling and Breast Cancer

The canonical Wnt or Wnt/β-catenin pathway plays a vital role in mammary gland development and tumorigenesis. It is involved in cell adhesion, signal transduction, and regulation of cell context-specific gene expression. β-Catenin corresponds to the principal regulator of this pathway. β-Catenin is coupled with a multiprotein destruction complex consisting of glycogen synthase kinase, adenomatous polyposis coli, casein kinase-1α, and Axin which directs its phosphorylation followed by ubiquitination and degradation by proteasome in the absence of a Wnt signal, while in the presence of a proper Wnt signal, the destruction complex becomes dormant, resulting in stabilization and accumulation of β-catenin in the cytoplasm (Huang et al. 2009; Emami et al. 2004). Consequently, β-catenin translocates to the nucleus where it binds to T-cell factor/lymphoid enhancer factor and stimulates transcription of target genes like cyclin D1, c-Myc, matrix metalloproteinase, and VEGF which play important roles in breast cancer pathogenesis. There is escalating evidence from various genetically engineered animal models about the dysregulation of Wnt/β-catenin signaling through stabilized and nucleus-bound β-catenin leading to mammary tumor initiation and development (Yang et al. 2006; Waaler et al. 2012). Considerable accumulation of β-catenin in the nucleus or cytoplasm has been discovered in human breast cancer samples which associates it with poor prognosis. Same kind of cytosolic and nuclear β-catenin accumulation has been found in ductal

carcinoma in situ and a basal-like in situ breast tumor which infers that Wnt/β--catenin pathway activation may be an early event in human breast cancer. Wnt/β--catenin signaling can be an impending target for prevention and novel therapy of human breast cancer since its abnormal activation is implicated in the pathogenesis of mammary carcinoma (Mandal et al. 2013).

Wnt/β-Catenin Signaling Targeting by Naturally Occurring Compounds

Phytochemicals represent appropriate agents for chemoprevention because of their unique and unmatchable characteristics like being less toxic, easily available, and less expensive than synthetic agents. Conversely, only few groups have directly estimated the ability of these agents to disrupt β-catenin-mediated Wnt signaling which include flavoinds like genistein, curcumin, epigallocatechin-3-gallate (EGCG), resveratrol, lupeol, retinoids, and lycopene.

Flavonoids These are low-molecular-weight natural products of plant origin universally distributed in foods. They are documented as dietary ingredients having an array of biological properties which helps in amelioration of various diseases like atherosclerosis or cancer (Khan et al. 2008; Stoner et al. 1995; Amado et al. 2011).

Genistein is an isoflavone found in a number of plants such as soybeans and soy products (Park et al. 2005). Park et al. demonstrated that β-catenin/Tcf-driven transcription was suppressed inhibited strongly by flavanone in AGS gastric cancer cell dose dependently. Studies by Sarkar et al. reported (Li et al. 2008) that genistein upregulated increased β-catenin phosphorylation, GSK-3b, and its binding to β-catenin signifying that genistein could inactivate Wnt/β-catenin signaling resulting in prostate cancer growth inhibition (Li et al. 2008; Sarkar et al. 2009). Genistein also lessened Wnt1-induced proliferation and diminished the expression of Wnt target genes like c-Myc and cyclin D1 (Sarkar et al. 2009; Su et al. 2009, 2007).

Curcumin is a yellow pigment found majorly in turmeric and a member of the ginger *Curcuma longa* (Zingiberaceae). Since ancient times, turmeric is used in Ayurvedic system of medicine in India (Narayan 2004). The therapeutic properties of curcumin have been reported as an anticoagulant, antibacterial, analgesic, antiviral, antiparasitic, antioxidant, antiarthritic, antihypercholesterolemic, and antihypertensive (Wilken et al. 2011; Zhou et al. 2011; Epstein et al. 2010). It is used in the treatment of a wide range of diseases from asthma (by reducing the activity of hyaluronidase activity) to Alzheimer's disease (decreasing expression of β-amyloid) and human immunodeficiency virus (Khan et al. 2008; Aggarwal et al. 2003; Kumar et al. 1998; Gupta et al. 2010). It has been reported to prevent cancer in the skin, forestomach, duodenum, and colon in mice and the tongue, colon, and mammary and sebaceous glands in rats (Kelloff et al. 1994a, b). Moreover, it has also been related with relapse of well-known malignancy in humans (Khan et al. 2008; Thomas et al. 2010; Son et al. 2010; Fu et al. 2010). It has been found to suppress

the expression of cyclooxygenase-2, which is an important player in colon carcinogenesis (Narayan 2004).

It is reported to be nontoxic to humans up to a dose of 8000 mg/day when taken orally for 3 months indicating its chemopreventive potential (Narayan 2004). Its treatment repressed the messenger RNA expression of Wnt target genes c-Myc, c-Fos, c-Jun, and iNOS in a variety of cancer cells (Prasad et al. 2009; Lin 2007). β-Catenin transcriptional activity in a variety of CRC cell lines in a dose-dependent manner (Jaiswal et al. 2002; Ryu et al. 2008) resulted in the increased degradation of cytoplasmic β-catenin and decreased nuclear β-catenin levels (Ryu et al. 2008). Ryu et al. demonstrated reduction in expression of nuclear p300 coactivator upon curcumin treatment dose dependently, and this is the best strategy since nuclear β-catenin forms a complex with Tcf4 and p300 coactivator to generate a transcriptional active complex (Karim et al. 2004).

EGCG and Green Tea Polyphenols Tea is the by-product obtained from plant *Camellia sinensis*, consumed by two-thirds of the world's population, and is the most well-liked drink (Khan et al. 2008). The three main commercial types are green, black, and oolong tea, and they differ in processing and fermentation. Green tea contains catechins which include epigallocatechin-3-gallate (EGCG), epigallocatechin, epicatechin-3-gallate, and epicatechin which are strong radical scavengers and metal chelators validated from various number cell lines, chemical-based assays, and in vivo as well (Lambert et al. 2010). Tea treatment has been shown to induce phase II metabolism and antioxidant enzymes in both animal models and humans (Tulayakul et al. 2007; Maliakal et al. 2001; Orner et al. 2004). Formation of 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine-induced colonic aberrant crypts in the rat as well as intestinal polyps in the APC-Min mouse was inhibited by green tea in one study (Carter et al. 2007; Melgarejo et al. 2010) along with downregulation of β-catenin expression in the intestine as well as reduced expression of β-catenin/Tcf target genes (c-Jun and cyclin D1) which resulted in decrease in tumor burden.

These findings suggest the opportunity for intercession because they suggest the possibility for intervention by tea against the early stages of human CRC, linking the β-catenin/APC pathway and the alteration of Wnt target genes, and hence seem to create a hope of therapeutic window (Korinek et al. 1997; Sparks et al. 1998; Orner et al. 2004).

Suppression of β-catenin/Tcf4 transcriptional activity in a concentration dependent upon treatment with EGCG in HEK293 cells induced with β-catenin/Tcf4 was observed (Dashwood et al. 2002). ECGC also inhibited Wnt/β-catenin signaling in breast cancer cells in a dose-dependent manner. There was upregulation of HMG-box containing protein 1 which is an important antagonist of Wnt signaling on treatment with EGCG (Sarkar et al. 2009; Kim et al. 2006). EGCG reduced both proliferation and invasiveness of breast cancer cells through induction of HMG-box containing protein 1 and the subsequent downregulation of Wnt/β-catenin signaling.

Resveratrol is a dietary polyphenol. It possesses strong antioxidant, anti-inflammatory properties and intercedes induction of antioxidant enzyme system. It also modulates lipid metabolism for attenuating hepatic lipid peroxidation (Baur et al. 2006; Ulrich et al. 2005). It increases hepatic glutathione content, quenches free radicals, and activates enzymes of phase II hepatic metabolism (Baur et al. 2006; Ulrich et al. 2005; Bishayee 2009). It has been found to attenuate nuclear translocation of NFkB (Tsai et al. 1999) and impedes with its transcription (Pendurthi et al. 1999). Furthermore, it reduces expression of numerous pro-inflammatory cytokines (Fremont 2000; Pendurthi et al. 1999).

Studies suggest that resveratrol restrains skin tumorigenesis through the regulation of phosphatidylinositol-3-kinase and Akt proteins, which are involved in the development and progression of cancer. Resveratrol has been found to reduce significantly the level of β-catenin in the nucleus of colon cancer cells which could be decreased (Hope et al. 2008). Besides solid tumors, resveratrol also inhibits proliferation and stimulates cell cycle arrest and apoptosis in Waldenstrom's macroglobulinemia cells. The downregulation of Akt, mitogen-activated protein kinase, and Wnt signaling pathways resulted in the abovementioned effects of resveratrol (Roccaro et al. 2008). Resveratrol-induced apoptosis is linked with the instigation of the p53 in a dose- and time-dependent manner (Roccaro et al. 2008). It has been found to modulate DNA double-strand break repair pathways in p53-dependent manner (Gatz et al. 2008), signifying the regulatory effect of resveratrol on p53 signaling.

Lupeol is a dietary triterpene found in several fruits (olives, figs, mangoes, strawberries, and grapes), vegetables (green peppers, white cabbage, and tomato), and medicinal plants (American ginseng, shea butter plant, *Tamarindus indica*, *Crataeva nurvala*, and *Bombax ceiba*), used extensively by native tribes of North America, China, Africa, and the Caribbean islands (Saleem 2009). It has various pharmacological activities reported from in vitro and in vivo studies. Some of which include its potential against inflammation, cancer, arthritis, diabetes, heart diseases, nephrotoxicity, and liver toxicity (Lee et al. 2007). It has also been reported to have strong antimutagenic activity in vitro and in vivo systems (Nigam et al. 2007; Lira Wde et al. 2008). Nigam et al. confirmed lupeol prevents 7,14-dimethylbenz(a)anthracene-induced DNA damage (DNA strand breaks) in mouse skin in their study (Nigam et al. 2007). Recently, lupeol also inhibited benzo(a)pyrene-induced genotoxicity in mouse model (Prasad et al. 2008), whereas significant induction of chromosomal aberrations and micronuclei was recorded in benzo(a)pyrene-treated animals, with a decrease in mitotic index, and benzo(a)pyrene-induced clastogenicity upon lupeol administration (Prasad et al. 2008). Lupeol was found to inhibit tumor promotion in a murine mouse. Lupeol (40 mg per animal per thrice a week) decreased tumor burden significantly in mice on topical application (Saleem et al. 2004). Lupeol modulated NFkB and phosphatidylinositol-3-kinase/Akt pathway which play an essential role in tumor progression, hence deciphering its antitumor effects (Saleem et al. 2004; Lira

Wde et al. 2008; Lee et al. 2007; Saleem et al. 2009a, b). Lupeol inhibited growth and proliferation of human prostate cancer cells irrespective of their androgen status by recent study reports (Saleem et al. 2009a). Recent studies from our laboratory have suggested that lupeol also inhibited human metastatic melanoma cells in vitro and in a xenograft mouse model (Saleem et al. 2008).

Conclusion

To summarize, abnormal Wnt/β-catenin signaling is found in an array of cancers and has a central function in cancer development. In vitro, in vivo, and preclinical human studies emphasize the value of natural products that implement their modulatory and suppressing effects on cancer growth and progression by downregulating Wnt/β-catenin signaling pathway. These modulatory natural products or dietary factors may be taken either individually or in amalgamation with traditional therapeutics for the anticipation and/or management of numerous human cancers. Suitable and significant studies in animal models as well as widespread clinical trials must be undertaken to fully elucidate the mechanisms behind their therapeutic properties to improve the quality of human health and thus saving millions of patients from disease and associated side effects of conventional treatment (Saleem et al. 2008, 2005a, 2009a, b). Lupeol inhibits growth of highly aggressive human metastatic melanoma cells in vitro and in vivo by inducing apoptosis. Approximately 80 % of all CRCs have mutations in Wnt components, which results in overactivation of the pathway in colon cells. The most common mutations result in APC loss of function which is a negative regulator of this signaling pathway and gain of function mutations in β-catenin, the main effector protein of this signaling. These mutations cause uncontrolled overexpression of several oncogenes and cell cycle genes, particularly in cells derived from the intestinal crypts (Miyoshi et al. 1992; Morin et al. 1997; Sparks et al. 1998).

References

Aggarwal BB et al (2003) Anticancer potential of curcumin: preclinical and clinical studies. Anticancer Res 23:363–398

Amado NG et al (2011) Flavonoids: potential Wnt/beta-catenin signaling modulators in cancer. Life Sci 89:545–554

Amado NG, Predes D, Moreno MM et al (2014) Flavonoids and Wnt/β-catenin signaling: potential role in colorectal cancer therapies. Int J Mol Sci 15:12094–12106

Baur JA et al (2006) Therapeutic potential of resveratrol: the *in vivo* evidence. Nat Rev Drug Discov 5:493–506

Beveridge TH, Li TS, Drover JC (2002) Phytosterol content in American ginseng seed oil. J Agric Food Chem 50:744–750

Bishayee A (2009) Cancer prevention and treatment with resveratrol: from rodent studies to clinical trials. Cancer Prev Res (Phila) 2:409–418

Brabletz T et al (2000) beta-Catenin induces invasive growth by activating matrix metalloproteinases in colorectal carcinoma. Verh Dtsch Ges Pathol 84:175–181

Carter O et al (2007) Comparison of white tea, green tea, epigallocatechin- 3-gallate, and caffeine as inhibitors of PhIP-induced colonic aberrant crypts. Nutr Cancer 58:60–65
Chandra SH, Wacker I, Appelt UK et al (2012) A common role for various human truncated adenomatous polyposis coli isoforms in the control of beta-catenin activity and cell proliferation. PLoS One 7:e34479
Chesire DR et al (2000) Detection and analysis of beta-catenin mutations in prostate cancer. Prostate 45:323–334
Chien AJ et al (2009) A Wnt survival guide: from flies to human disease. J Invest Dermatol 129:1614–1627
Clevers H (2006) Wnt/beta-catenin signaling in development and disease. Cell 127:469–480
Dashwood WM et al (2002) Inhibition of beta-catenin/Tcf activity by white tea, green tea, and epigallocatechin-3-gallate EGCG: minor contribution of H_2O_2 at physiologically relevant EGCG concentrations. Biochem Biophys Res Commun 296:584–588
De LA, Coste A et al (1998) Somatic mutations of the beta-catenin gene are frequent in mouse and human hepatocellular carcinomas. Proc Natl Acad Sci U S A 95:8847–8851
Elcheva I, Tarapore RS, Bhatia N et al (2008) Overexpression of mRNA-binding protein CRDBP in malignant melanomas. Oncogene 27:5069–5074
Emami KH, Nguyen C, Ma H et al (2004) Proc Natl Acad Sci U S A 101:12682–12687
Epstein J et al (2010) Curcumin as a therapeutic agent: the evidence from *in vitro*, animal and human studies. Br J Nutr 103:1545–1557
Fearon ER et al (1990) A genetic model for colorectal tumorigenesis. Cell 61:759–767
Fremont L (2000) Biological effects of resveratrol. Life Sci 66:663–673
Fu S et al (2010) Development of curcumin as an epigenetic agent. Cancer 116:4670–4676
Gatz SA et al (2008) Resveratrol modulates DNA double-strand break repair pathways in an ATM/ATR-p53- and -Nbs1-dependent manner. Carcinogenesis 29:519–527
Gerstein AV et al (2002) APC/CTNNB1 (beta-catenin) pathway alterations in human prostate cancers. Genes Chromosomes Cancer 34:9–16
Giles RH, van Es JH, Clevers H (2003) Caught up in a Wnt storm: Wnt signaling in cancer. Biochim Biophys Acta 1653:1–24
Goodall J et al (2004) Brn-2 expression controls melanoma proliferation and is directly regulated by beta-catenin. Mol Cell Biol 24:2915–2922
Gupta SC et al (2010) Regulation of survival, proliferation, invasion, angiogenesis, and metastasis of tumor cells through modulation of inflammatory pathways by nutraceuticals. Cancer Metastasis Rev 29:405–434
Hecht A et al (2000) The p300/CBP acetyltransferases function as transcriptional coactivators of beta-catenin in vertebrates. EMBO J 19:1839–1850
Hlubek F et al (2007) Heterogeneous expression of Wnt/beta-catenin target genes within colorectal cancer. Int J Cancer 121:1941–1948
Hope C et al (2008) Low concentrations of resveratrol inhibit Wnt signal throughput in colon-derived cells: implications for colon cancer prevention. Mol Nutr Food Res 52:S52–S61
Huang SM, Mishina YM, Liu SA et al (2009) Tankyrase inhibition stabilizes axin and antagonizes Wnt signalling. Nature 461:614–620
Iozzo RV et al (1995) Aberrant expression of the growth factor Wnt-5A in human malignancy. Cancer Res 55:3495–3499
Iwao K et al (1998) Activation of the beta-catenin gene by interstitial deletions involving exon 3 in primary colorectal carcinomas without adenomatous polyposis coli mutations. Cancer Res 58:1021–1026
Jaiswal AS et al (2002) Beta-catenin-mediated transactivation and cell cell adhesion pathways are important in curcumin diferuylmethane induced growth arrest and apoptosis in colon cancer cells. Oncogene 21:8414–8427
Jemal A et al (2009) Cancer statistics. CA Cancer J Clin 59:225–249
Karim R et al (2004) The significance of the Wnt pathway in the pathology of human cancers. Pathology 36:120–128

Kelloff GJ et al (1994a) Progress in cancer chemoprevention: perspectives on agent selection and short-term clinical intervention trials. Cancer Res 54:2015s–2024s
Kelloff GJ et al (1994b) Strategy and planning for chemopreventive drug development: clinical development plans. Chemoprevention Branch and Agent Development Committee National Cancer Institute. J Cell Biochem Suppl 20:55–62
Khan N et al (2008) Cancer chemoprevention through dietary antioxidants: progress and promise. Antioxid Redox Signal 10:475–510
Kim J et al (2006) Suppression of Wnt signaling by the green tea compound epigallocatechin 3-gallate EGCG in invasive breast cancer cells Requirement of the transcriptional repressor. J Biol Chem 281:10865–10875
Kinzler KW et al (1996) Lessons from hereditary colorectal cancer. Cell 87:159–170
Kitaeva MN et al (1997) Mutations in beta-catenin are uncommon in colorectal cancer occurring in occasional replication error-positive tumors. Cancer Res 57:4478–4481
Koch A et al (1999) Childhood hepatoblastomas frequently carry a mutated degradation targeting box of the beta-catenin gene. Cancer Res 59:269–273
Koesters R et al (1999) Mutational activation of the beta-catenin proto oncogene is a common event in the development of Wilms' tumors. Cancer Res 59:3880–3882
Korinek V et al (1997) Constitutive transcriptional activation by a beta catenin- Tcf complex in APC−/− colon carcinoma. Science 275:1784–1787
Kumar A et al (1998) Curcumin (diferuloylmethane) inhibition of tumor necrosis factor (TNF)-mediated adhesion of monocytes to endothelial cells by suppression of cell surface expression of adhesion molecules and of nuclear factor-kappa B activation. Biochem Pharmacol 55:775–783
Kusafuka T et al (2002) Codon 45 of the beta-catenin gene, a specific mutational target site of Wilms' tumor. Int J Mol Med 10:395–399
Lambert JD et al (2010) The antioxidant and pro-oxidant activities of green tea polyphenols: a role in cancer prevention. Arch Biochem Biophys 501:65–72
Larue L et al (2003) Beta-catenin in the melanocyte lineage. Pigment Cell Res 16:312–317
Larue L et al (2006) The Wnt/Beta-catenin pathway in melanoma. Front Biosci 11:733–742
Lee TK et al (2007) Lupeol suppresses cisplatin-induced nuclear factor kappa B activation in head and neck squamous cell carcinoma and inhibits local invasion and nodal metastasis in an orthotopic nude mouse model. Cancer Res 67:8800–8809
Li Y et al (2008) Regulation of Akt/FOXO3a/GSK-3beta/AR signaling network by isoflavone in prostate cancer cells. J Biol Chem 283:27707–27716
Lima LM, Perazzo FF, Tavares C et al (2007) Anti-inflammatory and analgesic activities of the ethanolic extracts from Zanthoxylum riedelianum (Rutaceae) leaves and stem bark. J Pharm Pharmacol 59:1151–1158
Lin JK (2007) Molecular targets of curcumin. Adv Exp Med Biol 595:227–243
Lira Wde M et al (2008) Modulatory effect of Byrsonima basiloba extracts on the mutagenicity of certain direct and indirect-acting mutagens in Salmonella typhimurium assays. J Med Food 11:111–119
Lustig B, Behrens J (2003) The Wnt signaling pathway and its role in tumor development. J Cancer Res Clin Oncol 129:199–221
Maiti S et al (2000) Frequent association of beta-catenin and WT1 mutations in Wilms tumors. Cancer Res 60:6288–6292
Maliakal PP et al (2001) Tea consumption modulates hepatic drug metabolizing enzymes inWistar rats. J Pharm Pharmacol 53:569–577
Mandal A, Bhatia D, Bishayee A (2013) Simultaneous disruption of estrogen receptor and Wnt/β--catenin signaling is involved in methyl amooranin-mediated chemoprevention of mammary gland carcinogenesis in rats. Mol Cell Biochem 384:239–250
Melgarejo E et al (2010) Targeting polyamines and biogenic amines by green tea epigallocatechin-3-gallate. Amino Acids 38:519–523
Michils G et al (2005) Large deletions of the APC gene in 15 % of mutation-negative patients with classical polyposis (FAP): a Belgian study. Hum Mutat 25:125–134

Miyoshi Y, Nagase H, Ando H et al (1992) Somatic mutations of the APC gene in colorectal tumors: mutation cluster region in the APC gene. Hum Mol Genet 1:229–233
Miyoshi Y et al (1998) Activation of the beta-catenin gene in primary hepatocellular carcinomas by somatic alterations involving exon 3. Cancer Res 58:2524–2527
Morin PJ (1997) Activation of β-catenin-Tcf signaling in colon cancer by mutations in β catenin or APC. Science 275:1787–1790
Morin PJ (1999) beta-catenin signaling and cancer. Bioessays 21:1021–1030
Morin PJ et al (1997) Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. Science 275:1787–1790
Munemitsu S et al (1995) Regulation of intracellular beta-catenin levels by the adenomatous polyposis coli (APC) tumor-suppressor protein. Proc Natl Acad Sci U S A 92:3046–3050
Murata M et al (2001) Accumulation of beta-catenin in the cytoplasm and the nuclei during the early hepatic tumorigenesis. Hepatol Res 21:126–135
Narayan S (2004) Curcumin, a multi-functional chemopreventive agent, blocks growth of colon cancer cells by targeting beta-catenin-mediated transactivation and cell-cell adhesion pathways. J Mol Histol 35:301–307
Nathália GA, Danilo P, Marcela M et al (2014) Flavonoids and Wnt/β-catenin signaling: potential role in colorectal cancer therapies. Int J Mol Sci 15:12094–12106
Nigam N et al (2007) Preventive effects of lupeol on DMBA induced DNA alkylation damage in mouse skin. Food Chem Toxicol 45:2331–2335
Nishisho I et al (1991) Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. Science 253:665–669
Orner GA et al (2004) Tumor-suppressing effects of antioxidants from tea. J Nutr 134:3177S–3178S
Park CH et al (2005) Inhibition of beta-catenin-mediated transactivation by flavanone in AGS gastric cancer cells. Biochem Biophys Res Commun 331:1222–1228
Pendurthi UR et al (1999) Resveratrol, a polyphenolic compound found in wine, inhibits tissue factor expression in vascular cells: a possible mechanism for the cardiovascular benefits associated with moderate consumption of wine. Arterioscler Thromb Vasc Biol 19:419–426
Phelps RA, Broadbent TJ, Stafforini DM et al (2009) New perspectives on APC control of cell fate and proliferation in colorectal cancer. Cell Cycle 8:2549–2556
Prasad S et al (2008) Protective effects of lupeol against benzo(a)pyrene induced clastogenicity in mouse bone marrow cells. Mol Nutr Food Res 52:1117–1120
Prasad CP et al (2009) Potent growth suppressive activity of curcumin in human breast cancer cells: modulation of Wnt/beta-catenin signaling. Chem Biol Interact 181:263–271
Rimm DL et al (1999) Frequent nuclear/cytoplasmic localization of beta-catenin without exon 3 mutations in malignant melanoma. Am J Pathol 154:325–329
Robbins PF et al (1996) A mutated beta-catenin gene encodes a melanoma-specific antigen recognized by tumor infiltrating lymphocytes. J Exp Med 183:1185–1192
Roccaro AM et al (2008) Resveratrol exerts antiproliferative activity and induces apoptosis in Waldenstrom's macroglobulinemia. Clin Cancer Res 14:1849–1858
Romagnolo B et al (1999) Intestinal dysplasia and adenoma in transgenic mice after overexpression of an activated beta-catenin. Cancer Res 59:3875–3879
Rubinfeld B et al (1997) Stabilization of beta-catenin by genetic defects in melanoma cell lines. Science 275:1790–1792
Ryu MJ et al (2008) Natural derivatives of curcumin attenuate the Wnt/ beta-catenin pathway through down-regulation of the transcriptional coactivator p300. Biochem Biophys Res Commun 377:1304–1308
Saleem M (2009) Lupeol, a novel anti-inflammatory and anti-cancer dietary triterpene. Cancer Lett 285:109–115
Saleem M et al (2004) Lupeol modulates NF-kappaB and PI3K/Akt pathways and inhibits skin cancer in CD-1mice. Oncogene 23:5203–5214

Saleem M et al (2005a) A novel dietary triterpene Lupeol induces fas mediated apoptotic death of androgen-sensitive prostate cancer cells and inhibits tumor growth in a xenograft model. Cancer Res 65:11203–11213

Saleem M et al (2005b) Lupeol, a fruit and vegetable based triterpene, induces apoptotic death of human pancreatic adenocarcinoma cells via inhibition of Ras signaling pathway. Carcinogenesis 26:1956–1964

Saleem M et al (2008) Lupeol inhibits growth of highly aggressive human metastatic melanoma cells *in vitro* and *in vivo* by inducing apoptosis. Clin Cancer Res 14:2119–2127

Saleem M et al (2009a) Lupeol inhibits proliferation of human prostate cancer cells by targeting beta-catenin signaling. Carcinogenesis 30:808–817

Saleem M et al (2009b) Lupeol triterpene, a novel diet-based microtubule targeting agent: disrupts survivin/cFLIP activation in prostate cancer cells. Biochem Biophys Res Commun 388:576–582

Sarkar FH et al (2009) Cellular signaling perturbation by natural products. Cell Signal 21:1541–1547

Siegel R, Naishadham D, Jemal A (2013) Cancer statistics. CA Cancer J Clin 63:11–30

Son M et al (2010) Ethanol extract of *Lycoris radiata* induces cell death in B16F10 melanoma via p38-mediated AP-1 activation. Oncol Rep 24:473–478

Sparks AB, Morin PJ, Vogelstein B et al (1998) Mutational analysis of the APC/β-catenin/Tcf pathway in colorectal cancer. Cancer Res 58:1130–1134

Stoner GD et al (1995) Polyphenols as cancer chemopreventive agents. J Cell Biochem Suppl 22:169–180

Su Y et al (2007) Expression profiling of rat mammary epithelial cells reveals candidate signaling pathways in dietary protection from mammary tumors. Physiol Genomics 30:8–16

Su Y et al (2009) Soy isoflavone genistein upregulates epithelial adhesion molecule E-cadherin expression and attenuates beta-catenin signaling in mammary epithelial cells. Carcinogenesis 30:331–339

Takemaru KI et al (2000) The transcriptional co activator CBP interacts with beta-catenin to activate gene expression. J Cell Biol 149:249–254

Tarapore RS, Siddiqui IA, Saleem M et al (2010) Specific targeting of Wnt/beta-catenin signaling in human melanoma cells by a dietary triterpene lupeol. Carcinogenesis 31:1844–1853

Thomas SL et al (2010) Activation of the p38 pathway by a novel monoketone curcumin analog, EF24, suggests a potential combination strategy. Biochem Pharmacol 80:1309–1316

Thomson JA et al (1995) The brn-2 gene regulates the melanocytic phenotype and tumorigenic potential of human melanoma cells. Oncogene 11:691–700

Tsai SH et al (1999) Suppression of nitric oxide synthase and the downregulation of the activation of NFkappaB in macrophages by resveratrol. Br J Pharmacol 126:673–680

Tulayakul P et al (2007) The effect of feeding piglets with the diet containing green tea extracts or coumarin on *in vitro* metabolism of aflatoxin B1 by their tissues. Toxicon 50:339–348

Ulrich S et al (2005) Molecular mechanisms of the chemopreventive effects of resveratrol and its analogs in carcinogenesis. Mol Nutr Food Res 49:452–461

Verras M et al (2006) Roles and regulation of Wnt signaling and beta catenin in prostate cancer. Cancer Lett 237:22–32

Voeller HJ et al (1998) Beta-catenin mutations in human prostate cancer. Cancer Res 58:2520–2523

Waaler J, Machon O, Tumova L et al (2012) Cancer Res 72:2822–2832

Weeraratna ATA (2005) Wnt-er wonderland--the complexity of Wnt signaling in melanoma. Cancer Metastasis Rev 24:237–250

Wharton KA Jr et al (2001) Vertebrate proteins related to Drosophila Naked Cuticle bind Dishevelled and antagonize Wnt signaling. Dev Biol 234:93–106

Wilken R et al (2011) Curcumin: a review of anti-cancer properties and therapeutic activity in head and neck squamous cell carcinoma. Mol Cancer 10:12

Yang F et al (2002) Linking beta-catenin to androgen-signaling pathway. J Biol Chem 277:11336–11344
Yang J, Zhang W, Evans PM et al (2006) Biol Chem 281:17751–17757
You YJ, Nam NH, Kim Y et al (2003) Antiangiogenic activity of lupeol from *Bombax ceiba*. Phytother Res 17:341–344
Zhou H et al (2011) The targets of curcumin. Curr Drug Targets 12:332–347

Modulation of Akt/mTOR Pathway Signaling by Chemoprevention

14

The PI3K/Akt/mTOR signaling pathway is one of the most repeatedly targeted pathways in all sporadic human cancers which accounts for 30 % of all well-known human cancers. Receptor tyrosine kinases (RTKs) or Ras activates PI3K which in turn activates Akt, its most chosen downstream target, and other several intracellular signaling molecules. Akt phosphorylates an array of substrates resulting in their activation, and the factors that are downstream factors of Akt control four wide processes—cell cycle progression, cell growth, cell metabolism, and cell survival (Luo et al. 2003; Ahmad et al. 2013). mTOR is activated when Akt alleviates the inhibitory action of tumor suppressor tuberin, namely, tuberous sclerosis complex 1 (TSC1) on mTOR. Therefore, the PI3K/Akt/mTOR pathway plays a vital role in the cell growth and metabolism once activated and eventually is involved in the invasion, metastasis, and aggressiveness of cancer cells. Hence this pathway could be targeted as a novel therapeutic option for better prognosis in cancer patients and its defined role in more than a quarter of known cancers (Manning and Cantley 2003; Falasca et al. 2011).

The mammalian target of rapamycin (mTOR) kinase is a conserved serine/threonine protein kinase which regulates many fundamental molecules in eukaryotes involved in cell growth and cell cycle progression in response to cellular signals (Houghton 2010). The mTOR signaling pathway has a central role in cellular processes such as cell survival, cell growth and proliferation, cell death, and tumor angiogenesis. It frequently causes hyper-activation in numerous human malignancies which made it an appealing and interesting therapeutic target for anticancer therapy (Shaw and Cantley 2006).

The mTOR is also known as rapamycin and FKBP12 target (RAFT) or rapamycin target (RAPT), FKBP12-rapamycin-associated protein (FRAP), or sirolimus effector protein (SEP). The mTOR gene is positioned on human chromosome 1. It is a 289 kDa serine/threonine kinase consisting of 2549 amino acids. It comprises of six functional domains which are:

S. Rashid, *Cancer and Chemoprevention: An Overview*,
DOI 10.1007/978-981-10-2579-2_14

- HEAT (huntingtin elongation factor 3, a subunit of protein phosphatase 2A and TOR1) domain mediating protein–protein interactions.
- FAT (FRAP-ATM- TRAPP) domain.
- FRB (FKBP12-rapamycin binding) domain mediating the inhibitory action of rapamycin on Raptor-bound mTOR.
- PIKK (PI3-kinase-related kinase) domain, serine phosphorylation sites (S2035 and S2481).
- RD (repressor domain).
- The carboxy-terminal FATC domain (Huang and Houghton 2003; Asnaghi et al. 2004) and all these structural domains are evolutionarily conserved (Luo et al. 2003).

Various cellular processes like cell growth, cell proliferation, cell survival, protein synthesis, and autophagy as well as transcription of ribosomal proteins and the synthesis of rRNA and tRNA are regulated by the activity of mTOR kinase. In general, insulin and other growth factors regulate the activity of mTOR via phosphatidylinositol-3-kinase (PI3K)–Akt pathway (Kadowaki and Kanazawa 2003).

mTOR exists as two separate complexes in eukaryotic cells: mTORC1, a rapamycin-sensitive complex defined by its association with Raptor (regulatory-associated protein of mTOR), and mTORC2, a rapamycin-insensitive complex defined by its association with Rictor (rapamycin-insensitive companion of mTOR). mTOR is mediated by Raptor (first protein) to regulate p70 ribosomal S6 kinase (p70S6K) and binds eukaryotic translation initiation factor 4E (4E-BP1) (Sarbassov et al. 2004). Alternatively, PRAS40 and Deptor are recognized as discrete negative regulators of mTORC1 (Peterson et al. 2009).

Rapamycin binds to FK506-binding protein of (12 kDa) FKBP12, and afterward the complex binds to the FRB domain of mTORC1 in the rapamycin-sensitive mTOR signaling pathway which leads to the weakening of correlation between mTOR and Raptor with subsequent inhibition of mTORC1 functions. Nevertheless, the mechanisms behind binding of rapamycin and several rapamycin derivatives to FKBP12 to inhibit mTORC1 signaling are not known. It may be attributed to starvation or lack of nutrients like amino acids or glucose that emerges to copy rapamycin treatment thereby resulting in rapid inactivation of p70S6K and hypophosphorylation of the 4E-BP1 (Dowling et al. 2010; Proud 2002).

Various growth factors include insulin-like growth factor 1 (IGF-1), epidermal growth factor (EGF), transforming growth factor (TGF), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), and platelet-derived growth factor (PDGF) (Gomez-Pinillos and Ferrari 2012). It has been reported to regulate the activity of mTOR. Growth factor-induced activation of mTOR is stimulated by Class I PI3K having distinctive capacity to produce oncogenic phosphatidylinositol 3,4,5-trisphosphate (PIP3). There are Class II and Class III PI3Ks which are deficient in this ability and thus are not linked to cancer. Class IA PI3Ks and Class IB PI3K are the two subclasses of Class I PI3Ks which are heterodimers having a

regulatory subunit (p85) and a catalytic subunit (p110α, β or δ) which are involved primarily in the pathogenesis of human cancer (Vogt et al. 2010; Rodon et al. 2013). When the growth factor binds to its cognate receptor tyrosine kinase (RTK), Class IA PI3Ks are deployed to the cell membrane by straight interaction of the p85 subunit with the activated receptors or by the inhibitory effect of p85 on p110 which is removed by binding, leading to the stimulation of p110 catalytic subunit which catalyzes the phosphorylation of phosphatidylinositol 4,5-bisphosphate (PIP2) to PIP3 at the membrane, which is an important secondary messenger in the cell and acts as docking site for signaling proteins having pleckstrin homology (PH) domain like Akt and 3-phosphoinositide-dependent kinase 1 (PDK1) and is the principal mediator of PI3K activity (Baselga 2011).

The serine/threonine protein kinase Akt called as protein kinase B (PKB), which is downstream of PI3K, is a vital mediator of mTOR activity. Akt is activated initially by translocation to the plasma membrane which is achieved by the docking of Akt to PIP3 on the membrane where it is phosphorylated by PDK1 to Thr308 and by PDK2 on Ser473. Integrin-linked kinase (ILK), protein kinase C β2, DNA-dependent protein kinase (DNA-PK), ataxia telangiectasia mutated (ATM), Akt, and mTORC2 are some of the potential PDK2s. Akt gets activated fully by both the phosphorylation efforts. Later on, it phosphorylates many other proteins which regulate an extensive range of cellular processes involved in protein-synthesizing machinery, evasion of apoptosis, cellular proliferation, and metabolism. mTOR is activated by Akt by directly phosphorylating mTOR at Ser2448 or by indirectly phosphorylating and inhibiting tuberous sclerosis complex 2 (TSC2) (Hay and Sonenberg 2004; Inoki et al. 2002), and later phosphorylation suppresses the activity of GTPase-activating protein (GAP) and allows GTP-bound active Ras homolog enriched in brain (Rheb) to stimulate mTOR. mTOR catalytic activity is activated by phosphorylation of mTOR at Ser2481 (an autophosphorylation site) (Soliman et al. 2010).

mTORC1 in activated state phosphorylates various substrates to promote anabolic processes (like biogenesis of ribosome, translation, and lipid and nucleotide synthesis) and represses catabolic processes like autophagy when conditions are favorable for cell growth. On the contrary, mTORC2 does not regulate protein translation directly, though it regulates cell cycle progression, cell survival, metabolism, and cytoskeletal organization via phosphorylation of serum and glucocorticoid-regulated kinase 1 (SGK1), protein kinase C (PKC), and Akt at Ser473 (Fruman and Rommel 2014).

Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) tumor suppressor is the most important negative regulator of the PI3K signaling pathway. PI3K activity is antagonized via dephosphorylating PIP3 by PTEN, which is a phosphatidylinositol-3 phosphatase that antagonizes PI3K activity that is generated by PI3K. PTEN loss results in an uncontrolled signaling of the PI3K pathway, leading to the formation of cancer. mTORC1 is regulated by another important protein, namely, tuberous sclerosis complex (TSC), and a heterodimer of two proteins, TSC1, namely, hamartin, and TSC2, namely, tuberin. Rheb that

is a small GTPase is negatively regulated by TSC1 and TSC2 which function as a GAP and convert Rheb into its inactive GDP-bound state to inhibit activation of mTOR (Abdulkareem and Blair 2013; Hay and Sonenberg 2004). In conclusion, an intrinsic mechanism of self-control acts as a regulatory feedback loop to deactivate additional instigation of mTOR pathway. Following mTOR phosphorylation, activated p70S6K phosphorylates and destabilizes insulin receptor substrate 1 (IRS1), thereby inhibiting PI3K activation is inhibited by mTOR phosphorylation in which p70S6K phosphorylates and destabilizes insulin receptor substrate 1 (IRS1) resulting in blocking upstream overactivation of the PI3K/Akt/mTOR cascade (Gomez-Pinillos and Ferrari 2012; Shimobayashi and Hall 2014). Autophagy is induced by nutrient depletion and starvation, or rapamycin leads to inhibition of mTOR. Comparatively little is known concerning the regulatory pathway of mTORC2 as compared to mTORC1. Unlike mTOR–Raptor, mTOR–Rictor complex does not bind to FRB domain and is insensitive to rapamycin treatment (Sarbassov et al. 2004). Activation of Akt which is a positive regulator of cell survival, proliferation, and metabolism is promoted by phosphorylation of mTORC2 complex. The molecular mechanism behind mTORC2 regulation of cytoskeletal organization is unknown, though many reports suggest that actin polymerization is done by knocking down mTORC2 components and disrupts cell morphology (Manning and Cantley 2007; Sarbassov et al. 2004). Exhaustion of mTOR and Rictor damages actin polymerization in neutrophils stimulated with chemoattractants and small Rho GTPases Rac and Cdc42 (He et al. 2013).

mTOR Signaling Pathway and Cancer

Various upstream activating elements and downstream effecting elements of mTOR are known to be deregulated in some cancers such as non-small cell lung cancer, breast cancer, renal cell carcinoma, colorectal sarcomas, and gastrointestinal tumors. The mTOR pathway is a key regulator of cell proliferation, and several upstream activators and downstream effectors of mTOR are known to be deregulated in some cancers such as renal cell carcinoma, non-small cell lung cancer, breast cancer, sarcomas, and colorectal and gastrointestinal tumors (Li et al. 2013; Takahashi et al. 2014; Wang and Zhang 2014). mTOR is an appealing target for cancer drug development and therapy since mTOR signaling is constitutively stimulated in various malignancies (Han et al. 2013; Pandurangan 2013). The mTOR signaling network consists of a number of tumor suppressor genes and proto-oncogenes that constitute the mTOR signaling network wherein abnormal activities of these genes will promote the development of cancerous cells. Cell cycle, survival, metabolism, motility, and genomic instability are the hallmarks of cancer which are controlled by the signaling network of PI3K, Akt, and mTOR (Tan et al. 2014) Fig. 14.1.

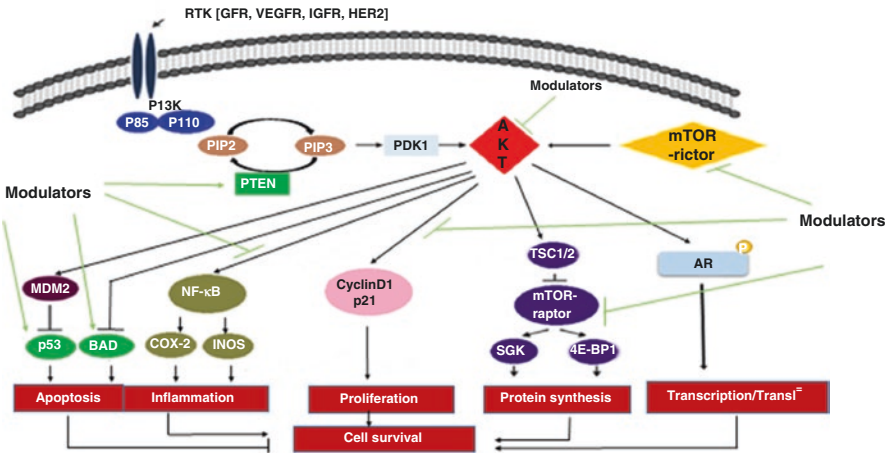


Fig. 14.1 Role of modulators or natural compounds against various signaling pathways like apoptosis, inflammation, proliferation, protein synthesis, transcription, and cell survival in deregulated cells which result into cancer cells (Adapted and modified from Zardavas et al. (2014))

Akt/mTOR Signaling in Cancer

Moreover, Akt triggers angiogenesis and incites epithelial–mesenchymal transition illustrated by activation of metallo-proteinases, morphological changes, loss of cell–cell adhesion, and augmented cell migration and invasion. 4E-BP1/eIF4-E axis additionally intervenes the effects of oncogenic Akt signaling on mRNA translation, cell growth, and tumor progression (Laplante et al. 2012). The prototypical PI3K inhibitors LY294002 and wortmannin restrain both mTOR and PI3K kinases because of their analogous ATP sites; the catalytic domains of mTOR and Class I PI3K exhibit similarity because such compounds have been developed which inhibit both kinases and diminish the phosphorylation of Akt, S6K1, and 4E-BP1 simultaneously. So dual PI3K/mTOR inhibitors NVP-BEZ235 (Novartis) and XL765 (Exelixis) are being investigated in patients with various types of tumors that are in phase I clinical trials (Serra et al. 2008).

Multiple cellular functions which include cell metabolism, cellular proliferation, and cell survival are being regulated by PTEN, and approximately half of all tumors have mutated tumor suppressor gene. It is one of the most frequently mutated tumor suppressor genes in human sporadic cancers, as reduced PTEN protein expression occurs in approximately half of all tumors. The major substrate of the lipid phosphatase activity of PTEN is PtdIns (3,4,5)P3, an important intracellular second messenger by dephosphorylating the D3 position of PtdIns (3,4,5)P3 (Song et al. 2012). PTEN is a negative regulator of PI3K pathway and Akt, and this inhibits cancer progression (Laplante and Sabatini 2012).

Role of Fisetin in Akt/mTOR Signaling Associated in Various Cancers

Lung Cancer Lung cancer is the leading cause of cancer-related deaths worldwide (Tong and Harpole 2012). Genetic and molecular alterations like autocrine signaling, activation of proto-oncogene into oncogenes, and loss of function of tumor suppressor genes have been reported toward its contribution; the mTOR, Akt, and MAPKs are the three important intracellular signaling proteins which seem to be appealing targets for lung cancer therapy (Papadimitrakopoulou and Adjei 2006). We examined the effect of fisetin in lung cancer that was studied by many investigators. PI3K/Akt and mTOR signaling in human lung cancer cells was inhibited by fisetin.

Treatment of A549 and H1792 human lung cancer cells with fisetin caused a decrease in cell viability and clonogenicity with negligible effect on normal bronchial cells (Khan et al. 2012). In A549 lung cancer cells, fisetin treatment resulted in increase in PTEN protein levels and decreased the expression of p85 subunit which is regulatory in function and p110 subunit which is catalytic in function of PI3K. Phosphorylation of Akt at both Ser473 and Thr308 in A549 cells was suppressed on treatment with fisetin and activated TSC ½ and reduced phosphorylation and stimulation of the mTOR kinase (Khan et al. 2012). Fisetin was found to actually interact with mTOR at two positions confirmed by in silico modeling of AMP-activated protein kinase (AMPK) which is a member of the protein kinase family which acts as cellular energy sensor in starvation conditions and was found to be upregulated by fisetin. Activation of AMPK restrains mTOR signaling resulting in inhibition of cancer cell growth and progression (Xu et al. 2012).

Raptor, Rictor, PRAS40, and GβL expressions were downregulated in lung cancer cells via decrease in mTOR phosphorylation upon fisetin treatment and suppressed mTOR complex. Additionally fisetin treatment downregulated downstream targets of mTOR like p70S6K1, eIF4-E, and 4E-BP1 which implicated in controlling ribosome protein synthesis, cell survival, and proliferation (Khan et al. 2012). The abovementioned findings clearly suggest fisetin-mediated growth inhibition of lung cancer cells via intervention of the Akt/mTOR signaling.

Prostate Cancer Prostate cancer is one of the most eminent medical problems faced by males of above 50 years of age. Other factors which are characteristic of PC are age, race, and familial history expanding the risk of disease (Brawley 2012). Remarkably, supplementation of a variety of nutrients of natural origin to the diet is found to have a prophylactic effect in the prevention and therapy of this lethal disease. Ingestion of at least five servings daily of fruits and vegetables has been recommended by the National Cancer Institute to prevent development and progression of prostate cancer (Higdon et al. 2007). The growth-inhibitory effect of dietary agents like silymarin, genistein, and epigallocatechin-3-gallate is being studied. Fisetin has been found to inhibit proliferation and human prostate cancer cell growth in in vitro and in vivo murine models (Khan et al. 2008a, b). Cell viability was decreased by the treatment of LNCaP, CWR22Rν1, and PC-3 prostate cancer cells with fisetin but had negligible effect on normal prostate epithelial cells. Fisetin

treatment on LNCaP cells induced caspase-dependent apoptosis, as well as arrested cells in the G0–G1 phase of the cell cycle. Moreover, in athymic nude mice injected with AR, positive CWR22Rν1 cancer cells upon treatment with fisetin led to the inhibition of tumor growth and decrease in serum prostate-specific antigen (PSA) levels (Khan et al. 2008a, b; Syed et al. 2013).

Fisetin had higher affinity and competed with the natural ligand dihydrotestosterone for the androgen receptor and physically interacted with its ligand-binding domain leading to reduced receptor stability as shown by cell culture studies resulting in reduced interaction. Interaction between the amino and carboxyl terminal ends of the receptor followed by poor receptor mediated transactivation of androgen receptor target genes like PSA (Khan et al. 2008b). Fisetin treatment decreased protein expression of PI3K at p85 subunit and phosphorylation of Akt at both Thr308 and Ser473 in LNCaP prostate cancer cells (Suh et al. 2010). Studies show that cell survival is influenced by Akt through various target proteins including inhibition of the pro-apoptotic Bcl-2 family member Bad and the forkhead family of transcription factors that normally activate apoptosis-related genes (Zhang et al. 2011).

In one study, it was observed that fisetin treatment silenced Akt which increased protein expressions of pro-apoptotic Bad and Bax and decreased anti-apoptotic Bcl-2 and Bcl-xL resulting in inhibition of cancer (Khan et al. 2008a). Fisetin mediates growth-inhibitory effects via suppression of Akt signaling that are consistent with other studies (Chien et al. 2010). Yet supplementary studies are required to demarcate whether fisetin-mediated inhibition of Akt-induced cell survival in prostate cancer cells is androgen dependent or if fisetin modulates these pathways independent of each other (Jang et al. 2012).

A premature response of the cellular metabolic modifications to nutrient starvation, stress, or deficit of growth factors leads to inhibition of growth and stimulus to autophagy (Jung et al. 2010). Autophagy is recognized not only as a survival response to growth factor or nutrient deficiency but also a chief mechanism for tumor cell suicide. It has been found that anticancer drug-induced cell death is enhanced via genetic or pharmacological methods by inhibition of cytoprotective autophagy. On the contrary, autophagy may limit necrosis and chronic inflammation thereby protecting against tumorigenesis (Yang et al. 2011). Among the abundant components involved in the regulation of autophagy, mTOR is the chief constituent that manages the cellular equilibrium between growth and autophagy in response to physiological conditions and environmental stress apart from numerous components involved in the regulation of autophagy (Jung et al. 2010). Cytotoxic autophagy was induced in androgen-independent, PTEN-negative human prostate cancer PC-3 cells upon treatment with fisetin. Raptor, Rictor, PRAS40, and GβL were downregulated with consequent reduction in the formation of mTORC1 and mTORC2 and inhibition of mTOR signaling pathway in PC-3 prostate cancer cells on treatment with fisetin. mTOR signaling pathway, ribosomal protein S6, and eukaryotic translation initiation factor eIF4-B were inhibited along with the decrease in the phosphorylation and activation of downstream kinase p70S6K upon fisetin treatment (Syed et al. 2012a, b).

Cap-dependent translation levels were decreased via conversion of 4E-BP1 from its hyperphosphorylated γ form to the hypo- or nonphosphorylated α form, which permits 4E-BP1 to sequester eIF4-E upon fisetin treatment (Suh et al. 2010). Negative feedback loop is observed in which mTORC2-mediated activation of Akt stimulates mTORC1 activity in response to potential mechanism of resistance to mTORC1 inhibitors which is observed in clinical trials. It was found that feedback loop was kept in check by fisetin-mediated inhibition of both mTORC1 and mTORC2 along with inhibition of Akt signaling (Suh et al. 2010).

Fisetin treatment to cells showed its growth-inhibitory and pro-apoptotic effects; inhibition of Akt signaling is found to be associated with decreased migration, invasion, and metastasis; fisetin has been linked to have an anti-invasive effect in the suppression of Akt/c-Jun N-terminal kinase (JNK) signaling in PC-3 cells by Chien et al. Fisetin decreased the nuclear translocation and activation of NFκB and AP-1 transcription factors and inhibited phosphorylation of JNK1/JNK2 and Akt. The study showed that fisetin inhibited the metastatic ability and matrix metalloproteinase (−2 and −9) expressions of PC-3 were downregulated through suppressing PI3K/Akt and JNK signaling pathways (Chien et al. 2010).

Myeloma Multiple myeloma may be defined as a neoplasm of plasma cells accounting for 15 % of lymphato-hematopoietic cancers approximately. Effects of polyphenols like EGCG, genistein, and butein have been investigated against myeloma cells in a few studies (Pandey et al. 2009). Cytotoxicity was elicited, depicted by increased fraction of the cells with sub-G1 content in multiple myeloma U266 cells on treatment with fisetin as reported by Jang et al. Fisetin was reported to upregulate pro-apoptotic Bax, Bim, Bad, and caspase-3 and downregulate Bcl-2 and Mcl-1 triggering apoptosis (Jang et al. 2012). Fisetin reduced phosphorylation of AKT and mTOR along with activation of AMPK and its substrate acetyl-CoA carboxylase in U266 cells triggering apoptosis (Jang et al. 2012).

Colon Cancer The efficacy of fisetin was investigated against radiation-induced toxicity in human colorectal cancer cells (Chen et al. 2010). Radiosensitivity of chemoresistant p53 mutant HT-29 human colorectal cancer cells was enhanced on treatment with fisetin which resulted in increased radiation-induced caspase-dependent apoptosis as well as cell growth in the G2-M phase. Reports reveal that fisetin-inhibited phosphorylation of AKT and ERK1/ERK2 after irradiation may be the mechanism behind induction of apoptosis in HT-29 cells with serious DNA damage (Chen et al. 2010).

Melanoma The effect of fisetin on melanoma growth and progression was studied (Syed et al. 2011). In one study, the role of Akt/mTOR suppression on melanoma progression from radial to vertical growth phase was studied by employing a three-dimensional human skin equivalent melanoma model encompassing A375 melanoma cells cultured with epidermal keratinocytes and dermal fibroblasts (Syed et al. 2012a, b). Fisetin treatment to melanoma reconstructs was given on every alternate day for 16 days, and cross sections were measured at 4 days interval. Lower mela-

nocytic lesions were obtained on fisetin treatment as compared to control samples as well as decreased phosphorylation of Akt and mTOR kinases in fisetin-treated tissue sections (Syed et al. 2012a, b), henceforth validating previously discussed results that fisetin regulated healthily the Akt/mTOR signaling axis. Therefore, great interest exists in the development of novel mTOR kinase inhibitors, which may restrain and inhibit both mTORC1 and mTORC2 and PI3K activities, thereby attenuating Akt activation (Xie et al. 2013).

References

Abdulkareem IH, Blair M (2013) Phosphatase and tensin homologue deleted on chromosome 10. Niger Med J 54:79–86

Ahmad A, Biersack B, Li Y et al (2013) Targeted regulation of PI3K/Akt/mTOR/NF-κB signaling by indole compounds and their derivatives, mechanistic details and biological implications for cancer therapy. Anticancer Agents Med Chem 13:1002–1013

Asnaghi L, Bruno P, Priulla M et al (2004) mTOR, a protein kinase switching between life and death. Pharmacol Res 50:545–549

Baselga J (2011) Targeting the phosphoinositide-3 (PI3) kinase pathway in breast cancer. Oncologist 16:12–19

Brawley OW (2012) Prostate cancer epidemiology in the United States. World J Urol 30:195–200

Chen MB, Shen WX, Yang Y et al (2010) Activation of AMP-activated protein kinase is involved in vincristine-induced cell apoptosis in B16 melanoma cell. J Cell Physiol 226:1915–1925

Chien CS, Shen KH, Huang JS et al (2010) Antimetastatic potential of fisetin involves inactivation of the PI3K/Akt and JNK signaling pathways with downregulation of MMP-2/9 expressions in prostate cancer PC-3 cells. Mol Cell Biochem 333:169–180

Dowling RJ, Topisirovic I, Fonseca BD et al (2010) Dissecting the role of mTOR, lessons from mTOR inhibitors. Biochim Biophys Acta 1804:433–439

Falasca M, Selvaggi F, Buus R et al (2011) Targeting phosphoinositide 3-kinase pathways in pancreatic cancer – from molecular signalling to clinical trials. Anticancer Agents Med Chem 11:455–463

Fruman DA, Rommel C (2014) PI3K and cancer, lessons, challenges and opportunities. Nat Rev Drug Discov 13:140–156

Gomez-Pinillos A, Ferrari AC (2012) mTOR signalling pathway and mTOR inhibitors in cancer therapy. Hematol Oncol Clin North Am 26:483–505

Han D, Li SJ, Zhu YT et al (2013) LKB1/AMPK/mTOR signalling pathway in non-small-cell lung cancer. Asian Pac J Cancer Prev 14:4033–4039

Hay N, Sonenberg N (2004) Upstream and downstream of mTOR. Genes Dev 18:1926–1945

He Y, Li D, Cook SL et al (2013) Mammalian target of rapamycin and Rictor control neutrophil chemotaxis by regulating Rac/Cdc42 activity and the actin cytoskeleton. Mol Biol Cell 24:3369–3380

Higdon JV, Delage B, Williams DE et al (2007) Cruciferous vegetables and human cancer risk, Epidemiologic evidence and mechanistic basis. Pharmacol Res 55:224–236

Houghton PJ (2010) mTOR Pathway and mTOR Inhibitors in Cancer Therapy, general principles. In: Polunovsky VA, Houghton PJ (eds). Humana Press/Springer, New York/Dordrecht/Heidelberg/London, pp 113–31

Huang S, Houghton PJ (2003) Targeting mTOR signalling for cancer therapy. Curr Opin Pharmacol 3:371–377

Inoki K, Li Y, Zhu T et al (2002) TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. Nat Cell Biol 4:648–657

Jang KY, Jeong SJ, Kim SH et al (2012) Activation of reactive oxygen species/AMP activated protein kinase signaling mediates fisetin-induced apoptosis in multiple myeloma U266 cells. Cancer Lett 319:197–202

Jung CH, Ro SH, Cao J et al (2010) mTOR regulation of autophagy. FEBS Lett 584:1287–1295
Kadowaki M, Kanazawa T (2003) Amino acids as regulators of proteolysis. J Nutr 133:2052–2056
Khan N, Afaq F, Syed DN et al (2008a) Fisetin, a novel dietary flavonoid, causes apoptosis and cell cycle arrest in human prostate cancer LNCaP cells. Carcinogenesis 29:1049–1056
Khan N, Asim M, Afaq F et al (2008b) A novel dietary flavonoid fisetin inhibits androgen receptor signaling and tumor growth in athymic nude mice. Cancer Res 68:8555–8563
Khan N, Afaq F, Khusro FH et al (2012) 2012. Dual inhibition of phosphatidylinositol 3-kinase/Akt and mammalian target of rapamycin signaling in human nonsmall cell lung cancer cells by a dietary flavonoid fisetin. Int J Cancer 130:1695–1705
Laplante M, Sabatini DM (2012) mTOR signaling in growth control and disease. Cell 149:274–293
Li JC, Zhu HY, Chen TX et al (2013) Roles of mTOR and p-mTOR in gastrointestinal stromal tumors. Asian Pac J Cancer Prev 14:5925–5928
Luo J, Manning BD, Cantley LC (2003) Targeting the PI3K-Akt pathway in human cancer, rationale and promise. Cancer Cell 4:257–262
Manning BD, Cantley LC (2003) United at last, the tuberous sclerosis complex gene products connect the phosphoinositide 3-kinase/Akt pathway to mammalian target of rapamycin (mTOR) signalling. Biochem Soc Trans 31:573–578
Manning BD, Cantley LC (2007) AKT/PKB signalling, navigating downstream. Cell 129: 1261–1274
Laplante M Sabatini DM (2012) mTOR signaling in growth control and disease. Cell. 149, 274–293.
Pandey MK, Sung B, Ahn KS et al (2009) Butein suppresses constitutive and inducible signal transducer and activator of transcription (STAT) 3 activation and STAT3-regulated gene products through the induction of a protein tyrosine phosphatase SHP-1. Mol Pharmacol 75:| 525–533
Pandurangan AK (2013) Potential targets for prevention of colorectal cancer, a focus on PI3K/Akt/mTOR and Wnt pathways. Asian Pac J Cancer Prev 14:2201–2205
Papadimitrakopoulou V, Adjei AA (2006) The Akt/mTOR and mitogen-activated protein kinase pathways in lung cancer therapy. J Thorac Oncol 1:749–751
Peterson TR, Laplante M, Thoreen CC et al (2009) DEPTOR is an mTOR inhibitor frequently overexpressed in multiple myeloma cells and required for their survival. Cell 137:873–886
Proud CG (2002) Regulation of mammalian translation factors by nutrients. Eur J Biochem 269:5338–5349
Rodon J, Dienstmann R, Serra V et al (2013) Development of PI3K inhibitors, lessons learned from early clinical trials. Nat Rev Clin Oncol 10:143–153
Sarbassov DD, Ali SM, Kim DH et al (2004) Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. Curr Biol 14:1296–1302
Serra V, Markman B, Scaltriti M et al (2008) NVPBEZ235, a dual PI3K/mTOR inhibitor, prevents PI3K signaling and inhibits the growth of cancer cells with activating PI3K mutations. Cancer Res 68(19):8022–8030
Shaw RJ, Cantley LC (2006) Ras, PI(3)K and mTOR signalling controls tumour cell growth. Nature 441:424–430
Shimobayashi M, Hall MN (2014) Making new contacts, the mTOR network in metabolism and signalling crosstalk. Nat Rev Mol Cell Biol 15:155–162
Soliman GA, Acosta-Jaquez HA, Dunlop EA et al (2010) mTOR Ser-2481 autophosphorylation monitors mTORC-specific catalytic activity and clarifies rapamycin mechanism of action. J Biol Chem 285:7866–7879
Song MS, Salmena L, Pandolfi PP (2012) The functions and regulation of the PTEN tumour suppressor. Nat Rev Mol Cell Biol 13(5):283–296
Suh Y, Afaq F, Khan N et al (2010) Fisetin induces autophagic cell death through suppression of mTOR signaling pathway in prostate cancer cells. Carcinogenesis 31:1424–1433

Syed DN, Afaq F, Maddodi N et al (2011) Inhibition of human melanoma cell growth by the dietary flavonoid fisetin is associated with disruption of Wnt/beta-catenin signaling and decreased mitf levels. J Invest Dermatol 131:1291–1299

Syed DN, Adhami VM, Chamcheu JC, et al (2012a) Tumor regression in a 3-D melanoma model by the dietary flavonoid fisetin is associated with inhibition of Akt/mTOR signaling. Annual meeting of the American Association for Cancer Research; Chicago, USA

Syed DN, Adhami VM, Chamcheu JC, et al (2012b) Tumor regression in a 3-D melanoma model by the dietary flavonoid fisetin is associated with inhibition of Akt/mTOR signaling. Annual meeting of the American Association for Cancer Research; Chicago, USA

Syed DN, Adhami VM, Khan MI et al (2013) Inhibition of Akt/mTOR signaling by the dietary flavonoid fisetin. Anticancer Agents Med Chem 13:995–1001

Takahashi Y, Kohashi K, Yamada Y et al (2014) Activation of the Akt/mammalian target of rapamycin pathway in myxofibrosarcomas. Hum Pathol 45:984–993

Tan HK, Moad A, Tan ML (2014) The mTOR signalling pathway in cancer and the potential mTOR inhibitory activities of natural phytochemicals. Asian Pac J Cancer Prev 15:6463–6475

Tong BC, Harpole DH (2012) Molecular markers for incidence, prognosis, and response to therapy. Surg Oncol Clin N Am 21:161–175

Vogt PK, Hart JR, Gymnopoulos M et al (2010) Phosphatidylinositol 3-kinase, the oncoprotein. Curr Top Microbiol Immunol 347:79–104

Wang XW, Zhang YJ (2014) Targeting mTOR network in colorectal cancer therapy. World J Gastroenterol 20:4178–4188

Xie H, Lee MH, Zhu F et al (2013) Discovery of the novel mTOR inhibitor and its antitumor activities *in vitro* and *in vivo*. Mol Cancer Ther 12:950–958

Xu J, Ji J, Yan XH (2012) Cross-talk between AMPK and mTOR in regulating energy balance. Crit Rev Food Sci Nutr 52:373–381

Yang ZJ, Chee CE, Huang S et al (2011) Autophagy modulation for cancer therapy. Cancer Biol Ther 11:169–176

Zardavas D, Phillips WA, Loi S (2014) PIK3CA mutations in breast cancer: reconciling findings from preclinical and clinical data. Breast Cancer Res 16:201

Zhang X, Tang N, Hadden TJ et al (2011) Akt, FoxO and regulation of apoptosis. Biochim Biophys Acta 1813:1978–1986

Novel Chemopreventive Agents

15

Dietary polyphenols are abundantly found in food ingredients and present in fruits, vegetables, cereals, and beverages which occur in nature. Presently more than 8000 compounds have been recognized based on their chemical structures and are called as the secondary metabolites of plants consisting of one or more hydroxyl (–OH) groups attached to *ortho*-, *meta*-, or *para*-positions on a benzene ring which are normally involved in protection of the plant against ultraviolet radiation, various environmental pollutants and pathogens, etc. These metabolites are normally involved in defense against radiation exposure, various environmental pollutants, and hostility from pathogens (Iranikhah et al. 2014). Dietary polyphenols consumed for a long time have deciphered promising results in prevention and treatment of various diseases which include cancer, neurodegenerative diseases, cardiovascular diseases (CVDs), neurodegenerative diseases, diabetes, cancer, and many others in epidemiological studies.

General Structure and Classes of Dietary Polyphenols

Polyphenols are polyhydroxylated plant-derived compounds which exhibit common chemical structures like conjugated closed rings and hydroxyl groups. Depending on their chemical structure and orientation and position of the number of phenol rings bound to one another, polyphenols may be classified into various groups. Polyphenols are classified based on several criteria in line with their source, biological function, or chemical structure as flavonoids and non-flavonoids. Flavonoids are further divided into subclasses in which flavonols, flavones, flavan-3-ols, anthocyanidins, flavanones, and isoflavones are the most representative ones (Bravo 1998).

Non-flavonoids constitute the following main classes: phenolic acids (benzoic acids and cinnamic acids), stilbenes, lignans, tannins, and other polyphenols (including curcumin, rosmarinic acid, and gingerol). Sixty percent constitute flavonoids and 30 % by phenolic acids among all polyphenol classes (Ramos 2008). The most representative group of polyphenols is flavonoids having 15 carbon atoms (C6–C3–C6) illustrated by two benzene rings joined by a three-carbon chain forming an oxygenated heterocycle.

S. Rashid, *Cancer and Chemoprevention: An Overview*,
DOI 10.1007/978-981-10-2579-2_15

The main dietary sources of flavonoids are fruits, vegetables, medicinal herbs, spices, tea, coffee, and wine. The daily intake of flavonoids is variable according to each subclass, namely, 0.1–1.2 mg (isoflavones), 0.3–1.6 mg (flavones), 5.4–27.4 mg (flavonols), 20.4–50.6 mg (flavanones), 12–189.2 mg (flavan-3-ols), and 180–215 mg (anthocyanins) (Fraga et al. 2010) (Table 15.1 and Fig. 15.1).

Table 15.1 Subclasses of dietary polyphenols

Phenolic acids	***Stilbenes***	***Curcuminoids***	***Flavoinoids***
◦ Derived form Cinnamic acid or Benzoic acid ◦ Anacardic acid ◦ Caffeic acid ◦ Ferulic acid ◦ p-Coumarinic acid ◦ Gentisic acid ◦ Gallic acid ◦ Ellagic acid	◦ Polyhydroxylated stilbenes -Resveratrol -Pterostilbene	◦ Curcumin and its analogs -Demethoxy-curcumin -Bisdemethoxy curcumin	◦ Flavones ◦ Flavonones ◦ Flavonols - Catechins - Proanthocyanidins - Condensed Tannins - Theaflavins - Theaarubigins ◦ Flavonols ◦ Anthocyanidins ◦ Chalcones + Dihydrocharcones ◦ Isoflavones + Isoflavanes

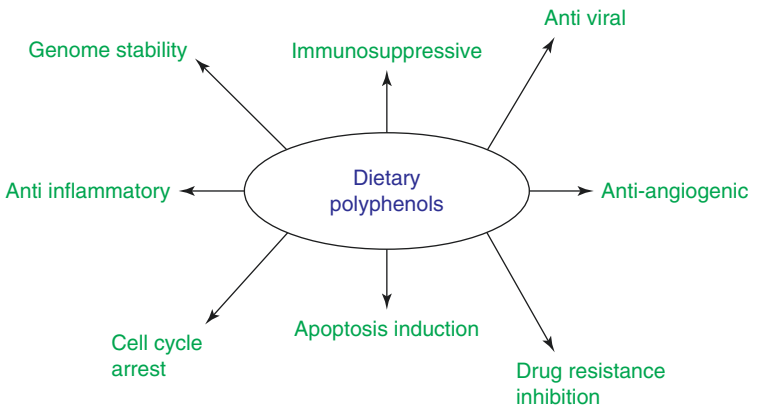


Fig. 15.1 Represents plethoric targets of dietary polyphenols (Adapted and modified from. Dietary polyphenols act on signaling molecules like growth factors, transcription factors, cytokines, enzymes, and genes regulating various signaling pathways. Dietary polyphenols play an imperative role in reducing inflammation, inducing apoptosis, blocking angiogenesis, and regulating autoimmune diseases

References

Bravo L (1998) Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. Nutr. Rev 56:317–333

Fraga CG, Galleano M, Verstraeten SV et al (2010) Basic biochemical mechanisms behind the health benefits of polyphenols. Mol. Asp. Med. 31:435–445

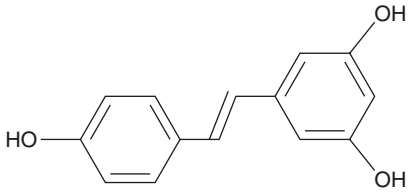
Iranikhah M, Stricker S, Freeman MK (2014) Future of bisphosphonates and denosumab for men with advanced prostate cancer. Cancer Manag. Res 6:217–224

Ramos S (2008) Cancer chemoprevention and chemotherapy: Dietary polyphenols and signaling pathways. Mol. Nutr. Food Res. 52:507–526

Chemoprevention by Resveratrol

16

Resveratrol is a naturally occurring polyphenol found abundantly in red grapes and red wine. It has been found to be antioxidant, anti-inflammatory, anti-carcinogenic, and antibacterial from the extensive research during the last two decades. It modulates various multiple biological pathways and hence exerts its chemopreventive and chemotherapeutic effects (Chung et al. 2013).



Resveratrol

Resveratrol in the Chemoprevention of Colon Cancer

Anticancer activity of resveratrol against colon cancer has been reported from experimental animal models in the laboratory as well as human clinical trials. A phase I pilot clinical trial to investigate the effect of resveratrol and grape powder on Wnt gene expression in colonic mucosa and colon cancer was conducted by Nguyen et al. It has been found that more than 85 % of colon cancer cases show aberrant Wnt signaling pathway. Administration of resveratrol decreased Wnt gene expression in normal mucosa cells, while it had no effect on cancer cells (Nguyen et al. 2009). Rats were given resveratrol at the dose of 200 μg/kg/day in an experimental model that resulted in decrease in large aberrant crypt foci (ACF) in rat colons, which is a precancerous lesion marker in colon cancer. It also regulated Bax and p21 expression in the ACF thereby deciphering its beneficial effect in colon carcinogenesis (Tessitore et al. 2000).

S. Rashid, *Cancer and Chemoprevention: An Overview*,
DOI 10.1007/978-981-10-2579-2_16

Resveratrol in the Chemoprevention of Skin Cancer

Resveratrol mitigated dimethylbenz(∝)anthracene (DMBA)-initiated skin cancer which is well observed by decreased tumor formation and induced apoptosis via resveratrol and was moderately effective in reducing DMBA which was mediated via upregulation of p53, Bax, and apoptotic protease-activating factor 1 (APAF-1) thereby releasing cytochrome c from the mitochondria and inhibiting Bcl-2 (Kalra et al. 2008; Soleas et al. 2002). Resveratrol decreased skin tumor formation induced by UVB exposure since ultraviolet radiation is the main causative agent of non-melanoma skin cancer. It repressed mRNA levels of survivin and augmented pro-apoptotic proteins like Smac thereby inducing apoptosis and decreasing cell survival (Aziz et al. 2005).

Resveratrol in the Chemoprevention of Breast Cancer

Resveratrol treatment against breast cancer has shown advantageous response in experimental models and various in vitro systems and hence has gathered an important evidence of the molecular mechanisms of resveratrol over the years. Resveratrol treatment in diet reduced DMBA-induced mammary tumors and prolonged the latency period in mice efficiently as reported by Banerjee et al. It also inhibited COX-2 and matrix metaloproteinase-9 (MMP-9), which are involved in the tumor metastasis and NFkB activation in breast tumor which mediates tumor cell proliferation hence deciphering its antitumor effect as reported by Bhat et al. (Banerjee et al. 2002; Bhat et al. 2001). Resveratrol when given with estrogen to breast cancer cells showed weak estrogenic potential and strong antagonism also as reported by Bhat and colleagues. The ratio of agonism to antagonism of resveratrol which seemed to be influenced by cell type that demonstrates a tissue-specific response signifies that resveratrol acts as a selective estrogen receptor modulator or SERM. Moreover it induced apoptosis, repressed angiogenesis, and modulated progesterone receptor expression thereby positively alleviating breast cancer development in mice (Garvin et al. 2006). Reduced mammary tumor formation was seen on supplementation of resveratrol in the drinking water of experimental mice as demonstrated by Provinciali et al. (2005). Resveratrol was reported to downregulate HER-2/neu expression and increased apoptosis in mammary gland tumors and in other tumor cell lines (Provinciali et al. 2005).

Resveratrol in the Chemoprevention of Prostate Cancer

Resveratrol at the dose of 625 mg per kg body weight inhibited prostate cancer progression in a male transgenic adenocarcinoma mouse prostate cancer model as reported by Harper and colleagues. The chemopreventive effects of resveratrol via mechanistic analysis were carried out in different studies. It reduced cell proliferation and insulin-like growth factor 1 and at the same time downregulated the

activated and phosphorylated MAP kinases called extracellular signal-regulated kinases, ERK1 and ERK2 (Harper et al. 2007), which are implicated in the pathogenesis of prostate cancer. It also suppressed prostate tumor growth by inducing apoptosis via downregulation of androgen receptor and also suppressed an ortholog of human prostate-specific antigen, namely, kallikrein, in transgenic rats as reported by Seeni et al. (2008).

Resveratrol in the Chemoprevention of Hepatocellular Carcinoma (HCC)

Resveratrol mediated in the progression of HCC in rat model via upregulation of Nrf2 expression, activation of apoptosis of tumor cells, and enhancement in cells in the G2-M phase (Carb'o et al. 1999; Bishayee et al. 2010). Carb'o et al. reported 5 % reduction in tumor cells in HCC on resveratrol treatment (Carb'o et al. 1999). Sprague–Dawley rats administered with resveratrol at 100 and 300 mg/kg reduced the emergence and number of hepatocyte nodules in hepatitis B-infected mice as Bishayee et al. report (Bishayee et al. 2010). In another study, the incidence of hepatitis B virus-mediated HCC was decreased by 5.3-fold upon resveratrol treatment at 30 mg/kg/day for 4 months in transgenic mice. Resveratrol was found to replace damaged cells in the transgenic liver and promoted regeneration by inhibiting ROS and increased hepatocyte proliferation. Additionally mice recovered from the liver pathology by hepatocyte proliferation upon resveratrol treatment within 30 days (Lin et al. 2012).

Resveratrol in the Chemoprevention of Pancreatic Cancer

Pancreatic cancer usually originates from pancreatic adenocarcinoma (originates from the ductal epithelium) apart from a variety of other cellular sources within the pancreas. Resveratrol reduced the activity of leukotriene A4 hydrolase, an inflammatory enzyme, and activated FOXO, a transcription factor which leads to the promotion of cell cycle arrest thereby deciphering its antitumor effects. Pancreatic tumor growth was declined in rats by resveratrol administration in a dose-dependent manner (Oi et al. 2010; Roy et al. 2011). Resveratrol induced apoptosis in pancreatic cancer cell lines via downregulation of Bcl-2 expression which is linked with mIR-21 expression (Liu et al. 2013).

Resveratrol in the Chemoprevention of Gastric Cancer

An experimental animal model, namely, congenic mice, contains mutated adenomatous polyposis coli (APC) genes because of which rats become predisposed to developing intestinal tumors that provides an excellent model. Congenic mice upon resveratrol treatment resulted in 70 % decrease in tumor formation in small

intestines via downregulation of cyclins D1 and D2, cell cycle progression genes, and the DP-1 transcription factor. Furthermore, resveratrol induced cytotoxic T cells, leukemia inhibitory factor receptor, and monocyte chemotactic protein-3 thereby inhibiting carcinogenesis process (Schneider et al. 2001). Resveratrol promoted apoptosis by alleviating expression BCL-2 and increased BAX gene expression thereby suppressing the growth of human primary gastric carcinoma cells in nude mice lacking the capability to show an immune response (Zhou et al. 2005).

Resveratrol in the Chemoprevention of Lung Cancer

Resveratrol administration at doses of 2.5 and 10 mg/kg in a lung cancer model condensed tumor volume (42 %) and weight (44 %) significantly. It also reduced the number of tumor cell colonies in Lewis lung carcinoma-bearing mice thereby decreasing metastasis (56 %) significantly (Kimura and Okuda 2001). Resveratrol might activate apoptosis via inhibition of neovascularization and decline in the S-phase population which may be the mechanistic approaches behind its antitumor activities (Kimura and Okuda 2001). It also activated caspase-mediated apoptosis via augmenting the caspase-9 and caspase-3 expressions significantly and repressed tumor progression by inhibiting basic fibroblast growth factor (BFGF), thereby deciphering its antitumor activity as reported by Lee et al. (2006). It has been reported that resveratrol given in combination with ionizing radiotherapy induced apoptosis in non-small cell lung cancer (NSCLC) cells through decrease in ROS generation and radio resistance and has been found to be an efficient approach in the treatment of cancer; however its use is often limited because of resistance in cancer cells (Luo et al. 2013). Likewise, there was an increase in in vivo antitumor effects seen when resveratrol was used in combination with 5-FU in mice inoculated with H22. Resveratrol alleviated the toxic effects of 5-FU and initiated S-phase arrest of H22 cells (Wu et al. 2004).

Resveratrol and Metastases

Micronized resveratrol (SRT501) administered to a patient with hepatic metastases for 14 days was detectable in hepatic tissue and significantly increased the levels of caspase-3 in malignant hepatic tissue as per results of phase I randomized double-blind clinical trials thereby signifying efficacy of resveratrol to activate mediated apoptosis in metastatic cancer cells. Brown et al. demonstrated that resveratrol treatment for 29 days in 40 healthy volunteers decreased insulin-like growth factor 1 (IGF-1) and IGFBP-3 in all volunteers since upregulation of IGF-1 and IGFBP-3 occurs in metastasis thereby inferring that resveratrol may be a prospective chemotherapeutic adjuvant (Brown et al. 2010). Therefore resveratrol has been found to be antitumor in action from results of various animal models and in vitro. Antitumor activity of resveratrol has been found to target similar intracellular oncogenic molecular pathways in view of similarities in the origin, pathogenesis, and

progression of carcinomas. In spite of having positive results of resveratrol against various cancer models in vivo and in vitro, limited human clinical trial data has shown contradictory results of resveratrol supplementation. But most of these clinical trials have small patient sample size, and different doses and different routes of resveratrol administration have been taken which leads to the difference in results (Popat et al. 2013). In summary both in vitro and in vivo data recommend a need for more widespread and large clinical trials to highlight the efficiency and safety of resveratrol as an anticancer agent. Presently, resveratrol is the most promising cancer chemopreventive agent (Malhotra et al. 2015).

References

Aziz MH, Afaq F, Ahmad N et al (2005) Prevention of ultraviolet-B radiation damage by resveratrol in mouse skin is mediated via modulation in survivin. Photochem Photobiol 81:25–31

Banerjee S, Bueso-Ramos C, Aggarwal BB (2002) Suppression of 7,12-dimethylbenz(a) anthracene-induced mammary carcinogenesis in rats by resveratrol: role of nuclear factor kappa B, cyclooxygenase 2, and matrix metalloprotease 9. Cancer Res 62:4945–4954

Bhat KPL, Lantvit D, Christov K et al (2001) Estrogenic and antiestrogenic properties of resveratrol in mammary tumor models. Cancer Res 61:7456–7463

Bishayee A, Barnes KF, Bhatia D et al (2010) Resveratrol suppresses oxidative stress and inflammatory response in diethylnitrosamine-initiated rat hepatocarcinogenesis. Cancer Prev Res 3:753–763

Brown VA, Patel KR, Viskaduraki M et al (2010) Repeat dose study of the cancer chemopreventive agent resveratrol in healthy volunteers: safety, pharmacokinetics, and effect on the insulin-like growth factor axis. Cancer Res 70:9003–9011

Carb'o N, Costelli P, Baccino FM et al (1999) Resveratrol, a natural product present in wine,decreases tumour growth in a rat tumour model. Biochem Biophys Res Commun 254:739–743

Chung MY, Lim TG, Lee KW (2013) Molecular mechanisms of chemopreventive phytochemicals against gastroenterological cancer development. World J Gastroenterol 19:984–993

Garvin S, Ollinger K, Dabrosin C (2006) Resveratrol induces apoptosis and inhibits angiogenesis in human breast cancer xenografts *in vivo*. Cancer Lett 231(113–122):2006

Harper CE, Patel BB, Wang J et al (2007) Resveratrol suppresses prostate cancer progression in transgenic mice. Carcinogenesis 28:946–1953

Kalra N, Roy P, Prasad S et al (2008) Resveratrol induces apoptosis involving mitochondrial pathways in mouse skin tumorigenesis. Life Sci 82:348–358

Kimura Y, Okuda H (2001) Resveratrol isolated from Polygonum cuspidatum root prevents tumor growth and metastasis to lung and tumor-induced neovascularization in lewis lung carcinoma-bearing mice. J Nutr 131:1844–1849

Lee EO, Lee HJ, Hwang HS et al (2006) Potent inhibition of Lewis lung cancer growth by heyneanol A from the roots of *Vitis amurensis* through apoptotic and anti-angiogenic activities. Carcinogenesis 27:2059–2069

Lin HC, Chen YF, Hsu WH et al (2012) Resveratrol helps recovery from fatty liver and protects against hepatocellular carcinoma induced by hepatitis B virus X protein in a mouse model. Cancer Prev Res (Phila) 5:952–962

Liu P, Liang H, Xia Q et al (2013) Resveratrol induces apoptosis of pancreatic cancers cells by inhibiting miR-21 regulation of BCL- 2 expression. Clin Transl Oncol 15:741–746

Luo H, Wang L, Schulte BA et al (2013) Resveratrol enhances ionizing radiation-induced premature senescence in lung cancer cells. Int J Oncol 43:1999–2006

Malhotra A, Bath S, Elbarbry F (2015) An organ system approach to explore the antioxidative, anti-inflammatory, and cytoprotective actions of resveratrol. Oxid Med Cell Longev 2015:803971

Nguyen AV, Martinez M, Stamos MJ et al (2009) Results of a phase I pilot clinical trial examining the effect of plant derived resveratrol and grape powder on wnt pathway target gene expression in colonic mucosa and colon cancer. Cancer Manag Res 1:25–37

Oi N, Jeong CH, Nadas J et al (2010) Resveratrol, a red wine polyphenol, suppresses pancreatic cancer by inhibiting leukotriene A(4) hydrolase. Cancer Res 70:9755–9764

Popat R, Plesner T, Davies F et al (2013) A phase 2 study of SRT501 (resveratrol) with bortezomib for patients with relapsed and or refractory multiple myeloma. Br J Haematol 160:714–717

Provinciali M, Re F, Donnini A et al (2005) Effect of resveratrol on the development of spontaneous mammary tumors in HER- 2/neu transgenic mice. Int J Cancer 115:36–45

Roy SK, Chen Q, Fu J et al (2011) Resveratrol inhibits growth of orthotopic pancreatic tumors through activation of FOXO transcription factors. PLoS One 6:e25166

Schneider Y, Duranton B, Goss'e F et al (2001) Resveratrol inhibits intestinal tumorigenesis and modulates host-defense-related gene expression in an animal model of human familial adenomatous polyposis. Nutr Cancer 39:102–107

Seeni A, Takahashi S, Takeshita K et al (2008) Suppression of prostate cancer growth by resveratrol in the transgenic rat for adenocarcinoma of prostate (TRAP) model. Asian Pac J Cancer Prev 9:7–14

Soleas GJ, Grass L, Josephy PD et al (2002) A comparison of the anticarcinogenic properties of four red wine polyphenols. Clin Biochem 35:119–124

Tessitore L, Davit A, Sarotto I et al (2000) Resveratrol depresses the growth of colorectal aberrant crypt foci by affecting bax and p21(CIP) expression. Carcinogenesis 21:1619–1622

Wu SL, Sun ZJ, Yu L et al (2004) Effect of resveratrol and in combination with 5-FU on murine liver cancer. World J Gastroenterol 10:3048–3052

Zhou HB, Chen JJ, Wang WX et al (2005) Anticancer activity of resveratrol on implanted human primary gastric carcinoma cells in nude mice. World J Gastroenterol 11:280–284

Chemoprevention by Genistein

17

Genistein (4,5,7-trihydroxyisoflavone) is a typical example of a phytoestrogenic compound, polyphenolic isoflavone, found in soy products, obtained primarily from *Genista tinctoria* L. in 1899 and named after the genus of this plant. Genistein is synthesized through transfer of (2S)-naringin into genistein by NAD(P)H in aerobic conditions and in the presence of cytochrome P450 in soybean cell suspension cultures (Kochs and Grisebach 1986). Furthermore, a metabolic engineering strategy to synthesize genistein was initiated by using genetically engineered *Saccharomyces cerevisiae* yeast cells having isoflavone synthase (IFS) gene obtained from *Glycyrrhiza echinata* L. (Koopman et al. 2012). Likewise, genistein was formed in *Nicotiana tabacum* L. leaves transformed with IFS by acting at phenylpropanoid pathway, although genistein production was enhanced by UVB treatment in *Arabidopsis* (Oliver et al. 2000).

It is antioxidant, anti-inflammatory, anti-carcinogenic, and anti-angiogenic. Genistein has been reported to have potent anticancer activity against prostate (Pavese et al. 2014; Adjakly et al. 2013), ovarian (Lee et al. 2012a; b), breast (Orlando et al. 2011), lung (Hess and Igal 2011), and pancreatic cancers (Wang et al. 2006). It may do so via modulation of cholesterol metabolism, NFkB activity, and decrease in AKT protein level, promotes apoptosis, amends polyamine metabolism (Jang et al. 1999), and downregulates androgen-mediated carcinogenesis and antioxidant activity (Kiao et al. 2008; Messina et al. 1994). It may also suppress HMGR, mevalonate, and protein prenylation leading to growth inhibition (Wang et al. 2006; Wong et al. 2002; Duncan et al. 2005; Sung et al. 2004).

It blocks EGF signaling through forkhead boxO3 and thus exerts antiproliferative activity (Qi et al. 2011; Pan et al. 2012). It inhibits catenin signaling in the transgenic adenocarcinoma of the mouse prostate model in prostate carcinogenesis (Shukla et al. 2007), reduces Wnt signaling in mammary epithelial cells (Su and Simmen 2009) and in a colon cancer cell line (Zhang and Chen 2011), inhibits catenin/TCF transcriptional activity, and upregulates E-cadherin thereby activating GSK3 leading to phosphorylation and degradation of β-catenin. It binds to ER leading to cell proliferation (Monroe et al. 2005) and cellular differentiation (Imamov

S. Rashid, *Cancer and Chemoprevention: An Overview*,
DOI 10.1007/978-981-10-2579-2_17

et al. 2004). It modulates ER gene transcription (Rietjens et al. 2013) which regulates proliferation and differentiation. It upregulates miR-200 as well as expression of various miRNAs. Its effect depends on concentration because genistein was found to inhibit cell proliferation at high concentrations and estrogen signaling activation at low concentrations. It is used in combination with a monoclonal antibody (B43) to treat patients with acute lymphocytic leukemia and non-Hodgkin's lymphoma (Uckun et al. 1995) as well as coupled with recombinant EGF to treat patients with EGFR-positive breast cancer (Uckun et al. 1998). Conversely, it exhibits tumor-promoting effect in some studies in vivo (Nakamura et al. 2011) which recommends the required careful selection of patients and safer planning in future clinical trials (Rietjens et al. 2013; Nakamura et al. 2011; Uckun et al. 1995, 1998).

It blocks AKT which is the downstream effector of Notch (Wang et al. 2011) and IGF-1 signaling in pancreatic cancer cells (Lee et al. 2012a, b), osteosarcoma (Liang et al. 2012), and breast cancer (Whyte et al. 2009) along with suppression of AKT effectors like FOXM1 in pancreatic cancer cells (Wang et al. 2011) and FOXO3 (Qi et al. 2011) in colon cancer cells. It restrains the formation of AKT complex with human TERT, heat shock protein 90, p70S6 kinase, and mTOR (Sundin and Hentosh 2012). It targets p21WAF1/CIP1 in BRCA1 mutant human breast cancer cell lines (Privat et al. 2010). It enhances apoptosis by modulating AKT with arsenic trioxide against human hepatocellular carcinoma (Ma et al. 2011), gefitinib against NSCLC (Zhu et al. 2012), gemcitabine against human osteosarcoma (Liang et al. 2012; Zhang et al. 2010), cisplatin against cervical cancer cells (Sahin et al. 2012), cetuximab against oral squamous cell carcinoma (Park et al. 2010), photoactivated hypericin against breast cancer cells (Ferenc et al. 2010), and indole-3-carbinol against human colon cancer (HT-29) cells (Nakamura et al. 2009). Carcinogenic effect of 17 beta-estradiol or bisphenol A was also inhibited by genistein via ER/IGF-1/AKT pathway in BG-1 ovarian cancer cells (Hwang et al. 2013) along with FOXO3 downregulation in colon cancer cells (Qi et al. 2011) and regulation of MAPKs/AKT in cervical cancer cells (Kim et al. 2009). It along with ceramide and lipid raft cholesterol inhibited cell viability in prostate cancer cells by regulation of EGFR/AKT/p70S6k pathway and of androgen receptor (Ji et al. 2012). Activation of membrane androgen receptors (mAR) by genistein in vitro and in vivo restrains the pro-survival signals, AKT/Bad, blocking the movement of colon cancer cells by regulating vinculin (cell adhesion protein) and reorganization of actin deciphering tumoristatic action of mAR receptors (Gu et al. 2011).There is an inverse correlation between genistein intake and breast cancer risk by case–control study and meta-analysis of various epidemiological studies and other clinical studies (Taylor et al. 2009). Normal dietary dose from 0.3 to 1 mg/kg body weight/day has been obtained from most phase I and phase II clinical trials of genistein (Pavese et al. 2010). In one study, patients prior to undergoing radical prostatectomy for localized prostate cancer were treated with 2 mg genistein/kg body weight and compared with no genistein treatment patients (Xu et al. 2009). Results obtained were that genistein decreased MMP-2 gene expression to 24 % when compared with control subjects (Xu et al. 2009). Genistein at the dose of 300 or 600 mg/d orally given as the purified soy extract G-2535, in a phase II randomized, placebo-controlled trial,

was efficient at lower dose on bladder cancer via EGFR phosphorylation, but the AKT pathway was unaltered in both in vivo conditions as reported by Messing et al. (2012). Subjects with progressive prostate cancer were treated with soy milk three times daily for 12 months at the dose of 1 mg genistein/kg/day and were found to attenuate serum PSA levels as compared to no treatment subjects in a phase II trial (Pendleton et al. 2008).

In another study, 6 mg genistein/kg/day for 6 months was given to men at different stages of prostate cancer (deVere et al. 2004) leading to decrease in their PSA levels in 17 % of the participants in a phase II trial. There were a wide variety of immunological studies done like 8 mg/kg/day of genistein injections in ovariectomized adult mice that led to the ER- and non-ER-mediated inhibition of thymocyte and CD4(+) and CD8(−) helper T-cell lineage maturation and a systemic lymphocytopenia as reported by Yellayi et al. (Hushmendy et al. 2009). It also competes with 17 beta-estradiol for ER with 4 % binding affinity for ER-α and 87 % for ER-β, thereby facilitating the treatment of hormone-related cancers (DeNardo et al. 2010), repression of Ag-specific immune responses, as well as downregulation of OVA-specific proliferation and IFN-γ and immunoglobulin (Ig) G1 levels without decreasing anti-CD3 monoclonal antibody (mab) and Ag-presenting activity of CD11c(+) dendritic cells (Kogiso et al. 2006). Genistein inhibits human NK cells activity to lyse breast cancer cells by stimulating the granzyme B inhibitor, proteinase inhibitor 9 (PI-9) in MCF-7 human breast cancer cells (Jiang et al. 2008). It also resulted in a significant therapeutic effect as compared to control group by augmenting lymphocyte proliferation and LDH release and IFN-γ levels in a mouse model of human papillomavirus-associated cervical cancer (Ghaemi et al. 2012) leading to antitumor effect in this study (Guo et al. 2007). Therefore immune-modulatory potential of genistein emerges to be reasonably healthy but needs further exploration to understand fully how these effects influence various cancers (Pendleton et al. 2008; deVere et al. 2004; Kogiso et al. 2006; Jiang et al. 2008).

Modulation of HCC Cells In Vitro by Genistein

Genistein has been found effective as an anticancer agent against liver cancer cells by inducing apoptosis in Bel-7402 (Gu et al. 2005), HuH-7 (Mansoor et al. 2011), Hep3B (Yeh et al. 2007), and HepG2 (Chodon et al. 2007a, b) which are liver cancer cell lines. It enhances apoptosis and cell cycle regulation (Gu et al. 2005, 2009) and inhibits metastasis in HCCs like in HepG2, SMMC-7721, and Bel-7402 cells (Dai et al. 2015). It acts against 12-O-tetradecanoylphorbol-13-acetate-mediated metastasis via modulating MMP-9 and EGFR and MAPK and PI3K/Akt signaling pathways (Wang et al. 2014).

It acts synergistically in combination with other anticancer drugs which is well demonstrated by MAPK inactivation leading to sensitization of human hepatocellular carcinoma Hep3B cells to TRAIL-mediated apoptosis (Jin et al. 2009a, b) and potentiates cytotoxic effect of arsenic trioxide (ATO) against human hepatocellular

carcinoma via inhibiting Akt and NFkB and triggering apoptosis in human HCC cell lines in vitro. 50 mg genistein/kg body weight has been found to considerably suppress cancer progression and angiogenesis in nude mice (Ma et al. 2011).

Modulation of HCC In Vivo by Genistein

50 mg genistein/kg body weight was administered in male BALB/C nu/nu mice injected with Bel-7402 cells. Tumor growth was significantly retarded when compared with control mice along with invasion of Bel-7402 cells into the renal parenchyma of nude mice with a xenograft transplant and was inhibited by modulating cell cycle, apoptosis, and angiogenesis (Gu et al. 2005). 15 mg genistein/kg body weight suspension in olive oil efficiently suppressed cell proliferation and induced apoptosis via mediating caspase-3 expression and PCNA in chemically induced HCC by single intraperitoneal injection of N-nitrosodimethylamine as a cancer initiator and promoted with phenobarbital (Chodon et al. 2007a, b).

Modulation of Gastric Cancer by Genistein via Epidemiological Data

High serum concentrations of isoflavones were linked with decreased risk of gastric cancer as reported from a case–control study from the Korean Multicenter Cancer Cohort (Ko et al. 2010), contrary to the Japan Public Health Center-based prospective study reports which show that there is no relation between isoflavone intake and gastric cancer risk among Japanese men and women (Hara et al. 2013).

Modulation of Gastric Cancer by Genistein In Vitro System

20 mM genistein for 24–72 h induced apoptosis in primary gastric cancer cells by mediating anti-apoptotic protein B-cell lymphoma 2 (Bcl-2) and pro-apoptotic Bcl-2-associated X protein (Bax) (Zhou et al. 2004) and modulating Bcl-2:Bax ratio that induced apoptosis in SG7901 cells transplanted into subcutaneous tissue of nude mice (Zhou et al. 2008). It was reported to regulate cell proliferation and apoptosis in a dose- and time-dependent manner in human gastric cancer cell line BGC-823 via inhibitory effect of NFkB, which reduced COX-2 protein concentrations (Li et al. 2011). It (20–80 mM) also induced G2/M cell cycle arrest in SGC-7901 and BGC-823 by Akt inactivation via upregulation of PTEN resulting in activation of cell division cycle protein 2 homolog/cyclin-dependent kinase 1 (CDC2/CDK1) and decreased phosphorylation of Wee1 on Ser642 leading to G2/M arrest (Liu et al. 2013).

40 mM genistein for 48 h to SGC-7901 cells induced the expression of 86 proteins involved in the regulation of G2/M transition, cellular growth, and proliferation and led to modulation in 49 proteins being upregulated and 37 being downregulated along with downregulation of four kinesins which include

kinesin-like protein (KIF)11, KIF20A, KIF22, and KIF23 and a KIF, centromere protein F (CENPF) with KIF20A, being the most important role player in genistein-induced mitotic arrest (Yan et al. 2012). A subpopulation of tumor cells having the capacity of self-renewal and resistance to chemotherapeutic drugs known as gastric cancer stem cells (GCSCs) leads to relapse of the disease. Genistein at the dose of (15 mM) inhibited the GCSC-like properties in gastric cancer cells like self-renewal ability, drug resistance, and tumorigenicity which were linked with the suppression of ATP-binding cassette subfamily G member 2 (ABCG2) expression and extracellular signal-regulated kinase (ERK) ½ activity (Huang et al. 2014) and increases cancer stemness and overexpression of CD44, a typical GCSC surface marker via inhibition of glioma-associated oncogene family zinc finger 1 (Gli1) which is an activator of Hedgehog signaling involved in oncin oncogenesis and found in MKN45, a human gastric cancer cell line. Besides, it also blocked high cell migration capacity of CD44+ cells inferring that it can target cancer stem cell-like features thereby an efficient drug for gastric cancer therapy (Yu et al. 2014).

Modulation of Lung Cancer by Genistein via Epidemiological Data

Estrogens interact with growth factor pathways in tumorigenesis and have mitogenic effects in lung cells, but there are contradictory results between lung cancer risk and isoflavone intake from epidemiologic studies (Schabath et al. 2005; Seow et al. 2009), whereas prospective studies in Asia demonstrated an inverse association in never smokers (Shimazu et al. 2010). There was an inverse relation between plasma isoflavone concentration and lung cancer risk in a nested case–control study in a large population-based prospective study in Japanese women with different isoflavone intakes and a high prevalence of never smokers (Shimazu et al. 2011).

Modulation of Lung Cancer by Genistein In Vitro

It demonstrated an anticancer effect on lung carcinogenesis in several in vitro and in vivo studies. Either this compound was used alone or in combination with other compounds (Zhu et al. 2012; Gadgeel et al. 2009), like on the small cell lung cancer (SCLC) cell line H446 by inducing cell cycle arrest and apoptosis, deregulated forkhead box protein M1 (FoxM1) and its target genes which include Cdc25B, cyclin B1, and survivin (Tian et al. 2014). Genistein (5–10 mM) used in combination with trichostatin A (TSA) in A549 lung cancer cells promoted apoptosis and upregulated death receptor TNF receptor 1 (TNFR-1) which executes extrinsic apoptosis pathways (Wu et al. 2012; Shiau et al. 2010). Patients acquired resistance to this therapy having non-SCLC which was treated with tyrosine kinase inhibitors. But when it was given in combination with gefitinib, an EGFR tyrosine kinase inhibitor, in a non-SCLC cell line carrying the T790 M mutation in EGFR, showed a synergistic anticancer effect via triggering apoptosis and inhibited key regulators of growth signaling pathways like Akt (Zhu et al. 2012) which was further confirmed in in vivo experiments.

The main drawback of the use of genistein is its low bioavailability in vivo which has switched an interest in its derivative, 7-difluoromethyl-5,49-dimethoxygenistein (dFMGEN), having better in vivo bioavailability. dFMGEN reduced the viability of lung carcinoma A549 cells through induction of G1 phase arrest in an in vitro study (Peng et al. 2010). Furthermore, dFMGEN was well tolerated and suppressed cancer progression in vivo suggesting its therapeutic index in the treatment of human lung cancer (Peng et al. 2010).

Modulation of Colorectal Cancer by Genistein via Epidemiological Data

There is a direct relation between decrease in colon cancer risk in human and animal studies by consuming soy products (Messina and Bennink 1998; Thiagarajan et al. 1998) especially phytoestrogens that reduce the development of colorectal cancer (Spector et al. 2003; Rossi et al. 2006) as obtained from a population-based Ontario Familial Colorectal Cancer Registry which mentioned the link between dietary phytoestrogen intake (isoflavones, lignans, and total phytoestrogens) and colorectal cancer risk among healthy subjects in a case–control study. Among these, dietary lignin and isoflavone intake was related with a significant decrease in colorectal cancer risk, besides polymorphic genes encoding enzymes involved in the metabolism of phytoestrogens like catechol-O-methyltransferase and UDP-glucuronosyltransferase (UGT). CYP and glutathione S-transferases (GSTs) were not subject to modifications (Cotterchio et al. 2006).

Modulation of Colorectal Cancer by Genistein In Vitro Data

Genistein has been found to reduce colorectal cancer from various in vitro studies, and the mechanistic approaches behind its working have been extensively explored. It has been found to attenuate PI3K/Akt pathway leading to colon cancer cell inhibition (Kim et al. 2005; Su et al. 2003) as well as ER and tumor suppressor gene expressions (Bielecki et al. 2011; Qi et al. 2011). It blocks uncontrolled cell growth via regulating Wingless and integration1 (Wnt) signaling pathway in a DLD-1 cell line (Zhang and Chen 2011) as well as antagonist, Dickkopf-related protein 1 (DKK1) via histone acetylation at the promoter region in SW480 human colon cancer cell line particularly (Wang et al. 2012).

Modulation of Colorectal Cancer by Genistein In Vitro

Rats fed with 140 mg genistein/kg body weight from gestation to 13 weeks of age to chemically induced colon cancer by azoxymethane male Sprague–Dawley rats showed downregulation of Wingless and integration of β-catenin (Wnt/β-catenin) signaling and a decrease in total aberrant crypts suggesting therapeutic role of isoflavone in mitigating the development of early colon neoplasia (Zhang et al. 2013).

It is used in the treatment of metastatic colorectal cancer in a phase I/II pilot study recruiting participants. It is expected to have positive results because of the promising results from in vitro and in vivo studies.

Modulation of Breast Cancer by Genistein via Epidemiological Data

There is an inverse relation between soy intake and breast cancer risk obtained from various case–control studies (Dai et al. 2001; Wu et al. 2004; Yamamoto et al. 2003). Frequent soup and isoflavone eating was coupled to the decrease in the risk of breast cancer in Japan from a prospective cohort study suggesting that the chemopreventive effects of soybeans and soy-containing foods are due to their isoflavone content.

Modulation of Colorectal Cancer by Genistein In Vitro

Genistein promoted inhibitory effects synergistically in combination with anticancer drugs and induced apoptosis in several breast cancer cell lines like low-invasive ER-positive MCF-7 and in the high-invasive ER-negative MDA-MB-231 breast cancer cell lines (10–100 mM) (Liu et al. 2005a, b; Hsieh et al. 1998). Genistein in combination with adriamycin, docetaxel, and tamoxifen exhibited synergistic pro-apoptotic effects in MDA-MB-231 cells (Satoh et al. 2003) and in BT-474 breast cancer cells, respectively (Mai et al. 2007), with NFkB (Li et al. 2005) and Akt pathways (Gong et al. 2003) as the main molecular targets. It induces expression of breast cancer growth suppressor protein, namely, BRCA1 and BRCA2 tumor suppressor genes and other genes involved in the regulatory pathways of BRCA1 and BRCA2 (Caëtano et al. 2006). Conversely, it has been found to activate proliferation and estrogen-sensitive gene expression at concentrations of 1–10 mM in ER-positive breast cancer cell lines (Seo et al. 2006). Its effect is null on ER-negative and tamoxifen-resistant breast cancer cells (Liu et al. 2005a, b) at these low concentrations, but genistein abrogates tamoxifen-associated mammary tumor prevention (Liu et al. 2005a, b).

Modulation of Colorectal Cancer by Genistein In Vivo

The risk of breast cancer has been found to be diminished by starting to take genistein early (Lamartiniere et al. 1998). On the contrary when there were low concentrations of circulating E2 and genistein was present which acted in an additive manner to activate estrogen-dependent tumor growth in a preclinical mouse model (Ju et al. 2006) which suggests consumption of genistein or the products having genistein may be toxic for postmenopausal women with estrogen-dependent breast cancer.

Modulation of Colorectal Cancer by Genistein in Clinical Trials

Genistein at the dose of 54 mg/d did not show any undesirable estrogenic effects on breast tissue in human clinical trials (Marini et al. 2008; Atteritano et al. 2008), whereas proestrogenic effects of dietary soy on breast tissue were observed by others (McMichael-Phillips et al. 1998; Khan et al. 2012). Signifying its agonist activity against ER-α and its use in women with ER-positive breast cancers, it must be carefully studied. A phase II study entitled Gemcitabine Hydrochloride and Genistein in Treating Women with Stage IV Breast Cancer and Genistein in Preventing Breast or Endometrial Cancer in Healthy Postmenopausal Women is a phase I study which has been completed although the results are not yet published (Jin et al. 2009a, b; Peng et al. 2013; Wang et al. 2012; Khan et al. 2012).

References

Adjakly M, Ngollo M, Boiteux JP et al (2013) Genistein and daidzein, different molecular effects on prostate cancer. Anticancer Res 33:39–44

Atteritano M, Pernice F, Mazzaferro S et al (2008) Effects of phytoestrogen genistein on cytogenetic biomarkers in postmenopausal women, 1 year randomized, placebo-controlled study. Eur J Pharmacol 589:22–26

Bielecki A, Roberts J, Mehta R et al (2011) Estrogen receptor-beta mediates the inhibition of DLD-1 human colon adenocarcinoma cells by soy isoflavones. Nutr Cancer 63:139–150

Caëtano B, Le Corre L, Chalabi N et al (2006) Soya phytonutrients act on a panel of genes implicated with BRCA1 and BRCA2 oncosuppressors in human breast cell lines. Br J Nutr 95:406–413

Chodon D, Banu SM, Padmavathi R et al (2007a) Inhibition of cell proliferation and induction of apoptosis by genistein in experimental hepatocellular carcinoma. Mol Cell Biochem 297:73–80

Chodon D, Ramamurty N, Sakthisekaran D (2007b) Preliminary studies on induction of apoptosis by genistein on HepG2 cell line. Toxicol In Vitro 21:887–891

Cotterchio M, Boucher BA, Manno M et al (2006) Dietary phytoestrogen intake is associated with reduced colorectal cancer risk. J Nutr 136:3046–3053

Dai Q, Shu XO, Jin F et al (2001) Population-based case-control study of soyfood intake and breast cancer risk in Shanghai. Br J Cancer 85:372–378

Dai W, Wang F, He L et al (2015) Genistein inhibits hepatocellular carcinoma cell migration by reversing the epithelial-mesenchymal transition, partial mediation by the transcription factor NFAT. Mol Carcinog 54:301–311

DeNardo DG, Andreu P, Coussens LM (2010) Interactions between lymphocytes and myeloid cells regulate pro- versus anti-tumor immunity. Cancer Metastasis Rev 29:309–316

deVere W,RW, Hackman RM, Soares SE et al (2004) Effects of a genistein-rich extract on PSA levels in men with a history of prostate cancer. Urology 63:259–263

Duncan RE, El-Sohemy A, Archer MC (2005) Regulation of HMG-CoA reductase in MCF-7 cells by genistein., EPA., and DHA., alone and in combination with mevastatin. Cancer Lett 224:221–228

Ferenc P, Solar P, Kleban J et al (2010) Down-regulation of Bcl-2and Akt induced by combination of photoactivated hypericin and genisteinin human breast cancer cells. J Photochem Photobiol 98:25–34

Ferreyra M,L,F, Sebastián PR et al (2012) Flavonoids , biosynthesis ., biological functions ., and biotechnological applications. Front Plant Sci 3:222

Gadgeel SM, Ali S, Philip PA et al (2009) Genistein enhances the effect of epidermal growth factor receptor tyrosine kinase inhibitors and inhibits nuclear factor kappa B in nonsmall cell lung cancer cell lines. Cancer 115:2165–2176

Ghaemi A, Soleimanjahi H, Razeghi S et al (2012) Genistein induces a protective immunomodulatory effect in a mouse model of cervical cancer. Iran J Immunol 9:119–127
Gong L, Li Y, Nedeljkovic-Kurepa A et al (2003) Inactivation of NF kappa B by genistein is mediated via Akt signaling pathway in breast cancer cells. Oncogene 22:4702–4709
Gu Y, Zhu CF, Iwamoto H et al (2005) Genistein inhibits invasive potential of human hepatocellular carcinoma by altering cell cycle., apoptosis., and angiogenesis. World J Gastroenterol 11:6512–6517
Gu Y, Zhu CF, Dai YL et al (2009) Inhibitory effects of genistein on metastasis of human hepatocellular carcinoma. World J Gastroenterol 15:4952–4957
Gu S, Papadopoulou N, Nasir O et al (2011) Activation of membrane androgen receptors in colon cancer inhibits the prosurvival signals Akt/bad *in vitro* and *in vivo* and blocks migration via vinculin/actin signaling. Mol Med (Cambridge., MA) 17:48–58
Guo TL, Chi RP, Hernandez DM et al (2007) Decreased 7,12-dimethylbenz(a)anthracene-induced carcinogenesis coincides with the induction of antitumor immunities in adult female B6C3F1 mice pretreated with genistein. Carcinogenesis 28:2560–2566
Hara A, Sasazuki S, Inoue M et al (2013) Plasma isoflavone concentrations are not associated with gastric cancer risk among Japanese men and women. J Nutr 143:1293–1298
Hess D, Igal RA (2011) Genistein downregulates de novo lipid synthesis and impairs cell proliferation in human lung cancer cells. Exp Biol Med (Maywood) 236:707–713
Hsieh CY, Santell RC, Haslam SZ et al (1998) Estrogenic effects of genistein on the growth of estrogen receptor-positive human breast cancer (MCF-7) cells *in vitro* and *in vivo*. Cancer Res 58:3833–3838
Huang W, Wan C, Luo Q et al (2014) Genistein-inhibited cancer stem cell-like properties and reduced chemoresistance of gastric cancer. Int J Mol Sci 15:3432–3443
Hushmendy S, Jayakumar L, Hahn AB et al (2009) Select phytochemicals suppress human T-lymphocytes and mouse splenocytes suggesting their use in autoimmunity and transplantation. Nutr Res 29:568–578
Hwang KA, Park MA, Kang NH et al (2013) Anticancer effect of genistein on BG-1 ovarian cancer growth induced by 17 beta-estradiol or bisphenol A via the suppression of the crosstalk between estrogen receptor alpha and insulin-like growth factor-1 receptor signaling pathways. Toxicol Appl Pharmacol 272:637–646
Imamov O, Morani A, Shim GJ et al (2004) Estrogen receptor beta regulates epithelial cellular differentiation in the mouse ventral prostate. Proc Natl Acad Sci U S A 101:9375–9380
Jang DS, Kang BS, Ryu SY et al (1999) Inhibitory effects of resveratrol analogs on unopsonized zymosan-induced oxygen radical production. Biochem Pharmacol 57:705–712
Ji C, Yang YL, He L et al (2012) Increasing ceramides sensitizes genistein-induced melanoma cell apoptosis and growth inhibition. Biochem Biophys Res Commun 421:462–467
Jiang X, Patterson NM, Ling Y et al (2008) Low concentrations of the soy phytoestrogen genistein induce proteinase inhibitor 9 and block killing of breast cancer cells by immune cells. Endocrinology 149:5366–5373
Jin CY, Park C, Kim GY et al (2009a) Genistein enhances TRAIL-induced apoptosis through inhibition of p38 MAPK signaling in human hepatocellular carcinoma Hep3B cells. Chem Biol Interact 180:143–150
Jin CY, Park C, Kim GY et al (2009b) Genistein sensitizes human hepatocellular carcinoma cells to TRAIL-mediated apoptosis by enhancing Bid cleavage. Anticancer Drugs 20:713–722
Ju YH, Allred KF, Allred CD et al (2006) Genistein stimulates growth of human breast cancer cells in a novel., postmenopausal animal model., with low plasma estradiol concentrations. Carcinogenesis 27:1292–1299
Khan SA, Chatterton RT, Michel N et al (2012) Soy isoflavone supplementation for breast cancer risk reduction, a randomized phase II trial. Cancer Prev Res (Phila) 5:309–319
Kiao CW, Mei J, Wood CM (2008) Effect of soy proteins and isoflavones on lipid metabolism and involved gene expression. Front Biosci 13:2660–2673
Kim EJ, Shin HK, Park JH (2005) Genistein inhibits insulin-like growth factor-I receptor signaling in HT-29 human colon cancer cells, a possible mechanism of the growth inhibitory effect of Genistein. J Med Food 8:431–438

Kim SH, Kim SH, Kim YB et al (2009) Genistein inhibits cell growth by modulating various mitogen-activated protein kinases and AKT in cervical cancer cells. Ann N Y Acad Sci 1171:495–500

Ko KP, Park SK, Park B et al (2010) Isoflavones from phytoestrogens and gastric cancer risk, a nested case-control study within the Korean Multicenter Cancer Cohort. Cancer Epidemiol Biomarkers Prev 19:1292–1300

Kochs G, Grisebach H (1986) Enzymic synthesis of isoflavones. Eur J Biochem 155:311–318

Kogiso M, Sakai T, Mitsuya K et al (2006) Genistein suppresses antigen-specific immune responses through competition with 17 beta estradiol for estrogen receptors in ovalbumin-immunized BALB/c mice. Nutrition 22:802–809

Koopman F, Beekwilder F, Crimi B et al (2012) De novo production of the flavonoid naringenin in engineered *Saccharomyces cerevisiae*. Microb Cell Fact 11:155

Lamartiniere CA, Zhang JX, Cotroneo MS (1998) Genistein studies in rats, potential for breast cancer prevention and reproductive and developmental toxicity. Am J Clin Nutr 68:1400S–1405S

Lee J, Ju J, Park S et al (2012a) Inhibition of IGF-1 signaling by genistein, modulation of E-cadherin expression and downregulation of beta-catenin signaling in hormone refractory PC-3 prostate cancer cells. Nutr Cancer 64:153–162

Lee JY, Kim HS, Song YS (2012b) Genistein as a potential anticancer agent against ovarian cancer. J Tradit Complement Med 2:96–104

Li Y, Ahmed F, Ali S et al (2005) Inactivation of nuclear factor kappa B by soy isoflavone genistein contributes to increased apoptosis induced by chemotherapeutic agents in human cancer cells. Cancer Res 65:6934–6942

Li YS, Wu LP, Li KH et al (2011) Involvement of nuclear factor kappa B (NF-kappa B) in the downregulation of cyclooxygenase-2 (COX-2) by genistein in gastric cancer cells. J Int Med Res 39:2141–2150

Liang C, Li H, Shen C et al (2012) Genistein potentiates the anti-cancer effects of gemcitabine in human osteosarcoma via the downregulation of Akt and nuclear factor-kappa B pathway. Anticancer Agents Med Chem 12:554–563

Liu B, Edgerton S, Yang X et al (2005a) Low-dose dietary phytoestrogen abrogates tamoxifen-associated mammary tumor prevention. Cancer Res 65:879–886

Liu Y, Zhang YM, Song DF et al (2005b) Effect of apoptosis in human breast cancer cells and its probable mechanisms by genistein. Wei Sheng Yan Jiu 34:67–69

Liu YL, Zhang GQ, Yang Y et al (2013) Genistein induces G2/M arrest in gastric cancer cells by increasing the tumor suppressor PTEN expression. Nutr Cancer 65:1034–1041

Ma Y, Wang J, Liu L et al (2011) Genistein potentiates the effect of arsenic trioxide against human hepatocellular carcinoma, role of Akt and nuclear factor-kappaB. Cancer Lett 301:75–84

Mai Z, Blackburn GL, Zhou JR (2007) Genistein sensitizes inhibitory effect of tamoxifen on the growth of estrogen receptor-positive and HER2-overexpressing human breast cancer cells. Mol Carcinog 46:534–542

Mansoor TA, Ramalho RM, Luo X et al (2011) Isoflavones as apoptosis inducers in human hepatoma HuH-7 cells. Phytother Res 25:1819–1824

Marini H, Bitto A, Altavilla D et al (2008) Breast safety and efficacy of genistein aglycone for postmenopausal bone loss, a follow up study. J Clin Endocrinol Metab 93:4787–4796

McMichael-Phillips DF, Harding C, Morton M et al (1998) Effects of soy-protein supplementation on epithelial proliferation in the histologically normal human breast. Am J Clin Nutr 68(6 Suppl): 1431S–1435S

Messina M, Bennink M (1998) Soyfoods., isoflavones and risk of colonic cancer, a review of the *in vitro* and *in vivo* data. Baillieres Clin Endocrinol Metab 12:707–728

Messina MJ, Persky V, Setchell KD et al (1994) Soy intake and cancer risk, a review of the *in vitro* and *in vivo* data. Nutr Cancer 21:113–131

Messing E, Gee JR, Saltzstein DR et al (2012) A phase 2 cancer chemoprevention biomarker trial of isoflavone G-2535 (genistein) in presurgical bladder cancer patients. Cancer Prev Res 5:621–630

Monroe DG, Secreto FJ, Subramaniam M et al (2005) Estrogen receptor alpha and beta heterodimers exert unique effects on estrogen and tamoxifen-dependent gene expression in human U2OS osteosarcoma cells. Mol Endocrinol 19:1555–1568

Nakamura Y, Yogosawa S, Izutani Y et al (2009) A combination of indol-3-carbinol and genistein synergistically induces apoptosis in human colon cancer HT-29 cells by inhibiting Akt phosphorylation and progression of autophagy. Mol Cancer 8:100

Nakamura H, Wang Y, Kurita T et al (2011) Genistein increases epidermal growth factor receptor signaling and promotes tumor progression in advanced human prostate cancer. PLoS One 6:e20034

Oliver Y, Woosuk J, Shi J et al (2000) Production of the isoflavones genistein and daidzein in non-legume dicot and monocot tissues. Plant Physiol 124:781–794

Orlando L, Schiavon P, Cinieri S (2011) Genistein, the future of prevention and treatment of breast cancer? Cancer Biol Ther 11:883–892

Pan H, Zhou W, He W et al (2012) Genistein inhibits MDA-MB-231 triple-negative breast cancer cell growth by inhibiting NF-kappaB activity via the Notch-1 pathway. Int J Mol Med 30:337–343

Park SJ, Kim MJ, Kim YK et al (2010) Combined cetuximab and genistein treatment shows additive anti-cancer effect on oral squamous cell carcinoma. Cancer Lett 292:54–63

Pavese JM, Farmer RL, Bergan RC (2010) Inhibition of cancer cell invasion and metastasis by genistein. Cancer Metastasis Rev 29:465–482

Pavese JM, Krishna SN, Bergan RC (2014) Genistein inhibits human prostate cancer cell detachment., invasion., and metastasis. Am J Clin Nutr 100:431S–436S

Pendleton JM, Tan WW, Anai S et al (2008) Phase II trial of isoflavone in prostate-specific antigen recurrent prostate cancer after previous local therapy. BMC Cancer 8:132

Peng B, Cao J, Yi S et al (2013) Inhibition of proliferation and induction of G1-phase cell-cycle arrest by dFMGEN., a novel genistein derivative., in lung carcinoma A549 cells. Drug Chem Toxicol 36:196–204

Peng X, Vaishnav A, Murillo G, et al (2010) Protection against cellular stress by 25-hydroxyvitamin D3 in breast epithelial cells. J Cell Biochem 110:1324–1333.

Privat M, Aubel C, Arnould S et al (2010) AKT andp21 WAF1/CIP1 as potential genistein targets in BRCA1-mutant human breast cancer cell lines. Anticancer Res 30:2049–2054

Qi W, Weber CR, Wasland K et al (2011) Genistein inhibits proliferation of colon cancer cells by attenuating a negative effect of epidermal growth factor on tumor suppressor FOXO3 activity. BMC Cancer 11:219–227

Rietjens IMCM, Sotoca AM, Vervoort J et al (2013) Mechanisms underlying thedualistic mode of action of major soy isoflavones in relation to cell proliferation and cancer risks. Mol Nutr Food Res 57:100–113

Rossi M, Negri E, Talamini R et al (2006) Flavonoids and colorectal cancer in Italy. Cancer Epidemiol Biomarkers Prev 15:1555–1558

Sahin K, Tuzcu M, Basak N et al (2012) Sensitization of cervical cancer cells to cisplatin by genistein, the role of NF kappa B and Akt/mTOR signaling pathways. J Oncol 2012:461562

Satoh H, Nishikawa K, Suzuki K et al (2003) Genistein., a soy isoflavone., enhances necrotic like cell death in a breast cancer cell treated with a chemotherapeutic agent. Res Commun Mol Pathol Pharmacol 113–114:149–158

Schabath MB, Hernandez LM, Wu X et al (2005) Dietary phytoestrogens and lung cancer risk. JAMA 294:1493–1504

Seo HS, DeNardo DG, Jacquot Y et al (2006) Stimulatory effect of genistein and apigenin on the growth of breast cancer cells correlates with their ability to activate ER alpha. Breast Cancer Res Treat 99:121–134

Seow A, Koh WP, Wang R et al (2009) Reproductive variables., soy intake., and lung cancer risk among nonsmoking women in the Singapore Chinese Health Study. Cancer Epidemiol Biomarkers Prev 18:821–827

Shiau RJ, Chen KY, Wen YD et al (2010) Genistein and b-carotene enhance the growth-inhibitory effect of trichostatin A in A549 cells. Eur J Nutr 49:19–25

Shimazu T, Inoue M, Sasazuki S et al (2010) Isoflavone intake and risk of lung cancer, a prospective cohort study in Japan. Am J Clin Nutr 91:722–728
Shimazu T, Inoue M, Sasazuki S et al (2011) Plasma isoflavones and the risk of lung cancer in women, a nested case-control study in Japan. Cancer Epidemiol Biomarkers Prev 20:419–427
Shukla S, MacLennan GT, Flask CA et al (2007) Blockade of beta-catenin signaling by plant flavonoid apigenin suppresses prostate carcinogenesis in TRAMP mice. Cancer Res 67:6925–6935
Spector D, Anthony M, Alexander D et al (2003) Soy consumption and colorectal cancer. Nutr Cancer 47:1–12
Su Y, Simmen RC (2009) Soy isoflavone genistein upregulates epithelial adhesion molecule E-cadherin expression and attenuates beta-catenin signaling in mammary epithelial cells. Carcinogenesis 30:331–339
Su SJ, Chow NH, Kung ML et al (2003) Effects of soy isoflavones on apoptosis induction and G2-M arrest in human hepatoma cells involvement of caspase-3 activation., Bcl-2 and Bcl-XL downregulation., and Cdc2 kinase activity. Nutr Cancer 45:113–123
Sundin T, Hentosh P (2012) InTERTesting association between telomerase., mTOR and phytochemicals. Expert Rev Mol Med 14:e8
Sung BH, Lee SJ, Park KH et al (2004) Isoflavones inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase *in vitro*. Biosci Biotechnol Biochem 68:428–432
Taylor CK, Levy RM, Elliott JC et al (2009) The effect of genistein aglycone on cancer and cancer risk, a review of *in vitro*., preclinical., and clinical studies. Nutr Rev 67:398–415
Thiagarajan DG, Bennink MR, Bourquin LD et al (1998) Prevention of precancerous colonic lesions in rats by soy flakes., soy flour., genistein., and calcium. Am J Clin Nutr 68:1394S–1399S
Tian T, Li J, Li B et al (2014) Genistein exhibits anti-cancer effects via down-regulating FoxM1 in H446 small-cell lung cancer cells. Tumour Biol 35:4137–4145
Uckun FM, Evans WE, Forsyth CJ et al (1995) Biotherapy of B-cell precursor leukemia by targeting genistein to CD19-associated tyrosine kinases. Science 267:886–891
Uckun FM, Narla RK, Zeren T et al (1998) *In vivo* toxicity pharmacokinetics., and anticancer activity of Genistein linked to recombinant human epidermal growth factor. Clin Cancer Res 4:1125–1134
Wang Z, Zhang Y, Banerjee B et al (2006) Inhibition of nuclear factorkappaB activity by genistein is mediated via Notch-1 signaling pathway in pancreatic cancer cells. Int J Cancer 118:1930–1936
Wang Z, Li Y, Ahmad A et al (2011) Down-regulation ofNotch-1 is associated with Akt and FoxM1 in inducing cell growth inhibition and apoptosis in prostate cancer cells. J Cell Biochem 112:78–88
Wang H, Li Q, Chen H (2012) Genistein affects histone modifications on Dickkopf-related protein 1 (DKK1) gene in SW480 human colon cancer cell line. PLoS One 7:e40955
Wang SD, Chen BC, Kao ST et al (2014) Genistein inhibits tumor invasion by suppressing multiple signal transduction pathways in human hepatocellular carcinoma cells. BMC Complement Altern Med 14:26
Whyte J, Bergin O, Bianchi A et al (2009) Key signalling nodes in mammary gland development and cancer. Mitogen-activated protein kinase signalling in experimental models of breast cancer progression and in mammary gland development. Breast Cancer Res 11:209
Wong WW, Dimitroulakos L, Minden MD et al (2002) HMG-CoA reductaseinhibitors and the malignant cells, the statin family of drugs and triggers of tumor-specific apoptosis. Leukemia 16:508–519
Wu AH, Yu MC, Tseng CC et al (2004) Plasma isoflavone levels versus self-reported soy isoflavone levels in Asian-American women in Los Angeles County. Carcinogenesis 25:77–81
Wu TC, Yang YC, Huang PR et al (2012) Genistein enhances the effect of trichostatin A on inhibition of A549 cell growth by increasing expression of TNF receptor-1. Toxicol Appl Pharmacol 262:247–254
Xu L, Ding Y, Catalona WJ et al (2009) MEK4 function., genistein treatment., and invasion of human prostate cancer cells. J Natl Cancer Inst 101:1141–1155

Yamamoto S, Sobue T, Kobayashi M et al (2003) Soy., isoflavones., and breast cancer risk in Japan. J Natl Cancer Inst 95:906–913

Yan GR, Zou FY, Dang BL et al (2012) Genistein induced mitotic arrest of gastric cancer cells by downregulating KIF20A, a proteomics study. Proteomics 12:2391–2399

Yeh TC, Chiang PC, Li TK et al (2007) Genistein induces apoptosis in human hepatocellular carcinomas via interaction of endoplasmic reticulum stress and mitochondrial insult. Biochem Pharmacol 73:782–792

Yu D, Shin HS, Lee YS et al (2014) Genistein attenuates cancer stem cell characteristics in gastric cancer through the downregulation of Gli1. Oncol Rep 2014(31):673–678

Zhang Y, Chen H (2011) Genistein attenuates WNT signaling by up-regulating sFRP2 in a human colon cancer cell line. Exp Biol Med (Maywood) 2011(236):714–722

Zhang B, Shi ZL, Liu B et al (2010) Enhanced anticancer effect of gemcitabine by genistein in osteosarcoma, the role of Akt and nuclear factor-kappaB. Anticancer Drugs 21:288–296

Zhang Y, Li Q, Zhou D et al (2013) Genistein., a soya isoflavone., prevents azoxymethane- induced up-regulation of WNT/beta-catenin signaling and reduces colon pre-neoplasia in rats. Br J Nutr 109:33–42

Zhou HB, Chen JJ, Wang WX et al (2004) Apoptosis of human primary gastric carcinoma cells induced by genistein. World J Gastroenterol 10:1822–1825

Zhou HB, Chen JM, Cai JT et al (2008) Anticancer activity of genistein on implanted tumor of human SG7901 cells in nude mice. World J Gastroenterol 14:627–631

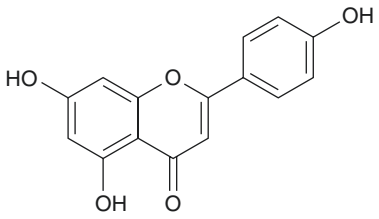
Zhu H, Cheng H, Ren Y et al (2012) Synergistic inhibitory effects by the combination of gefitinib and genistein on NSCLC with acquired drug-resistance *in vitro* and *in vivo*. Mol Biol Rep 39:4971–4979

Chemoprevention by Apigenin

18

Phytoestrogens are compounds derived from plants with steroid-like structures. They are also called as plant hormones and bind to estrogen receptor (ER). The five main classes of phytoestrogens include flavones, isoflavones, lignans, coumestans, and stilbenes. Phytoestrogens are found to restrain tumor growth and act as regulatory molecules in various processes (Merabishvili and Voprosy 2013; Scherbakov and Andreeva 2015).

Apigenin (4′,5,7-trihydroxyflavone) belongs to the flavone class that is the aglycone of several naturally occurring glycosides. It is a yellow crystalline solid utilized to dye wool. Apigenin inhibited nuclear factor NFκB activation in acute carrageenan-induced paw edema in mice. It is also reported to reduce the levels of TNF-α, PGE2, MPO (myeloperoxidase activity), and LPO in acetic acid-induced colitis in mice model as reported by Ganjare et al. It also attenuated morphological signs and biochemical markers in both trinitrobenzene sulfonic acid- and dextran sulfate sodium (DSS)-induced acute colitis models on oral administration (Márquez-Flores et al. 2016; Funakoshi-Tago et al. 2011; Ganjare et al. 2011).



Apigenin

It has been found to reduce the risk of various cancers like cancers of the breast, digestive tract, skin, prostate, lung, and ovary and hematological malignancies from epidemiological reports (Bhukhai et al. 2012). It inhibits PI3K/Akt/FOXO signaling pathway thereby decreasing prostate tumorigenesis in transgenic adenocarcinoma

S. Rashid, *Cancer and Chemoprevention: An Overview*,
DOI 10.1007/978-981-10-2579-2_18

of the mouse prostate (TRAMP) mice in in vivo models. It also inactivated Akt and JNK inhibiting tumor growth in U937 xenografts. It suppressed HIF-1α and VEGF expression in nude mice. It also inhibited cancer progression by decreasing cell survival and inducing apoptosis in breast, lung, colon, prostate, leukemia, and pancreatic cells (Patisaul and Jefferson 2010; Bhukhai et al. 2012; Knekt et al. 1997; Mourouti and Panagiotakos 2013).

It has been found to be a strong inhibitor of UVB light or 7,12-dimethylbenz(a) anthracene (DMBA) and TPA skin tumors (Birt et al. 1997) . It was found to downregulate COX-2 which is an important pro-tumorigenic agent in cultured keratinocytes exposed to UVB and TPA (Van Dross et al. 2005; Tong et al. 2007). There was a decrease in the incidence and the size of tumors in mouse models of skin carcinogenesis induced by chemical carcinogens and UV exposure on treatment with apigenin. It suppresses angiogenesis and regulates p53, downregulates COX-2 expression, and triggers cell cycle arrest and induction of apoptosis. It stimulates AMP-activated protein kinase (AMPK) in keratinocytes which suppresses mTOR signaling pathway. Therefore it is a promising chemopreventive agent against various malignancies (Bridgeman et al. 2016; Kiraly et al. 2016 and Tong et al. 2007).

References

Bhukhai K, Suksen K, Bhummaphan N et al (2012) A phytoestrogen diarylheptanoid mediates estrogen receptor/Akt/glycogen synthase kinase 3β protein-dependent activation of the Wnt/β--catenin signaling pathway. J Biol Chem 287:36168–36178

Birt DF, Mitchell D, Gold B et al (1997) Inhibition of ultraviolet light induced skin carcinogenesis in SKH-1 mice by apigenin, a plant flavonoid. Anticancer Res 17:85–91

Bridgeman BB, Wang P, Ye B et al (2016) Inhibition of mTOR by apigenin in UVB-irradiated keratinocytes. A new implication of skin cancer prevention. Cell Signal 28:460–468

Funakoshi-Tago M, Nakamura K, Tago K et al (2011) Anti-inflammatory activity of structurally related flavonoids, apigenin, luteolin and fisetin. Int Immunopharmacol 11:1150–1159

Ganjare AB, Nirmal SA, Patil AN (2011) Use of apigenin from *Cordia dichotoma* in the treatment of colitis. Fitoterapia 82:1052–1056

Kiraly AJ, Soliman E, Jenkins A et al (2016) Apigenin inhibits COX-2, PGE2, and EP1 and also initiates terminal differentiation in the epidermis of tumor bearing mice. Prostaglandins Leukot Essent Fatty Acids 104:44–53

Knekt P, Jarvinen R, Seppanen R (1997) Dietary flavonoids and the risk of lung cancer and other malignant neoplasms. Am J Epidemiol 146(3):223–230

Márquez-Flores YK, Villegas I, Cárdeno A et al (2016) Apigenin supplementation protects the development of dextran sulfate sodium-induced murine experimental colitis by inhibiting canonical and non-canonical inflammasome signaling pathways. J Nutr Biochem 30:143–152

Merabishvili VM, Voprosy O (2013) Morbidity, mortality and analysis of the effectiveness of the organisation of oncology care for patients with esophageal cancer. Problems in oncology 59:314–319

Mourouti N, Panagiotakos DB (2013) Soy food consumption and breast cancer. Maturitas 76:118–122

Patisaul HB, Jefferson W (2010) The pros and cons of phytoestrogens. Front Neuroendocrinol 31:400–419

Scherbakov AM, Andreeva OE (2015) Apigenin Inhibits Growth of Breast Cancer Cells. The Role of ERα and HER2/neu. Acta Naturae 7(3):133–139

Tong X, Van Dross RT, Abu-Yousif A et al (2007) Apigenin prevents UVB-induced cyclooxygenase 2 expression. coupled mRNA stabilization and translational inhibition. Mol Cell Biol 27:283–296

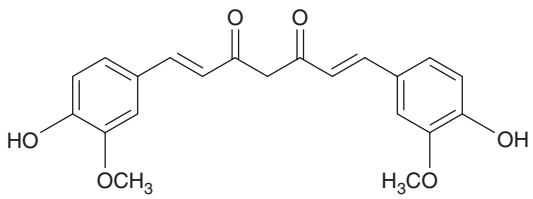
Van Dross RT, Hong X, Pelling JC (2005) Inhibition of TPA-induced cyclooxygenase-2 (COX-2) expression by apigenin through downregulation of Akt signal transduction in human keratinocytes. Mol Carcinog 44:83–91

Chemoprevention by Curcumin

19

Curcumin (diferuloylmethane or 1, 7-bis-(4-hydroxy-3-methoxylphenyl)-1, 6-heptadiene-3, 5-dione), a phenolic compound and a yellow spice, is the active ingredient of the turmeric spice from the plant *Curcuma longa* which has been used for thousands of years in traditional medicine for various ailments like inflammatory diseases in Asia. It is used in treating inflammtory diseases and also in cooking from centuries. In Ayurveda, Unani, and Siddha medicine, turmeric powder is used as a home remedy for various diseases.

Isolated curcuminoid complex contains curcumin (80 %), demethoxycurcumin (17 %), and bisdemethoxycurcumin (3 %). It has been reported to show antitumor, anti-inflammatory, and pro-apoptotic activity in various human cancer cell lines. Curcumin regulates various intracellular signaling pathways resulting in alleviation of various diseases like cancers, diabetic nephropathy, and neurocognitive disorders (Boyanapalli and Tony 2015; Aggarwal and Sung 2009; Berlowitz and Pallotta 1972; Choudhuri et al. 2010).



Curcumin regulates epigenetically the activation of important genes by reversing DNA methylation and varying histone modifications and by targeting numerous miRNAs that have significant role in diseases. It covalently blocks the catalytic thiol group of Cys1226 binding site and suppresses DNMT1 activity (Nephew and Huang 2003). Prostate LNCaP cells treated with curcumin demethylates CpG islands of the NEUROG1 and NRF1 genes when given to prostate LNCaP cells. It upregulates HDAC1, HDAC4, HDAC5, and HDAC8 and decreases HDAC3 (Shu et al. 2011).

S. Rashid, *Cancer and Chemoprevention: An Overview*,
DOI 10.1007/978-981-10-2579-2_19

It upregulates SOCS1 and SOCS3 genes via restraining HDAC8 expression to increase the acetylation of histone in the regions of SOCS1 and SOCS3 promoters in leukemia K562 and HEL cells (Chen et al. 2013). It also inhibits HAT activity leading to suppression of histone protein p300/CBPB and nonhistone protein p53 acetylation. Trichostatin A, an HDAC inhibitor which acts as anticancer agent, can be augmented by curcumin in breast cancer cells. It also elevates miR-15 and miR-16 expression in MCF-7 breast cancer cells and leukemia cells leading to apoptosis (Yan et al. 2013). It increases miR-22 and inhibits miR-199a* in human pancreatic cells (Liu et al. 2009; Chang and Yu 2016).

It has been found to restrain EGFR; VEGFR-1, VEGFR-2, and VEGFR-3; and Src and FAK kinase activity which switches on angiogenic genes, endothelial cell polarity, and migration. MMP-2 and MMP-9 expression, invasiveness of tumor cells in culture, and xenograft experiments are downregulated by curcumin (Arbiser et al. 1998). In nude mice xenografted with hepatocarcinoma, cells led to significant lowering of tumor neo-capillary density upon oral administration of curcumin. COX-2 and VEGF in serum were significantly decreased by curcumin treatment (Yoysungnoen et al. 2006). Both VEGF and VEGFR inhibition in diverse cancers have ignited the enthusiasm in curcumin for anti-angiogenesis (Wang et al. 2015).

There is an inverse relation between curcumin consumptions in diet with numerous human malignancies (Basnet and Skalko-Basnet 2011). Signaling pathways like Wnt, NFκB, STAT3, PI3K/Akt, and mTOR are regulated by curcumin. miR-21 expression has been found to be downregulated and reduced the expression of Bcl-2 by upregulating miR-15a and miR-15b by curcumin and difluorinated curcumin, its synthetic analog (Yang et al. 2010; Bao et al. 2012) inferring that curcumin impacts upon proliferation, partly, by epigenetic mechanisms. However its low bioavailability and stability are its main problems prompting the generation of its structural analogs which may be stabilized with adjuvants like piperine or in liposomal and nanoparticle form and the generation of curcumin phospholipid complexes which might augment its bioavailability besides maintaining antitumor properties (Feitelson et al. 2015).

Curcumin suppresses tumor initiation and promotion, angiogenesis, and metastasis. VEGF along with MMP9 is downregulated by curcumin in prostate cancer cells. CDF, a curcumin-derived analog in vitro and in vivo, inhibits VEGF, IL-6, and cancer stem cell signature genes like Nanog, Oct4, and EZH2 in prostate cancer cells (Gupta et al. 2013). Curcumin inhibited invasiveness and migratory movement of human lung cancer cells by suppressing MMP-2, MMP-9, and VEGF, and migration and invasion of human lung cancer cells were inhibited by curcumin. IL-1 signaling is vital to inflammatory and malignancy processes as IL-6. IL-1 induced IkB alpha phosphorylation and inhibits NFκB which is involved in cell proliferation, invasion, and angiogenesis. IL-1 and VEGF were blocked in chondrosarcoma cells upon treatment with curcumin. It also suppressed NFκB-related gene expression and IL-1 beta-induced angiogenesis (Lin et al. 2007; Yamazaki et al. 2009). Using up to 8000 mg of curcumin per day for 3 months was found to be safe having no toxicity from a phase I human trial with 25 subjects besides from reports of six other human trials (Casey et al. 2015).

Liposomal formulation of curcumin leads to cancer growth suppression and NFκB reduction thereby inferring that it hits NFκB target genes like cyclin D1, COX-2, MMP-9, Bcl-2, Bcl-xL, Mcl-1 L, and Mcl-1S (Wang et al. 2008). PAC, a novel curcumin analog, reduced tumor size, triggered apoptosis in breast cancer tumor xenografts by mitigating survivin and NFκB expression and its downstream elements, and strongly upregulated p21 (WAF1) as reported by Al-Hujaily et al. (Al-Hujaily et al. 2011). Furthermore it has been found to downregulate NFκB and COX-2 in peripheral blood mononuclear cells from patients with pancreatic cancer in clinical trials thereby regulating NFκB signaling in vitro and in vivo.

Curcumin inhibits phosphorylation of mTOR and Akt as well as their downstream effectors dose- and time-dependently in prostate cancer cells. There was suppression of SCC40 xenografts growth via inhibition of Akt/mTOR pathway upon curcumin treatment. In head and neck squamous cell carcinoma induced by 4-nitroquinoline 1-oxide, survival increases by taking 15 mg curcumin as reported from a survival study (Wilken et al. 2011).

In cisplatin-resistant ovarian cancer cells, curcumin inhibited the proliferation by Akt inactivation. Curcumin has molecular targets within the Akt signaling pathways, and Akt inactivity inhibits proliferation and apoptosis in cancer cells as reported. Diphenyl difluoroketone, a curcumin derivative, mitigated the colon cancer xenograft Akt and ERK phosphorylation in mice (Subramaniam et al. 2008). In other study, solubilized curcumin effectively blocked development of brain tumors in the mice which received an intracerebral administration of mouse melanoma cells; namely, B16F10 was blocked by curcumin via alleviating p-Akt, cyclin D1, p-NFκB, Bcl-xL, and VEGF expressions (Purkayastha et al. 2009; Kuno et al. 2012).

Chemoprevention of Esophageal Cancer by Curcumin

Curcumin reduces the growth of *H. pylori* infection in mice. Curcumin downregulates NFκB and its target genes like Bcl-2 and Bcl-xL and cyclin D1 in human gastric cancer SGC 7901 cell line and, therefore, protects against chemoresistance in human gastric cancer cells (Yu et al. 2011). Curcumin additionally attenuates EGFR and its downstream regulator, p21-activated kinase (PAK)-1, in an esophageal epithelial cell line (HET-1A). Curcumin reversed inhibition of superoxide dismutase (SOD)-1 and initiation of COX-2 gene expression. Therefore increased activity of antioxidant enzymes like SOD and decreased prooxidant enzymes like COX-2 may prevent esophageal cancer. Its anticancer activities are mediated via its anti-inflammatory activity besides its antioxidant capacity (Rayet and Gélinas 1999). Curcumin inhibits NFκB activity which has been demonstrated in esophageal adenocarcinoma since increased NFκB has been associated with cell proliferation, invasion, angiogenesis, metastasis, suppression of apoptosis, and chemoresistance in various types of cancer (Hartojo et al. 2010).

A 500 mg curcumin tablet daily for 7 days to the patients with Barrett's esophagus had decreased IL-8 mRNA expression signifying that curcumin may be a chemopreventive agent against esophageal cancer. It stimulates cell cycle arrest and

apoptosis by blocking Notch signaling pathways which is found to be upregulated in esophageal cancer and therefore can prove to be a therapeutic target for esophageal cancer because it plays an important role in tumor cell proliferation, apoptosis, stem cell maintenance, and renewal (Mendelson et al. 2011). Curcumin inhibited Notch signaling which in turn downregulated NFκB and its target genes like Bcl-2, cyclin D1, VEGF, and MMP-9 in oral squamous carcinoma cells (Liao et al. 2011).

Chemoprevention of Esophageal Cancer by Curcumin

Gastric cancer is the seventh most common cause of cancer-related death in the world. *Helicobacter pylori* (*H. pylori*) infection or exposure to chemical carcinogens may lead to the development of gastric cancer by infiltration of neutrophils and macrophages into the gastric mucosa in case of *H. pylori* which leads to the generation of free radicals like superoxide and nitric oxide resulting in gastric mucosal injury, ulcers, and ultimately gastric cancer which may be mitigated by antioxidants by scavenging ROS or enhancing antioxidant capacity (Kuipers 1999).

Curcumin inhibits *H. pylori* infection in mice by reducing its growth (De et al. 2009). The mechanisms behind the growth of cells and prospective therapeutic capability of curcumin have been additionally explored by various in vitro studies using multiple gastric cancer cell lines. Curcumin is reported to demonstrate protection against chemoresistance in human gastric cancer cells by downregulating NFκB and subsequent NFκB-mediated anti-apoptotic genes which include Bcl-2 and Bcl-xL in the human gastric cancer SGC 7901 cell line (Yu et al. 2011). Moreover, curcumin reduces PAK-1 activity which is a downstream regulator of EGFR as well as EGFR expression. Curcumin also diminishes NFκB activity which is regulated by PAK-1 resulting in decrease in cell proliferation by reducing mRNA and protein expression of cyclin D1 and suppressing cell cycle progression from G1 to S phase thereby inhibiting cellular proliferation and invasiveness of various gastric cancer cells (Cai et al. 2009; Chung et al. 2013).

References

Aggarwal BB, Sung B (2009) Pharmacological basis for the role of curcumin in chronic diseases: an age-old spice with modern targets. Trends Pharmacol Sci 30:85–94

Al-Hujaily EM, Mohamed AG, Al-Sharif I et al (2011) PAC, a novel curcumin analogue, has anti-breast cancer properties with higher efficiency on ER-negative cells. Breast Cancer Res Treat 128:97–107

Arbiser JL, Klauber N, Rohan R et al (1998) Curcumin is an *in vivo* inhibitor of angiogenesis. Mol Med 4:376–383

Bao B, Ali S, Banerjee S et al (2012) Curcumin analogue CDF inhibits pancreatic tumor growth by switching on suppressor microRNAs and attenuating EZH2 expression. Cancer Res 72:335–345

Basnet P, Skalko-Basnet N (2011) Curcumin. an anti-inflammatory molecule from a curry spice on the path to cancer treatment. Molecules 16:4567–4598

Berlowitz L, Pallotta D (1972) Acetylation of nuclear protein in the heterochromatin and euchromatin of mealy bugs. Exp Cell Res 71:45–48

Boyanapalli SS, Tony KAN (2015) Curcumin, the King of Spices. Epigenetic Regulatory Mechanisms in the Prevention of Cancer, Neurological, and Inflammatory Diseases. Curr Pharmacol Rep 1:129–139

Cai XZ, Wang J, Li XD et al (2009) Curcumin suppresses proliferation and invasion in human gastric cancer cells by downregulation of PAK1 activity and cyclin D1 expression. Cancer Biol Ther 8:1360–1368

Casey SC, Amedei A, Aquilano K et al (2015) Cancer prevention and therapy through the modulation of the tumor microenvironment. Semin Cancer Biol 35:S199–S223

Chang LC, Yu YL (2016) Dietary components as epigenetic-regulating agents against cancer. Biomedicine (Taipei) 6:2

Chen CQ, Yu K, Yan QX et al (2013) Pure curcumin increases the expression of SOCS1 and SOCS3 in myeloproliferative neoplasms through suppressing class I histone deacetylases. Carcinogenesis 34:1442–1449

Choudhuri S, Cui Y, Klaassen CD (2010) Molecular targets of epigenetic regulation and effectors of environmental influences. Toxicol Appl Pharmacol 245:378–393

Chung MY, Lim TG, Lee KW (2013) Molecular mechanisms of chemopreventive phytochemicals against gastroenterological cancer development. World J Gastroenterol 19:984–993

De R, Kundu P, Swarnakar S et al (2009) Antimicrobial activity of curcumin against Helicobacter pylori isolates from India and during infections in mice. Antimicrob Agents Chemother 53:1592–1597

Feitelson MA, Arzumanyan A, Kulathinal RJ et al (2015) Sustained proliferation in cancer. Mechanisms and novel therapeutic targets. Semin Cancer Biol 35(Suppl):S25–S54

Gupta A, Zhou CQ, Chellaiah MA (2013) Osteopontin and MMP9 association with VEGF expression/secretion and angiogenesis in PC3 prostate cancer cells. Cancers (Basel) 5:617–638

Hartojo W, Silvers AL, Thomas DG et al (2010) Curcumin promotes apoptosis, increases chemosensitivity, and inhibits nuclear factor kappaB in esophageal adenocarcinoma. Transl Oncol 3:99–108

Kuipers EJ (1999) Review article: exploring the link between Helicobacter pylori and gastric cancer. Aliment Pharmacol Ther 13:3–11

Kuno T, Tsukamoto T, Hara A et al (2012) Cancer chemoprevention through the induction of apoptosis by natural compounds. J Biophys Chem 3:156–173

Liao S, Xia J, Chen Z et al (2011) Inhibitory effect of curcumin on oral carcinoma CAL-27 cells via suppression of Notch-1 and NF-κB signaling pathways. J Cell Biochem 112:1055–1065

Lin YG, Kunnumakkara AB, Nair A et al (2007) Curcumin inhibits tumor growth and angiogenesis in ovarian carcinoma by targeting the nuclear factor-kappaB pathway. Clin Cancer Res 13:3423–3430

Liu Z, Xie Z, Jones W et al (2009) Curcumin is a potent DNA hypomethylation agent. Bioorg Med Chem Lett 19:706–709

Mendelson J, Song S, Li Y et al (2011) Dysfunctional transforming growth factor-β signaling with constitutively active Notch signaling in Barrett's esophageal adenocarcinoma. Cancer 117:3691–3702

Nephew KP, Huang TH (2003) Epigenetic gene silencing in cancer initiation and progression. Cancer Lett 190:125–133

Purkayastha S, Berliner A, Fernando SS et al (2009) Curcumin Blocks Brain Tumor Formation. Brain Res 1266:130–138

Rayet B, Gélinas C (1999) Aberrant rel/NFkB genes and activity in human cancer. Oncogene 18:6938–6947

Shu L, Khor TO, Lee JH et al (2011) Epigenetic CpG demethylation of the promoter and reactivation of the expression of Neurog1 by curcumin in prostate LNCaP cells. AAPS J 13:606–614

Subramaniam D, May R, Sureban SM (2008) Diphenyl difluoroketone: a curcumin derivative with potent *in vivo* anticancer activity. Cancer Res 68:1962–1969

Wang D, Veena MS, Stevenson K et al (2008) Liposome-encapsulated curcumin suppresses growth of head and neck squamous cell carcinoma *in vitro* and in xenografts through the inhibition of nuclear factor kappaB by an AKT-independent pathway. Clin Cancer Res 14:6228–6236

Wang Z, Dabrosin C, Yin X et al (2015) Broad targeting of angiogenesis for cancer prevention and therapy. Semin Cancer Biol 35(Suppl):S224–S243

Wilken R, Veena MS, Wang MB et al (2011) Curcumin: a review of anticancer properties and therapeutic activity in head and neck squamous cell carcinoma. Mol Cancer 10:12

Yamazaki K, Gohda J, Kanayama A et al (2009) Two mechanistically and temporally distinct NF-kappaB activation pathways in IL-1 signaling. Sci Signal 2:ra66

Yan G, Graham K, Lanza-Jacoby S (2013) Curcumin enhances the anticancer effects of trichostatin A in breast cancer cells. Mol Carcinog 52:404–411

Yang J, Cao Y, Sun J et al (2010) Curcumin reduces the expression of Bcl-2 by upregulating miR-15a and miR-16 in MCF-7 cells. Med Oncol 27:1114–1118

Yoysungnoen P, Wirachwong P, Bhattarakosol P et al (2006) Effects of curcumin on tumor angiogenesis and biomarkers, COX-2 and VEGF, in hepatocellular carcinoma cell-implanted nude mice. Clin Hemorheol Microcirc 34:109–115

Yu LL, Wu JG, Dai N et al (2011) Curcumin reverses chemoresistance of human gastric cancer cells by downregulating the NF-κB transcription factor. Oncol Rep 26:1197–1203

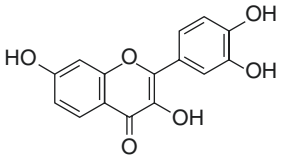
Chemoprevention by Fisetin

20

Fisetin (3,7,3′,4′-tetrahydroxyflavone) is a naturally occurring flavonoid that belongs to the flavonol subgroup together with quercetin, myricetin, and kaempferol. It is found in many edible fruits and vegetables like apples, grapes, kiwis, persimmons, strawberries, cucumbers, and onions having various pharmacological properties which include antioxidant, anti-inflammatory, anti-proliferative, pro-apoptotic, anti-tumorigenic, anti-invasive, and antimetastatic activity (Pal et al. 2015). Reports suggest that it inhibits Wnt/β-catenin, PI3K/AKT/ mTOR, and NFκB signaling pathways (Pal et al. 2013) thus revealing anti-proliferative, pro-apoptotic, and anti-tumorigenic activities against various cancers. It has also been reported to diminish melanoma cell invasion and transition from epithelial to mesenchymal (Pal et al. 2014). It is found to be quickly absorbed and detectable in serum by some murine investigations (Shia et al. 2009).

Strawberries are the richest source of fisetin having 160 μg/g in wet food. It was observed that 45 mg/kg of fisetin administration in mice reduced tumor growth by 74.8 % through androgen receptor inhibition as well as induced apoptosis in LNCaP human prostate cancer cells (Khan et al. 2008a, b, c). Fisetin at the same dose regulated Wnt/β-catenin signaling and suppressed melanoma cell growth by 66.6 %. Moreover, it has been reported to regulate NF*κ*B, Aurora B kinase (Salmela et al. 2009), mTOR, and PI3K/AKT signaling (Murtaza et al. 2009a, b) along with induction of apoptosis in human NSCLC by downregulating Bcl-2 expression as Kang and colleagues reported (Kim et al. 2015). It mediates inflammation through attenuation of COX-2, iNOS, and NO levels in RAW 264.7 cells and mice (Geraets et al. 2009; Kim et al. 2012). It has been found to have chemotherapeutic effect against human epidermoid carcinoma A431 cells and hence may be used in the treatment of non-melanoma skin cancer.

S. Rashid, *Cancer and Chemoprevention: An Overview*,
DOI 10.1007/978-981-10-2579-2_20



Structure of Fisetin (3,7,3′,4′-Tetrahydroxyflavone)

It prevents growth of the lung fibroblast cells by activation of nonenzymic antioxidant system to scavenge cellular ROS (Kang et al. 2014). Additionally it shows cytotoxicities by modulating tumor-associated biochemical/molecular targets like inhibition of CDKs, by downregulating NFkB, inhibiting PI3K/Akt pathway in prostate cancer cells (Adhami et al. 2012), and impeding angiogenesis in the endothelial cells as well as its ROS scavenging ability. It downregulates Bcl2 therefore triggering in an HCC cell line, namely, Huh7 cells. However its anticancer properties against HCC in vivo must be evaluated. It deciphers anticancer activity against benzopyrene-induced lung carcinoma in mice via mediating oxidative stress. Fisetin has been found to stabilize GST-pi (glutathione S-transferase, placental type) levels; HCC marker mitigates HCC lesions in aflatoxin-intoxicated liver tumors (Maurya and Trigun 2016).

It has been reported to demonstrate cell death in the NSCLC cell line NCI-H460 via mitochondria-mediated apoptosis; besides that, fisetin has shown anticancer activity against various human cancers via antiproliferation, anti-invasion, or antimetastasis (Kang et al. 2016). It has been found to prevent colon cancer (Lim and Park 2009), prostate cancer (Szliszka et al. 2011), pancreatic cancer (Murtaza et al. 2009a, b), lung cancer (Khan et al. 2012), and multiple myeloma which might be by growth inhibition via activating death receptor and intrinsic pathways of apoptosis, alterations in cell cycle regulatory genes, and regulation of signaling pathways like PI3K/Akt and NFκB (Adhami et al. 2012). It has been found to induce apoptosis in human acute monocytic leukemia cells via activation of NO production resulting in double-strand breaks (Ash et al. 2015). In another study, it was shown that sorafenib-induced apoptosis in melanoma cells was potentiated by fisetin by inhibiting PI3K signaling pathway by mediating PI3K expression and phosphorylation of MEK1/2, ERK1/2, AKT, and mTOR (Adan and Baran 2016).

It is an effective anticancer agent against extensively varied tumor cell lines at physiologically relevant concentrations and mostly does not affect normal cells like human bronchial epithelial (NHBE) cells (Klimaszewska-Wisniewska et al. 2016) and normal prostate epithelial cells. Cell cycle arrest in PC3 cells was in G2/M phase, while the LNCaP cells showed a different cell cycle profile as per flow cytometric analysis. Fisetin induced arrest in the G1 phase of the cell cycle, decreased level of cyclins and cdks and simultaneous induction of p21 and p27 in LNCaP cells, as well as mediated apoptosis by releasing cytochrome *c* from mitochondria into the cytosol (Khan et al. 2008a; b, c). A supramolecular complex called apoptosome formed by the combination of cytochrome *c*, Apaf-1, ATP, and procaspase-9 activates caspase-9 through autocatalysis. Fisetin treatment activated caspase-3,

caspase-8, and caspase-9 in CaP cells. Inhibitors of apoptosis protein (IAP) regulate caspases in apoptosis, which are nullified by high-temperature requirement protein A2 and Smac, a direct IAP-binding protein with low pI DIABLO released from mitochondria. There was downregulation of XIAP and upregulation of Smac/DIABLO upon fisetin treatment associated by modulation in the critical regulatory genes of the apoptotic pathway, namely, Bcl2 family proteins. Therefore it mediates the mitochondrial membrane function of CaP cells triggering apoptosis which is the most critical molecular target for chemoprevention of cancer (Haddad et al. 2006). It also inhibited uPA in the advancing capillary vessels surrounding the tumor which may be the plausible mechanism behind anti-angiogenesis and tumor growth retardation. It is also found to inhibit five α-reductase activities which may be used for the prevention or treatment of androgen-dependent disorders like prostate cancer (Jankun et al. 2006). Hence it may be consumed as part of the normal diet or in supplements suggesting that fisetin may be a promising chemopreventive agent against prevention of prostate cancer (Syed et al. 2008).

References

Adan A, Baran Y (2016) Fisetin and hesperetin induced apoptosis and cell cycle arrest in chronic myeloid leukemia cells accompanied by modulation of cellular signaling. Tumour Biol. 37:5781–5795

Adhami VM, Syed DN, Khan N et al (2012) Dietary flavonoid fisetin, a novel dual inhibitor of PI3K/Akt and mTOR for prostate cancer management. Biochem Pharmacol. 84:1277–1281

Ash D, Subramanian M, Surolia A et al (2015) Nitric oxide is the key mediator of death induced by fisetin in human acute monocytic leukemia cells. Am J Cancer Res. 5:481–497

Bhat TA, Nambiar D, Pal A et al (2011) Fisetin inhibits various attributes of angiogenesis *in vitro* and *in vivo*-implications for angioprevention. Carcinogenesis. 33:385–393

Geraets L, Haegens A, Brauers K et al (2009) Inhibition of LPS-induced pulmonary inflammation by specific flavonoids. Biochem. Biophys. Res. Commun. 382:598–603

Haddad AQ, Venkateswaran V, Viswanathan L et al (2006) Novel anti proliferative flavonoids induce cell cycle arrest in human prostate cancer cell lines. Prostate Cancer Prostatic Dis. 9:68–76

Jankun J, Selman SH, Aniola J et al (2006) Nutraceutical inhibitors of urokinase, potential applications in prostate cancer prevention and treatment. Oncol Rep. 16:341–346

Kang KA, Piao MJ, Kim KC et al (2014) Fisetin attenuates hydrogen peroxide-induced cell damage by scavenging reactive oxygen species and activating protective functions of cellular glutathione system. In vitro Cell Dev Biol Anim 50:66–74

Kang KA, Piao MJ, Madduma HSR et al (2016) Fisetin induces apoptosis and endoplasmic reticulum stress in human non-small cell lung cancer through inhibition of the MAPK signaling pathway. Tumour Biol 37(7):9615–9624 (Epub ahead of print)

Khan N, Afaq F, Syed DN, et al (2008a) Fisetin, a novel dietary flavonoid causes apoptosis and cell- cycle arrest in human prostate cancer LNCaP cells. Annual Meeting of the American Association for Cancer Research; San Diego, CA, USA. p 08-AB-2152-AACR

Khan N et al (2008b) Fisetin, a novel dietary flavonoid, causes apoptosis and cell cycle arrest in human prostate cancer LNCaP cells. Carcinogenesis. 29:1049–1056

Khan N et al (2008c) A novel dietary flavonoid fisetin inhibits androgen receptor signaling and tumor growth in athymic nude mice. Cancer Res 68:8555–8563

Khan N, Afaq F, Khusro FH et al (2012) Dual inhibition of phosphatidylinositol 3-kinase/Akt and mammalian target of rapamycin signaling in human nonsmall cell lung cancer cells by a dietary flavonoid fisetin. Int J Cancer 130:1695–1705

Kim HJ, Kim SH, Yun JM (2012) Fisetin inhibits hyperglycemiainduced proinflammatory cytokine production by epigenetic mechanisms. Evid Based Complement Alternat Med 2012:639469

Kim JA, Lee S, Kim DE et al (2015) Fisetin, a dietary flavonoid, induces apoptosis of cancer cells by inhibiting HSF1 activity through blocking its binding to the hsp70 promoter. Carcinogenesis 36:696–706

Klimaszewska-Wisniewska A, Halas-Wisniewska M, Tadrowski T et al (2016) Paclitaxel and the dietary flavonoid fisetin, a synergistic combination that induces mitotic catastrophe and autophagic cell death in A549 non-small cell lung cancer cells. Cancer Cell Int 16:16,10

Lim DY, Park JH (2009) Induction of p53 contributes to apoptosis of HCT-116 human colon cancer cells induced by the dietary compound fisetin. Am J Physiol Gastrointest Liver Physiol 296:G1060–G1068

Lu X, Jae IJ, Han JC et al (2005) Fisetin inhibits the activities of cyclin-dependent kinases leading to cell cycle arrest in HT-29 human colon cancer cells. J Nutr 135:2884–2890

Maurya BK, Trigun SK (2016) Fisetin modulates antioxidant enzymes and inflammatory factors to inhibit aflatoxin-B1 induced hepatocellular carcinoma in rats. Oxid Med Cell Longev 2016:1972793 9 pages

Murtaza I, Adhami VM, Hafeez BB et al (2009a) Fisetin, a natural flavonoid, targets chemoresistant human pancreatic cancer AsPC-1 cells through DR3 mediated inhibition of NF- κB. Int J Cancer 2:2465–2473

Murtaza I et al (2009b) Fisetin, a natural flavonoid, targets chemoresistant human pancreatic cancer AsPC-1 cells through DR3-mediated inhibition of NF-kappaB. Int J Cancer 125:2465–2473

Pal HC, Sharma S, Elmets CA et al (2013) Fisetin inhibits growth, induces G2 /M arrest and apoptosis of human epidermoid carcinoma A431 cells, role of mitochondrial membrane potential disruption and consequent caspases activation. Exp Dermatol 22:470–475

Pal HC, Sharma S, Strickland LR et al (2014) Fisetin inhibits human melanoma cell invasion through promotion of mesenchymal to epithelial transition and by targeting MAPK and NFκB signaling pathways. PLoS One 9:e86338

Pal HC, Baxter RD, Hunt KM et al (2015) Fisetin, a phytochemical, potentiates sorafenib-induced apoptosis and abrogates tumor growth in athymic nude mice implanted with BRAF-mutated melanoma cells. Oncotarget 6:28296–28311

Salmela AL et al (2009) Dietary flavonoid fisetin induces a forced exit from mitosis by targeting the mitotic spindle checkpoint. Carcinogenesis 30:1032–1040

Shia CS, Tsai SY, Kuo SC et al (2009) Metabolism and pharmacokinetics of 3,3′,4′,7-tetrahydroxyflavone (fisetin), 5-hydroxyflavone, and 7-hydroxyflavone and antihemolysis effects of fisetin and its serum metabolites. J Agric Food Chem 57:83–89

Syed DN, Suh Y, Afaq F et al (2008) Dietary agents for chemoprevention of prostate cancer. Cancer Lett 265:167–176

Szliszka E, Helewski KJ, Mizgala E et al (2011) The dietary flavonol fisetin enhances the apoptosis-inducing potential of TRAIL in prostate cancer cells. Int J Oncol 39:771–779

Chemoprevention by Epigallocatechin-3-Gallate (EGCG)

21

Tea that is obtained from leaves of the plant *Camellia sinensis* is subsequent to water and is the most extensively consumed drink in the world. Black, green, and oolong tea are the forms which are produced by the different processing and manufacturing techniques making them chemically different from each other resulting for around 75 %, 23 %, and 2 % of the global production, respectively (Kavanagh et al. 2001). Black tea is a rich source of complex antioxidants called theaflavins and thearubigins, whereas steamed and dried green tea contains simple antioxidants called catechins. Green tea has been used for centuries to treat and prevent chronic diseases in traditional Chinese medicine, but it is only lately known as an efficient chemopreventive agent against various cancers (Sueoka et al. 2001).

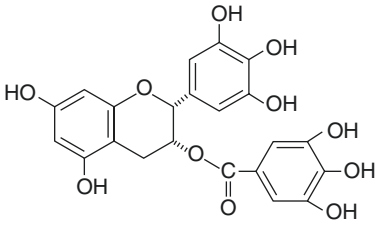
Green tea has been found to contain polyphenols like flavonols, flavandiols, flavonoids, and phenolic acids which account for about 30 % of its dry weight. Flavonols make up most of the green tea polyphenols (GTPs), commonly known as catechins, which are present in four types which constitute epicatechin (EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epigallocatechin-3-gallate (EGCG). The preparation methods influence the catechins both quantitatively and qualitatively. Most attention has been given to EGCG as it shows chemopreventive and potential anticarcinogenic activity and comprises of 50 % of the total catechin content and has a higher antioxidant potential than vitamin C and E (Chowdhury et al. 2016).

Polyphenols in tea have been reported to be anti-angiogenic through diverse signaling pathways like EGCG at low concentrations which directly inhibit capillary endothelial cell proliferation deciphering its antitumor activity. It inhibits VEGF in MDA-MB231 breast cancer cells, and human umbilical vein endothelial cells mediate the suppression of protein kinase C, c-fos, and c-jun RNA transcripts, signifying that AP-1 responsive regions in the human VEGF promoter might be involved (Maeda-Yamamoto et al. 1999). EGCG restrained MMP-2 and MMP-9 and induced the activity of their inhibitors TIMP-1 and TIMP-2 in neuroblastoma, glioblastoma, prostate cancer, fibrosarcoma, and human gastric cancer cells. EGCG treatment reduced MMP-2 activity and focal adhesion kinase (FAK), membrane

S. Rashid, *Cancer and Chemoprevention: An Overview*,
DOI 10.1007/978-981-10-2579-2_21

type-1-MMP (MT1-MMP), NFkB, VEGF, and the adhesion of cells to the ECM in human breast cancer cells, and parallel signaling pathways have also been confirmed in animal studies (Slivova et al. 2005). uPA has been targeted by EGCG resulting in downregulation of VEGF production in tumor cells following suppression of AP-1, NFkB, and signal transducer and activator of transcription (STAT)-1 transcription factor pathway and aryl hydrocarbon receptor (AhR)-mediated transcription by binding to HSP90 (Ramos 2007; Yang et al. 2010).

GTP infusion in transgenic adenocarcinoma of the mouse prostate (TRAMP) mice inhibited angiogenesis and metastasis, like VEGF, uPA, MMP-2, and MMP-9 (Hastak et al. 2003). Black tea polyphenols decreased incidence of invasion, tumor hypoxia, and angiogenesis in a dimethylaminoazobenzene (DAB)-induced hepatoma model. Tissue plasminogen activator (tPA) is one of the vital proteases that facilitate tumors to metastasize which is inhibited by EGCG. Chemoprevention by tea is not only limited to suppression of VEGF, NFkB, c-fos, and cyclin D1 activity but also the reduced Bcl-XL and the stabilization of p53 as reported from various cell culture and animal models (Wang et al. 2015).



EGCG modulates various molecular processes like initiation and execution of apoptosis and suppression of cancer progression and angiogenesis like p27, Bcl-2 or Bcr-Abl oncoproteins, Bax, MMP-2 and MMP-9, AR, EGFT, AP1, and some cell cycle regulatory proteins (Nam et al. 2001; Liang et al. 1999). EGCG induces apoptosis, cell growth inhibition, and cyclin kinase inhibitor WAF-1/p21-mediated cell cycle dysregulation in GTE in cell culture systems by Adhami et al. (2007). It resulted in activation of genes that functionally demonstrate growth inhibitory effects and suppression of genes belonging to the G protein signaling network in prostate cancer cells by cDNA microarrays signifying that GTEs have strong and selective pro-apoptotic activity in vitro and in vivo in prostate cancer (Mohammad et al. 2015).

EGCG which is a green tea polyphenol demonstrates suppression of tumor formation and progression in animal models. The wide spectrum of anticancer properties of this polyphenol, like inhibition of proliferation, inflammation, angiogenesis, progression, and pro-apoptosis, are mechanistic pathways behind the chemopreventive and therapeutic effects of EGCG (Yang et al. 2009). It inhibits the expression of IDO which mediates T cells and induces immune tolerance to tumor cells via tryptophan depletion. It obstructs JAK/STAT-regulated IDO activation leading to suppression of IDO and IDO-related downstream gene expression in human cancer cells as well as inhibits IDO expression via suppression of IFN-γ induction in

human oral cancer cell lines. It also inhibited the transcriptional activation of IDO by blocking translocation of STAT1 into the nucleus.

Therefore inhibition of IFN-γ stimulated STAT1 phosphorylation along with inhibition of the PKC as reported by Cheng et al. (2010). Likewise it blocks IDO expression in human colorectal cancer through transcriptional inhibition of STAT1 phosphorylation leading to suppression of STAT1-activated sequence elements of the IDO promoter, IFN-stimulated response element (ISRE), and IFN-γ activation sequence (GAS) as reported by another group. Anti-IDO activities in murine bone marrow-derived dendritic cells (BMDCs) were also regulated by EGCG (Jeong et al. 2007). In response to IFN-γ activation, EGCG obstructed binding of phosphorylated STAT1 to INF regulatory factor-1(IRF-1) promoter.

EGCG treatment to murine BMDCs resulted in downregulation of prostaglandin E2 (PGE2), a bioactive lipid, and COX-2, the key enzyme in prostaglandin biosynthesis which is frequently coupled with immune surveillance, and cancer was also seen in human prostate carcinoma and colon carcinoma cell lines (Peng et al. 2006). Ogawa et al. (2012) investigated the effect of EGCG on azoxymethane (AOM)-induced preneoplastic lesions in F344 rat through IDO expression suppression and found that EGCG decreased aberrant crypt foci, which had overexpression of IDO, and COX-2 mRNA expression was also downregulated. EGCG regulates JAK/STAT signaling pathway leading to inhibition of STAT1 phosphorylation and IRF-1 expression on various cancer cell lines like mammary carcinoma, cervical carcinoma, and hepatocarcinoma. STAT3 is associated with constitutive IDO expression in human cancer cells (Tedeschi et al. 2002).

EGCG has been found to suppress phosphorylation and expression of both JAK3 and STAT3 proteins in pancreatic cancer cells in which STAT3 is coupled with constitutive IDO expression in human cancer cells. Insulin-like growth factors (IGFs) in hepatocellular carcinoma cells stimulate phosphorylated STAT3 proteins which are decreased by EGCG treatment probably by inhibiting the bioavailability of IGFs (Shimizu et al. 2008). STAT3 in head, neck, and breast cancers is inhibited by EGCG (Masuda et al. 2003). The anticancer property of EGCG could be well evidenced by its regulation of JAK/STAT-mediated IDO as well as JAK/STAT signaling. EGCG in combination with chemotherapeutic drugs like tamoxifen and paclitaxel has shown synergistic effects thus suggesting that EGCG is a promising chemopreventive agent by targeting IDO (Casey et al. 2015).

Dietary and lifestyle modifications are responsible for causing one third of the human cancers. Chemoprevention has opened a new practical strategy to lessen cancer incidences, thereby decreasing mortality and morbidity associated with this dreadful disease. Tea has gained global positive reception in the last 25 years as a cancer chemopreventive agent (Deng and Lin 2011). There is inverse relation between tea consumption and progression of various cancers as reported from epidemiological and laboratory studies. Polyphenols in tea selectively induce apoptotic cell death and cell cycle arrest via various mechanisms in tumor cells and not in their normal counterparts (Alexander et al. 2011). Individuals drinking green tea regularly have less frequent or less severe cancer in various areas of the body like the ovary and prostate as per epidemiological reports.

Regular intake of more than three cups of green tea daily may decrease lung cancer in smokers. Polyphenols in tea have an inhibitory effect on oral cavity, esophagus, stomach, small intestine, and colon tumorigenesis (Jihyeung et al. 2007). There is a correlation between tea polyphenols and colorectal cancer. Conversion of high-grade prostate intraepithelial neoplasia to cancer was inhibited by the consumption of tea catechin capsules after 1 year in comparison to placebo (Susanne et al. 2011). EGCG has been found to intervene at the cellular level against several cancers like breast, pancreas, mouth, colon, and prostate by inhibiting mitogen-activated protein (MAPK) and AP-1, NFkB signaling pathway, EGFR-mediated pathways, IGF-1-mediated signal transduction pathways, proteosome activities, MMP activity, uPA activities, and induction of apoptosis and cell cycle arrest as reported by Khan and Mukhtar (Eiman et al. 2012; Kanwar et al. 2012).

EGCG enhances the sensitization of chemo/radiation therapy to activate cancer cell death while protecting the normal cells. A novel targeted therapy for breast cancer has been a combination therapy of curcumin and EGCG that suppresses breast cancer stem cells (BCSCs) by mediating STAT3 and NFkB signaling pathways serving as targets for reducing CSCs (Chung and Vadgama 2015). EGCG along with curcumin induces inhibition in cell growth and activation of apoptosis in resistant breast cancer cells (Thangapazham et al. 2007, Wang et al. 2014). EGCG inhibits HIF-1α and VEGF, thereby preventing cell growth and proliferation of MCF-7 breast cancer cells. Another study demonstrates that EGCG restrains growth, migration, and invasion of human triple-negative breast cancer cells by blocking VEGF expression (Braicu et al. 2013).

The activity of tamoxifen has been enhanced by its combinational effect with EGCG in ER-negative breast cancer as well as may reduce the dose in ER-positive breast cancer and in breast cancer chemoprophylaxis resulting in upgradation of the safety profile. Combinational treatment of 5 mM EGCG and 200 nM tamoxifen had a synergistic effect in the inhibition of MCF-7 and AU565 breast cancer cell growth by modulating Skp2 protein, an S-phase kinase protein 2 (Skp2), a component of the Skp1-cullin 1-Fbox protein (SCF) ubiquitin ligase complex regulating the p27 proteolysis, and a key regulator of G1-to-S phase progression as reported by Huang et al. (2008). High intakers of EGCG have less chances of developing breast cancer as it was found to be less prevalent among Asian women as reported by Zhou et al. Retardation of the growth of human lung cancer cells in test tubes by EGCG has been reported (Zhou et al. 2004). There has been an experimental report deciphering the anticarcinogenic activity of EGCG to inhibit lung cancer. Leptomycin B (LMB) and EGCG given in combination augment LMB-induced cytotoxicity via scavenging ROS and regulating drug metabolism and p21/survivin pathways, thereby preventing the development of lung cancer and acting as a promising anti-lung cancer drug (Cromie and Gao 2015). EGCG inhibits angiogenesis and epithelial–mesenchymal transition (EMT) in nicotine-induced migration and invasion thus preventing lung tumor growth. EGCG inhibited proliferation of human NSCLC A549 cells by suppressing the expression of anti-apoptotic gene, Bcl-xL (Sonoda et al. 2014).

EGCG downregulates Axl and Tyro 3 expression thereby obstructing proliferation of lung cancer cells and their chemoresistant variants. Moreover, EGCG binds

with Ras-GTPase-activating protein SH3 domain-binding protein 1 (G3BP1) thereby suppressing lung tumorigenesis (Shim et al. 2010). EGCG inhibited the EGFR signaling pathway thereby exhibiting anti-lung cancer activity as reported by Ma et al. (2014). EGCG has antiproliferative effect on A549 lung cancer tumor growth and angiogenesis. It inhibits MDM2-mediated p53 ubiquitination which may be the anti-lung cancer activity of EGCG (Li et al. 2013). It has been reported to restrain transforming growth factor-b1 (TGF-b1)-mediated EMT by blocking acetylation of Smad2 and Smad3 thereby hindering lung tumorigenesis as reported by Ko et al. (2013). The invasion of highly invasive CL1-5 lung cancer cells is inhibited by EGCG by mediating MMP-2 expression via JNK signaling pathway thereby thwarting lung cancer via inducing G2/M arrest. Tea polyphenols, particularly EGCG, inhibit prostate cancer in an animal experimental model as reported by Gupta et al. (2001) and Adhami et al. (2004).

EGCG that inhibits prostate cancer cell growth by mediating apoptosis is the most effective catechin as evidenced by changes in nuclear morphology and DNA fragmentation reported by Paschka et al. (1998). EGCG induces apoptosis via inhibition of fatty acid synthase (FAS) activity thus preventing prostate cancer progression and thereby may be a potent chemopreventive and therapeutic antineoplastic agent for the prevention of prostate cancer as reported by Brusselmans et al. (2003). SENCAR mouse was pretreated with a dose of 15 mmol EGCG/mouse for 7 days prior to initiation with DMBA and promotion by 12-O-tetradecanoylphorbol-13-acetate (TPA), and twice-weekly treatment led to significant prevention against skin tumor initiation. Prophylactic treatment of EGCG to the SENCAR mouse (Katiyar et al. 1992) led to 30 % inhibition in carcinogen metabolite binding to epidermal DNA as compared with the only carcinogen treatment signifying that EGCG modulates the metabolism of procarcinogen (Chowdhury et al. 2016).

EGCG induces apoptosis in LNCaP, DU145, and PC-3 CaP cells as reported recently. Besides, it mediates regulation of G1 phase cyclin kinase inhibitors (cki) inhibiting the cyclin–CDK complexes operative in G0/G1 phase of the cell cycle leading to cell cycle arrest, irreversibly resulting in apoptosis, and finally, thereby, downregulating cell proliferation (Gupta et al. 2003). It impedes intracellular communication pathways as well as obstructs cell-to-cell contact adhesion required for cell division. Proteasome inhibition of the ester bond containing EGCG accumulates proteasome substrates p27 and IkBα leading to growth arrest by inhibition of proteasome in the G1 phase of cell cycle resulting in cancer preventive effects of tea. Another study suggests that cell proliferation is inhibited by EGCG by regulating constitutive activation of PI3K/Akt signaling pathway (Siddiqui et al. 2004) and MEK-independent ERK1/ERK2 activation (Albrecht et al. 2008) via EGCG which targets kinase CK2 since CK2 downregulation sensitizes CaP cells to EGCG-induced apoptosis as reported by Ahmad et al. thereby mediating its cellular activity (Ahmad et al. 2007).

EGCG modulates both intrinsic and extrinsic apoptotic pathways, sensitizing TRAIL-resistant LNCaP cells to undergo apoptosis. Moreover, synergistic inhibition was deciphered by the combinational treatment of cells with EGCG and TRAIL resulting in inhibition of biomarkers linked with invasion, angiogenesis, and

metastasis (Siddiqui et al. 2007). Laminin receptor is a 67 kD protein through which EGCG binds to the cell surface of cancer cells deciphering its preventive strength. Its expression is coupled with the risk of tumor invasion and metastasis and is expressed on a variety of tumor cells. eEF1A, a G protein, is upregulated by EGCG through the laminin receptor that brings aminoacyl-tRNA to the elongating ribosome, and this has been found to be very important for cancer prevention by EGCG as reported by Umeda et al. (2007). There was induction of clusterin with cleavage of both procaspase-8 and procaspase-3 upon EGCG treatment to CaP cells, but not normal cells, resulting in apoptosis of cancer cells. There was an overexpression of clusterin in the TRAMP mouse model, revealing chemopreventive properties of catechins as reported by Caporali et al. (2004).

EGCG has shown inhibiting activity on 5α-reductase in cell-free assays resulting in its capacity to regulate androgen action in target organs. Strong 5α-reductase inhibitors that actively act in both cell-free and whole cell assay systems are formed by replacement of the gallate ester in EGCG with long-chain fatty acids. EGCG attenuates AR and AR-regulated PSA, and hK2 genes in cell culture studies have also been reconfirmed in in vivo models. GTPs when given to TRAMP mice demonstrated significant suppression of IGF-1 and restoration of IGFBP-3 with marked delay in CaP progression (Adhami et al. 2004). EGCG-treated mice showed downregulation of AR and insulin-like growth factor-1, inflammation biomarkers, and regulation of MAPK signaling which contribute to the decrease in cell proliferation and induction of apoptosis leading to suppression of CaP without explicit toxicity. GTPs have been found to inhibit testosterone-mediated induction of ornithine decarboxylase leading to CaP development in both in vitro and in vivo (Gupta et al. 1999).

S100A4, an important calcium binding protein, represents a promising marker for CaP progression. S100A4 deciphers an increase in its expression at mRNA and protein level in the dorsolateral prostate of TRAMP, but not in non-transgenic, mice as reported by Saleem et al. TRAMP mice fed with GTPs resulted in marked reduction of S100A4, restoration of E-cadherin, and inhibition of CaP progression (Saleem et al. 2005). S100A4 may control the invasive potential of human CaP cells via regulation of MMP-9 and its tissue inhibitor TIMP-1 and is usually overexpressed in the progression of CaP in humans (Saleem et al. 2006). There may be many varied mechanisms and pathways which may influence its anticancer activity in vivo in animal models and humans. However anticarcinogenic properties of EGCG seem promising in vitro results. EGCG suppresses early stage CaP but not late-stage CaP in TRAMP mice as per current data. Therefore it may be suggested that the chemopreventive potential of green tea decreases with escalating tumor grade and emphasizes the requirement to discover the stage of CaP development which is most susceptible to chemopreventive intervention (Adhami et al. 2008).

Current data that are inconsistent along with few more limitations in study design interfere in an exact elucidation of the published observations. Else epidemiological studies suggest that green tea compounds could protect CaP. Existing data are incoherent, and limitations in study design thwart full interpretation of the published observations like when a report revealed that increasing frequency, duration, and quantity of green tea consumption decreases CaP risk from a case–control study

conducted in southeast China during 2001 to 2002 (Jian et al. 2004). In another study, progressive PSA elevations were evaluated after ingestion of 6 g of green tea per day to the patients without symptoms of having androgen-independent metastatic prostate carcinoma in which just one patient had a decline in serum PSA, and none of the patients showed a noticeable tumor response on radiographic assessment or physical examination.

Therefore just a maximum response rate of 2 % that was observed with green tea reveals limited antineoplastic effect (Jatoi et al. 2003). Same kind of results was obtained in patients in a clinical trial where green tea extract capsules were given at a dose of 250 mg twice daily which demonstrated minimal clinical activity against hormone refractory CaP (Choan et al. 2005). Green tea may work efficiently if given either in the early stages of the disease or in patients at high risk as a prophylactic treatment since both the abovementioned studies were conducted in end-stage disease patients where it had minimal effects. Green tea catechins were given orally for a year to a group of 32; only one man with high-grade PIN developed CaP as compared to nine out of 30 in the control group which signifies a rate of only 3 % in men developing the disease versus the expected rate of 30 % in men treated with placebo as reported by Bettuzzi et al. (2006). Conversely, there is an urgent requirement for large-scale, forthcoming, and randomized trials to test the effectiveness of green tea for the prevention and treatment of CaP (Deeba et al. 2008).

References

Adhami VM, Siddiqui IA, Ahmad N et al (2004) Oral consumption of green tea polyphenols inhibits insulin-like growth factor-I-induced signaling in an autochthonous mouse model of CaP. Cancer Res 64:8715–8722

Adhami VM, Malik A, Zaman N et al (2007) Combined inhibitory effects of green tea polyphenols and selective cyclooxygenase-2 inhibitors on the growth of human prostate cancer cells both *in vitro* and *in vivo*. Clin Cancer Res 13:1611–1619

Adhami VM, Siddiqui IA, Sarfaraz S, et al (2008) Efficacious prostate cancer chemopreventive intervention with green tea polyphenols decreases with increasing tumor grade in the autochthonous transgenic TRAMP model. Annual meeting of the American Association for Cancer Research. San Diego, p 08-AB-5532-AACR

Ahmad KA, Harris NH, Johnson AD et al (2007) Protein kinase CK2 modulates apoptosis induced by resveratrol and epigallocatechin-3-gallate in prostate cancer cells. Mol Cancer Ther 6:1006–1012

Albrecht DS, Clubbs EA, Ferruzzi M et al (2008) Epigallocatechin-3-gallate (EGCG) inhibits PC-3 prostate cancer cell proliferation via MEK-independent ERK1/2 activation. Chem Biol Interact 171:89–95

Alexander G, Li en Jao D, Mou-Tuan H et al (2011) Effects of the black tea polyphenol theaflavin-2 on apoptotic and inflammatory pathways *in vitro* and *in vivo*. Mol Nutr Food Res 55:198–208

Bettuzzi S, Brausi M, Rizzi F et al (2006) Chemoprevention of human prostate cancer by oral administration of green tea catechins in volunteers with high-grade prostate intraepithelial neoplasia, a preliminary report from a one-year proof-of-principle study. Cancer Res 66: 1234–1240

Braicu B, Gherman CD, Irimie A et al (2013) Epigallocatechin-3- Gallate (EGCG) inhibits cell proliferation and migratory behaviour of triple negative breast cancer cells. J Nanosci Nanotechnol 13:632–637

Brusselmans K, De SE, Heyns WG et al (2003) Epigallocatechin-3-gallate is a potent natural inhibitor of fatty acid synthase in intact cells and selectively induces apoptosis in prostate cancer cells. Int J Cancer 106:856–862

Caporali A, Davalli P, Astancolle S et al (2004) The chemopreventive action of catechins in the TRAMP mouse model of prostate carcinogenesis is accompanied by clusterin over-expression. Carcinogenesis 25:2217–2224

Casey SC, Amedei A, Aquilano K et al (2015) Cancer prevention and therapy through the modulation of the tumor microenvironment. Semin Cancer Biol 35:S199–S223

Cheng CW, Shieh PC, Lin YC et al (2010) Indoleamine 2,3-dioxygenase, an immunomodulatory protein, is suppressed by (−)-epigallocatechin-3-gallate via blocking of gamma-interferon-induced JAK-PKC-delta-STAT1 signaling in human oral cancer cells. J Agric Food Chem 58:887–894

Choan E, Segal R, Jonker D et al (2005) A prospective clinical trial of green tea for hormone refractory CaP, an evaluation of the complementary/alternative therapy approach. Urol Oncol 23:108–113

Chowdhury A, Sarkar J, Chakraborti T et al (2016) Protective role of epigallocatechin-3-gallate in health and disease. A perspective. Biomed Pharmacother 78:50–59

Chung SS, Vadgama JV (2015) Curcumin and epigallocatechin gallate inhibit the cancer stem cell phenotype via down-regulation of STAT3-NFkB signaling. Anticancer Res 35:39–46

Cromie MM, Gao W (2015) Epigallocatechin-3-gallate enhances the therapeutic effects of leptomycin B on human lung cancer a549 cells. Oxid Med Cell Longev 2015:217304

Deeba NS, Yewseok S, Farrukh A (2008) Dietary agents for chemoprevention of prostate cancer. Cancer Lett 265:167–176

Deng YT, Lin JK (2011) EGCG inhibits the invasion of highly invasive CL1-5 lung cancer cells through suppressing MMP-2 expression via JNK signaling and induces G2/M arrest. J Agric Food Chem 59:13318–13327

Eiman M, Adhami VM, Naghma K et al (2012) Apoptosis and autophagy induction as mechanism of cancer prevention by naturally occurring dietary agents. Curr Drug Targets 13:1831–1841

Gupta S, Ahmad N, Mohan RR et al (1999) CaP chemoprevention by green tea, *in vitro* and *in vivo* inhibition of testosterone-mediated induction of ornithine decarboxylase. Cancer Res 59: 2115–2120

Gupta S, Hastak K, Ahmad N et al (2001) Inhibition of prostate carcinogenesis in TRAMP mice by oral infusion of green tea polyphenols. Proc Natl Acad Sci U S A 98:10350–10355

Gupta S, Hussain T, Mukhtar H (2003) Molecular pathway for (−)-epigallocatechin-3-gallate-induced cell cycle arrest and apoptosis of human prostate carcinoma cells. Arch Biochem Biophys 410:177–185

Hastak K, Gupta S, Ahmad N et al (2003) Role of p53 and NF-kappaB in epigallocatechin-3-gallate-induced apoptosis of LNCaP cells. Oncogene 22:4851–4859

Huang HC, Way TD, Lin CL (2008) EGCG stabilizes p27kip1 in E2- stimulated MCF-7 cells through down-regulation of the Skp2 protein. Endocrinology 149:5972–5983

Jatoi A, Ellison N, Burch PA et al (2003) A phase II trial of green tea in the treatment of patients with androgen independent metastatic prostate carcinoma. Cancer 97:1442–1446

Jeong Y, Jung ID, Lee JS et al (2007) (−)-Epigallocatechin gallate suppresses indoleamine 2,3-dioxygenase expression in murine dendritic cells, evidences for the COX-2 and STAT1 as potential targets. Biochem Biophys Res Commun 354:1004–1009

Jian L, Xie LP, Lee AH et al (2004) Protective effect of green tea against CaP, a case-control study in southeast China. Int J Cancer 108:130–135

Jihyeung J, Gang L, Joshua D et al (2007) Inhibition of carcinogenesis by tea constituents. Semin Cancer Biol 17:395–402

Kanwar J, Taskeen M, Mohammad I et al (2012) Recent advances on tea polyphenols. Front Biosci (Elite Ed) 4:111–131

Katiyar SK, Agarwal R, Wang ZY (1992) (−)-Epigallocatechin-3-gallate in Camellia sinensis leaves from Himalayan region of Sikkim, inhibitory effects against biochemical events and tumor initiation in SENCAR mouse skin. Nutr Cancer 18:73–83

Kavanagh KT, Hafer LJ, Kim DW et al (2001) Green tea extracts decrease carcinogen-induced mammary tumor burden in rats and rate of breast cancer cell proliferation in culture. J Cell Biochem 82:387–398

Ko H, So Y, Jeon H et al (2013) TGF-b1-induced epithelial-mesenchymal transition and acetylation of Smad2 and Smad3 are negatively regulated by EGCG in human A549 lung cancer cells. Cancer Lett 335:205–213

Li X, Feng Y, Liu J, Tang X et al (2013) Epigallocatechin-3-gallate inhibits IGF-I-stimulated lung cancer angiogenesis through downregulation of HIF-1a and VEGF expression. J Nutrigenet Nutrigenomics 6:169–178

Liang YC, Lin-Shiau SY, Chen CF et al (1999) Inhibition of cyclin-dependent kinases 2 and 4 activities as well as induction of Cdk inhibitors p21 and p27 during growth arrest of human breast carcinoma cells by (−)-epigallocatechin-3- gallate. J Cell Biochem 75:1–12

Ma YC, Li C, Gao Y et al (2014) Epigallocatechin gallate inhibits the growth of human lung cancer by directly targeting the EGFR signaling pathway. Oncol Rep 31:1343–1349

Maeda-Yamamoto M, Kawahara H, Tahara N et al (1999) Effects of tea polyphenols on the invasion and matrix metalloproteinases activities of human fibrosarcoma HT1080 cells. J Agric Food Chem 47:2350–2354

Masuda M, Suzui M, Lim JT et al (2003) Epigallocatechin-3-gallate inhibits activation of HER-2/neu and downstream signaling pathways in human head and neck and breast carcinoma cells. Clin Cancer Res 9:3486–3491

Mohammad RM, Muqbil I, Lowe L et al (2015) Broad targeting of resistance to apoptosis in cancer. Semin Cancer Biol 35:S78–103

Nam S, Smith DM, Dou QP (2001) Ester bond-containing tea polyphenols potently inhibit proteasome activity *in vitro* and *in vivo*. J Biol Chem 276:13322–13330

Ogawa K, Hara T, Shimizu M et al (2012) Suppression of azoxymethane-induced colonic preneoplastic lesions in rats by 1-methyltryptophan, an inhibitor of indoleamine 2,3-dioxygenase. Cancer Sci 103:951–958

Paschka AG, Butler R, Young CY (1998) Induction of apoptosis in prostate cancer cell lines by the green tea component, mepigallocatechin-3-gallate. Cancer Lett 130:1–7

Peng G, Dixon DA, Muga SJ et al (2006) Green tea polyphenol (−)-epigallocatechin-3-gallate inhibits cyclooxygenase-2 expression in colon carcinogenesis. Mol Carcinog 45:309–319

Ramos S (2007) Effects of dietary flavonoids on apoptotic pathways related to cancer chemoprevention. J Nutr Biochem 18:427–442

Saleem M, Adhami VM, Ahmad N et al (2005) Prognostic significance of metastasis-associated protein S100A4 (Mts1) in CaP progression and chemoprevention regimens in an autochthonous mouse model. Clin Cancer Res 11:147–153

Saleem M, Kweon MH, Johnson JJ et al (2006) S100A4 accelerates tumorigenesis and invasion of human prostate cancer through the transcriptional regulation of matrix metalloproteinase 9. Proc Natl Acad Sci U S A 103:14825–14830

Shim JH, Su ZY, Chae JI et al (2010) Epigallocatechin gallate suppresses lung cancer cell growth through Ras-GTPase-activating protein SH3 domain-binding protein 1. Cancer Prev Res 3:670–679

Shimizu M, Shirakami Y, Sakai H et al (2008) EGCG inhibits activation of the insulin-like growth factor (IGF)/IGF-1 receptor axis in human hepatocellular carcinoma cells. Cancer Lett 262: 10–18

Siddiqui IA, Adhami VM, Afaq F et al (2004) Modulation of phosphatidylinositol-3-kinase/protein kinase B- and mitogen-activated protein kinase-pathways by tea polyphenols in human prostate cancer cells. J Cell Biochem 91:232–242

Siddiqui IA, Malik A, Adhami VM et al (2007) Green tea polyphenol EGCG sensitizes human prostate carcinoma LNCaP cells to TRAIL-mediated apoptosis and synergistically inhibits biomarkers associated with angiogenesis and metastasis. Oncogene. 27(14): 2055–2063

Slivova V, Zaloga G, DeMichele SJ et al (2005) Green tea polyphenols modulate secretion of urokinase plasminogen activator (uPA) and inhibit invasive behavior of breast cancer cells. Nutr Cancer 52:66–73

Sonoda JI, Ikeda R, Baba Y et al (2014) Green tea catechin, epigallocatechin- 3-gallate, attenuates the cell viability of human non-small-cell lung cancer A549 cells via reducing Bcl-xL expression. Exp Ther Med 8:59–63

Sueoka N, Suganuma M, Sueoka E et al (2001) A new function of green tea, prevention of lifestyle-related diseases. Ann N Y Acad Sci 928:274–280

Susanne MH, Piwen W, David H (2011) Chemopreventive effects of tea in prostate cancer, Green Tea vs. Black Tea. Mol Nutr Food Res 55:905–920

Tedeschi E, Suzuki H, Menegazzi M (2002) Antiinflammatory action of EGCG, the main component of green tea, through STAT-1 inhibition. Ann N Y Acad Sci 973:435–437

Thangapazham RL, Passi N, Maheshwari RK (2007) Green tea polyphenol and epigallocatechin gallate induce apoptosis and inhibit invasion in human breast cancer cells. Cancer Biol Ther 6:1938–1943

Umeda D, Yano S, Yamada K et al (2007) Green tea polyphenol epigallocatechin-3-gallate (EGCG) signaling pathway through 67-kDa laminin receptor. J Biol Chem. 283(6):3050–3058

Wang S, Chen R, Zhong Z et al (2014) Epigallocatechin-3- gallate potentiates the effect of curcumin in inducing growth inhibition and apoptosis of resistant breast cancer cells. Am J Chin Med 42:1279–1300

Wang Z, Dabrosin C, Yin X et al (2015) Broad targeting of angiogenesis for cancer prevention and therapy. Semin Cancer Biol 35:S224–S243

Yang CS, Wang X, Lu G et al (2009) Cancer prevention by tea, animal studies, molecular mechanisms and human relevance. Nat Rev Cancer 9:429–439

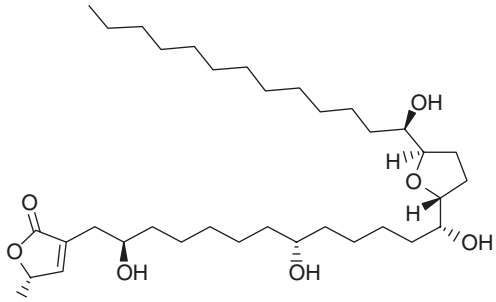
Yang H, Sun DK, Chen D et al (2010) Antitumor activity of novel fluoro-substituted (−)-epigallocatechin-3-gallate analogs. Cancer Lett 292:48–53

Zhou JR, Yu LY, Mai ZM (2004) Combined inhibition of estrogen dependent human breast carcinoma by soy and tea bioactive components in mice. Int J Cancer 108:8–14

Chemoprevention by Graviola

22

Annona muricata is a tropical evergreen tree belonging to the family Annonaceae, commonly named as soursop, custard apple, and guanabana whose leaves are identified as graviola, extensively grown and consumed around the world. It is native to the Amazon basin in South America and Southeast Asia. Graviola pulp is consumed as juice and used in making smoothies and as a flavoring agent of ice creams. Graviola has also long been used for the treatment of widespread human diseases which include bacterial and fungal infections, fever, digestive problems, inflammatory diseases (rheumatism), neuralgia, diabetes, hypertension, insomnia, cystitis, parasitic infections, and cancer in traditional medicine systems (Taylor 2002). Annonaceous acetogenins are the main bioactive compounds that are obtained from various parts of the plant. The roots, stems, leaves, and fruits of graviola are rich sources of flavonoids, isoquinoline alkaloids, and annonaceous acetogenins (de Sousa et al. 2010). These are derivatives of long-chain (C35 or C37) fatty acids extracted from polyketide pathway which are selectively toxic to cancer cells and are MDR against various cancer cell lines (Chang and Wu 2001).



Annonaceous acetogenins have been reported to stimulate cytotoxicities by blocking the mitochondrial complex I, which plays an important role in ATP synthesis, and these mitochondrial inhibitors could be of great significance in cancer

S. Rashid, *Cancer and Chemoprevention: An Overview*,
DOI 10.1007/978-981-10-2579-2_22

therapeutics since cancer cells have an elevated demand for ATP than the normal cells (McLaughlin 2008). The efficacy of *A. muricata* have been reported in few in vivo studies in which two reports demonstrate the efficacy of the leaf extract to regenerate pancreatic islet B cells in diabetic rats. Besides this, they have reported that this leaf extract could be used as a preventive agent since diabetes is known as a risk factor in pancreatic malignancy (Magruder et al. 2011; Adewole and Caxton-Martins 2006). One study was published showing antitumor efficacy of *A. muricata* recently in which the extract downregulated EGFR expression and thus had a direct anti-tumorigenic effect on breast cancer cells. The doses used in the experimental design were not properly standardized. Though this study exhibits the prospective anti-tumorigenic effect of graviola because the extract was mixed with the feed of mice, the accurate amount ingested by each mice could not be calculated correctly. It has been found to have anticancer activity on various cell lines which include Ehrlich ascites carcinoma cells (EACC), breast cancer cell lines (MD Anderson (MDA) and SKBR3 (breast adenocarcinoma cell line), and pancreatic cancer cell lines (FG/COLO357 and CD18/HPAF)) (Torres et al. 2012).

Powdered graviola fruit and leaf/stem powder have shown anticancer efficacy against breast and pancreatic cancer cells, in vitro and in vivo, respectively (Torres et al. 2012). Conversely, graviola pulp extract (GPE) was investigated for the first time for its NOX inhibitory efficacy. The potential of GPE, as graviola fruit pulp, was investigated on the basis of rationale that it is consumed by humans' for many centuries. Therefore it will be practical and straightforward for translational studies due to its beneficial effects into clinics in the future. GPE strongly inhibited NOX activity leading to downregulation of cell proliferation and clonogenic expansion in PCa cells selectively targeting cancer cells and without any cytotoxicity toward nonneoplastic prostate epithelial cells. Graviola has been reported to target multiple molecular pathways regulating metabolism, cell cycle, cell survival, and metastasis of pancreatic cancer cells (Dai et al. 2011). Treatment with graviola in pancreatic cancer cells mediated processes which include hypoxia and glycolysis involving HIF-1α, NFkB, GLUT1, GLUT4, hexokinase II, and lactate dehydrogenase-A molecules. Graviola fruit extract downregulated EGFR, suppressing the growth of human breast cancer cells both in vitro and in vivo as reported by Dai et al. (2011) (Deep et al. 2016).

The National Cancer Institute (NCI) encourages numerous initiatives like the significance of taking vegetables and fruits on a daily basis in our diet as a measure of cancer chemoprevention (Jansen et al. 2011). Phytochemicals which include fruits and vegetables have been reported to target multiple signaling pathways, molecules, and stages of cancer resulting in mitigation of overall cancer burden. Cell death has been shown to be induced by the active annonaceous acetogenins in cancer cells which are resistant even to chemotherapeutic drugs thereby modulating them (Oberlies et al. 1995). Annonaceous acetogenins have been accredited with devastating side effects which include neurotoxicity by mediating the blood–brain barrier and easily crossing it and are also identified to cause atypical Parkinson's disease in spite of their significant antiproliferative potential. As a result, it limits its usage and development as a new drug. On the other hand, plausible

chemotherapeutic potential of whole graviola extract against cancer has been reported (Chang and Wu 2001). Therapeutic potential of whole graviola in pancreatic cancer models has been studied via investigating its effect in both in vitro and in vivo on cancer cell proliferation, metabolism, and tumor growth inhibition (Stan et al. 2010).

Whole foods offer health-promoting and disease-fighting beneficial effects either by additive or synergistic interaction of constituent phytochemicals (Smith et al. 2009). The mechanism behind the whole foods like graviola must be understood by bioactivity-based complexity which must be studied urgently so that phytochemical therapeutic window is a practical approach in the treatment and prevention of various diseases.

References

Adewole SO, Caxton-Martins EA (2006) Morphological changes and hypoglycemic effects of *Annona muricata* Linn. (Annonaceae) leaf aqueous extract on pancreatic B- Cells of streptozotocin-treated diabetic rats. Afr J Biomed Res 9:173–180

Chang FR, Wu YC (2001) Novel cytotoxic annonaceous acetogenins from Annona muricata. J Nat Prod 64:925–931

Dai Y et al (2011) Selective growth inhibition of human breast cancer cells by graviola fruit extract *in vitro* and *in vivo* involving downregulation of EGFR expression. Nutr Cancer 63:795–801

de Sousa OV, Vieira GD, de Jesus RG et al (2010) Antinociceptive and anti-inflammatory activities of the ethanol extract of Annona muricata L. leaves in animal models. Int J Mol Sci 11: 2067–2078

Deep G, Kumar R, Jain AK et al (2016) Graviola inhibits hypoxia-induced NADPH oxidase activity in prostate cancer cells reducing their proliferation and clonogenicity. Sci Rep 6:23135

Jansen RJ, Robinson DP, Stolzenberg-Solomon RZ et al (2011) Fruit and vegetable consumption is inversely associated with having pancreatic cancer. Cancer Causes Control 22:1613–1625

Magruder JT, Elahi D, Andersen DK (2011) Diabetes and pancreatic cancer, chicken or egg? Pancreas 40:339–351

McLaughlin JL (2008) Paw paw and cancer, annonaceous acetogenins from discovery to commercial products. J Nat Prod 71:1311–1321

Oberlies NH, Jones JL, Corbett TH et al (1995) Tumor cell growth inhibition by several Annonaceous acetogenins in an *in vitro* disk diffusion assay. Cancer Lett 96:55–62

Smith BD, Smith GL, Hurria A et al (2009) Future of cancer incidence in the United States, burdens upon an aging, changing nation. J Clin Oncol 27:2758–2765

Stan SD, Singh SV, Brand RE (2010) Chemoprevention strategies for pancreatic cancer. Nat Rev Gastroenterol Hepatol 7:347–356

Taylor, L (2002) Herbal secrets of the rainforest. 2nd edn. Sage Press, Inc; Technical Data Report for Graviola, Annona Muricata

Torres MP, Rachagani S, Purohit V et al (2012) Graviola, a novel promising natural-derived drug that inhibits tumorigenicity and metastasis of pancreatic cancer cells *in vitro* and *in vivo* through altering cell metabolism. Cancer Lett 323:29–40

Cancer Chemoprevention: Hurdles and Future Prospects and Considerations

23

The disappointing results of chemoprevention trials in human are certainly of concern. Negative results should not discourage us from looking into the positive effects of chemoprevention. However slight advances in cancer chemotherapy catch the attention of the world, and feeble positive results are also measured considerably, unlike in cancer chemoprevention. Another factor is that expectations from chemoprevention are too high and success is barely observed. If chemoprevention is proposed to be the future therapy for cancer, various other factors are required to be calculated seriously.

Additionally, the approach of the pharmaceutical industry toward cancer chemoprevention is indeed worrying. Exemestane, an aromatase inhibitor, has been reported to reduce by 65 % the tumor risk in breast cancer chemoprevention as compared to placebo (Goss et al. 2011). The pharmaceutical industry shows lack of interest in following up on these reports despite such promising results. The National Breast Cancer Coalition (NBCC) and many support organizations consider that cancer chemoprevention is not a practical and promising approach and illustrate programs and market chemopreventive agents as not remarkable and efficient enough to be tested further (Schmidt 2011).

The lack of attentiveness of caregivers and the public's attitude toward cancer chemoprevention are other issues which could be evidenced by usually primary physicians who do not have much knowledge as well as do not pay much attention toward cancer chemoprevention. They are not aware of the strategies that the chemoprevention offers for high-risk groups resulting in few patients enrolling for chemoprevention trials which form another limiting factor for chemoprevention trails. One more factor about the population who are at high risk for developing cancer is their uncertainty regarding results of cancer chemopreventive interventions which makes them to worry about its long-term use and side effects associated with it.

Although more than two million women are suitable for tamoxifen treatment. Besides, the decreases in the number of women on tamoxifen from 2002 to 2005 were 120,000 and 60,000, respectively (Schmidt 2011). In a nutshell I must say that, like other fields of intervention, cancer chemoprevention is also dependent on

S. Rashid, *Cancer and Chemoprevention: An Overview*,
DOI 10.1007/978-981-10-2579-2_23

federal money for support. It has been witnessed over the past decade or more that the share committed to cancer prevention research has declined steadily, despite increasing the National Cancer Institute's budget largely.

Chemoprevention seems to open a new therapeutic window for the treatment of cancer. It is well evident from its preclinical and primary clinical trial results which include a few examples like selective estrogen receptor modulators for breast cancer, Cox-2 inhibitors for colon cancer, finasteride for prostate cancer, retinoic acid for head and neck cancer, and vaccine for cervical cancer which are all showing promising results (Baron et al. 2006). Cox-2 inhibitors have been found to be useful in the prevention of non-melanoma skin cancers in a recent clinical trial (Elmets et al. 2010). Patients were given 200 mg of celecoxib or placebo orally twice daily for 9 months who had actinic keratosis in which incidence of non-melanoma skin cancer was lower in the celecoxib group than in the placebo after 11 months post-randomization. Celecoxib might have been useful for the individuals who are at high risk for the development of non-melanoma skin cancers and was found to prevent squamous cell and basal cell carcinomas in individuals having widespread actinic damage which further need to be assessed in human trials.

But on the other hand, the complexities associated with the chemoprevention decrease its potentiality as a new treatment approach. Nutrient metabolism is influenced by the differences in human genomes which mediate the form. A particular cancer-fighting molecule is handled in the body which could be well understood by the example that people in whom the catechol-O-methyltransferase (COMT) gene is less efficient are less active, have less capacity to remove tea catechins, and therefore get benefited more from tea drinking which causes an increase in bioavailability (Lu et al. 2003; Wu et al. 2003). Another example is of sulforaphane metabolism which is affected by glutathione S-transferase M1 gene (GSTM1), and the quicker it happens, the less benefit we may obtain from eating broccoli (Gasper et al. 2005).

Phytonutrient metabolism in the intestines varies considerably like genomes, i.e., human microbiomes vary considerably. Phytonutrient types and contents differ significantly between individuals eating the identical food, depending on the type of intestinal flora that act upon it (Lampe 2009). Some phytonutrients may be present in small quantities in large foods, some are intricate to access being present in seasonal and expensive food items, and some have significant problems with oral bioavailability (De 2011).

Hopefully, such obstacles may be overcome by extensive research and help from the food industry. Colon cancers and its precursor lesions have elevated levels of prostaglandins, metabolic products of arachidonic acid pathway (Bennett et al. 1977). Aspirin and NSAIDs are coupled with a reduced risk of developing or dying from colon cancer via inhibition of the arachidonic acid pathway (Fournier and Gordon 2000; Rothwell et al. 2010). NSAIDs have been reported from human trials to decrease the size and number of polyps in individuals with FAP. An important enzyme involved in the metabolism of arachidonic acid is found to be upregulated in inflammatory states and in many cancers as well as premalignant lesions (Fournier and Gordon 2000). Mitogens and growth factors are found to upregulate the levels

of COX-2. COX-2-specific NSAIDs are being suggested in the prevention of colon and other malignancies in subjects having high prostaglandin levels (Fournier and Gordon 2000).

Reduced vitamin D levels are associated with risk factors for prostate cancer like advancing age and African-American ethnicity (Guyton et al. 2003). Individualized approaches can be developed for populations which show positive results from NSAIDs and vitamin D intervention along with age. Higher soy food consumption has been associated with lower breast cancer risk as reported from various epidemiological studies (Lampe 2009, 2010). The efficiency of green tea depends on the stage of the disease at which it is taken (Adhami et al. 2013; Harper et al. 2007) as well as decreases with advancing stage of the disease. Intervention of prostate cancer by green tea is quite beneficial when started early in life as data obtained from preclinical and clinical studies. Therefore one must highlight the need to devise suitable chemoprevention clinical trials taking these observations into consideration.

Future Prospects

Numerous approaches must be intended to recognize the exact patient population, and the right agent needs to be explored concurrently to make chemoprevention a practical approach for cancer control. Various signaling molecules and biomarkers have been recognized that might provide exceptional targets for chemoprevention from the results obtained from several in vitro and in vivo studies. On the basis of these findings which promote the use of synthetic agents as well as natural agents. As most of them decipher pleiotrophic effects in cancer chemoprevention. We must identify the high-risk population that will benefit from chemoprevention, and to do this, we must establish risk factors and gene signatures of the population. Now the obtained high-risk population must be tested and investigated at individual level to categorize responders from nonresponders, i.e., those who show positive effects from those who show null effects against chemopreventive agents.

Efficient prevention trials must be done since all individuals vary depending on their genetic makeup which mediates a particular response as a one-size-fits-all strategy appears improper for cancer chemoprevention. Therefore personalized therapies and interventions may be an effective strategy for prevention of various cancers. Cancer cells work by molecular multiple pathways to survive. Therefore chemopreventive agents must interfere and influence various signaling pathways to be effective as compared to targeting just one pathway. They may work efficiently either if multiple agents are investigated in combination or single agents having numerous targets need to be developed.

The concerns related to bioavailability may be defeated by nanotechnology, and simultaneously it may deliver constant levels of bioactive agents as well as reducing toxicity (Siddiqui et al. 2009). Cancer chemopreventive regimes produce unexpected and serious side effects and usually run over a long course of time. Toxicity issues caused due to long-term usage of a chemopreventive agent could be managed and counteracted by a short-term discontinuous approach (Wu and Lippman 2011).

Supplementary data in animal models may be required before designing and genetically modifying commonly used foods which contain cancer-fighting ingredients like anthocyanin-rich tomatoes, and this is an efficient way to move forward in cancer prevention. Probiotics may also be used to influence and mediate intestinal microbial flora through which redundant molecular degradation of bioactive phytonutrients could be escaped (Mukhtar 2012).

Cancer chemoprevention in case of an established cancer has been reported to be an impossible task, whereas during the process of carcinogenesis, chemoprevention is possible. The best way could be increasing the time of either onset or progression of disease, and it emerges out to be a feasible approach for cancer control and is suitable for most solid malignancies. Chemopreventive agents are being identified as target specific and less toxic. Individual genotyping must be carried out for cancer prevention which will require many more years of thorough case–control studies (Mukhtar 2012).

Cancer chemoprevention promotional campaigns which are awareness programs need to be started on the same scale as statins for cardiovascular health. At the same time, both caregivers and the public need to be educated and made aware about the benefits of cancer chemoprevention. It is high time that the pharmaceutical industry and funding agencies must recognize the benefits of this approach as per cost, efficiency, and efforts are concerned. Most importantly, we must lower our expectations and settle for reasonable effects from cancer chemopreventive trials. Cancer chemoprevention has become a promising field in less than four decades (Wu et al. 2011).

Cancer chemoprevention could be a practical approach for high-risk populations if the trails are designed carefully keeping all the limitations in mind. The efficiency of inhibition of process of cancer development could be doubled either by removing foods causing cancer and increasing consumption of foods that slow the process of carcinogenesis which would increase the onset of disease around 80–100 years of age rather than prevailing 40–50 years of age. Most solid malignancies which include breast, colon, lung, and bladder could be delayed by slowing the disease progression concept which appears to be valid (Mukhtar 2012).

References

Adhami VM et al (2013) Human cancer chemoprevention, hurdles and challenges. Top Curr Chem 329:203–220

Baron JA, Sandler RS, Bresalier RS et al (2006) A randomized trial of rofecoxib for the chemoprevention of colorectal adenomas. Gastroenterology 131:1674–1682

Bennett A, Tacca MD, Stamford IF et al (1977) Prostaglandins from tumours of human large bowel. Br J Cancer 35:881–884

De S (2011) Food, the omnivore's labyrinth. Nature 471:S22–S24

Elmets CA, Viner JL, Pentland AP et al (2010) Chemoprevention of nonmelanoma skin cancer with celecoxib, a randomized, double-blind, placebo-controlled trial. J Natl Cancer Inst 102:1835–1844

Fournier DB, Gordon GB (2000) COX-2 and colon cancer, potential targets for chemoprevention. J Cell Biochem 34:97–102

Gasper AV, Al-Janobi A, Smith JA et al (2005) Glutathione S-transferase M1 polymorphism and metabolism of sulforaphane from standard and high glucosinolate broccoli. Am J Clin Nutr 82:1283–1291

Goss PE, Ingle JN, Ales-Martinez JE et al (2011) Exemestane for breast-cancer prevention in postmenopausal women. N Engl J Med 364:2381–2391

Guyton KZ, Kensler TW, Posner GH (2003) Vitamin D and vitamin D analogs as cancer chemopreventive agents. Nutr Rev 61:227–238

Harper CE, Patel BB, Wang J et al (2007) Epigallocatechin-3-Gallate suppresses early stage, but not late stage prostate cancer in TRAMP mice, mechanisms of action. Prostate 67:1576–1589

Lampe JW (2009) Is equal the key to the efficacy of soy foods? Am J Clin Nutr 89:1664S–1667S

Lampe JW (2010) Emerging research on equol and cancer. J Nutr 140:1369S–1372S

Lu H, Meng X, Yang CS (2003) Enzymology of methylation of tea catechins and inhibition of catechol-O-methyltransferase by –.-epigallocatechin gallate. Drug Metab Dispos. The Biological Fate of Chemicals 31:572–579

Mukhtar H (2012) Chemoprevention, making it a success story for controlling human cancer. Cancer Lett 326(2):123–127

Rothwell PM, Wilson M, Elwin CE et al (2010) Long-term effect of aspirin on colorectal cancer incidence and mortality, 20-year follow-up of five randomised trials. Lancet 376:1741–1750

Schmidt C (2011) The breast cancer chemoprevention debate. J Natl Cancer Inst 103:1646–1647

Siddiqui IA, Adhami VM, Bharali DJ et al (2009) Introducing nanochemoprevention as a novel approach for cancer control, proof of principle with green tea polyphenol epigallocatechin-3-gallate. Cancer Res 69:1712–1716

Wu AH, Tseng CC, Van Den Berg D et al (2003) Tea intake, COMT genotype, and breast cancer in Asian-American women. Cancer Res 63:7526–7529

Wu X, Lippman SM (2011) An intermittent approach for cancer chemoprevention. Nat Rev Cancer 11:879–885

Wu X, Patterson S, Hawk E (2011) Chemoprevention-history and general principles. Best Pract Res Clin Gastroenterol 25:445–459

Clinical Trials of Chemoprevention

24

Since the 1980s, numerous large randomized clinical chemoprevention trials have been carried out. Breast and prostate cancer and FAP demonstrated positive results, while lung and colon cancer demonstrated negative results for the agents under investigation which taught some important lessons in the domains of trial design, selection of chemopreventive agents, and doses for future trials.

Some of the important positive trials are enlisted as:

The first trial for chemoprevention was carried out on breast cancer. Tamoxifen was given as a chemopreventive agent for five long years to 413,000 women at increased risk of breast cancer (Fisher et al. 1998) which demonstrated a 49 % decrease in invasive breast cancer and a 50 % reduction in noninvasive disease as compared to placebo by the Breast Cancer Prevention Trial (BCPT). But thromboembolic events and the risk of endometrial carcinoma got doubled. There were two more breast cancer trials as International Breast Cancer Intervention Study (IBIS)-1 which compared tamoxifen treatment against placebo and IBIS-2 which compared tamoxifen versus anastrozole. IBIS-1 (Cuzick et al. 2015) further affirmed the results obtained in BCPT. It also revealed that the toxicity decreased overall and was comparable to the patients on placebo for 10 years or tamoxifen discontinuation after 5 years. Because of mild toxicity associated with the use of tamoxifen, it led to its fewer acceptances and use among women. Another study was designed in which efficacy of tamoxifen and raloxifene was evaluated in terms of prevention and associated toxicities by the Study of Tamoxifen and Raloxifene (STAR).

Encouragingly, it was reported that the efficacy of raloxifene and tamoxifen was equivalent in reducing invasive breast cancer and it didn't increase the risk of endometrial tumors which was another important point. Further results from STAR trial (Vogel et al. 2010) revealed that raloxifene increased median follow-up from 47 to 81 months but simultaneously raloxifene deciphered less potency than tamoxifen in reducing invasive cancer along with its better and extensive safety profile and both the drugs are FDA approved for breast cancer prevention. Exemestane which is an aromatase inhibitor deciphered chemopreventive potential for women having one

S. Rashid, *Cancer and Chemoprevention: An Overview*,
DOI 10.1007/978-981-10-2579-2_24

risk factor for disease. There has been found to be 65 % relative reduction in the annual incidence of invasive disease as per recent analysis (Goss et al. 2011).

There were two large positive trials carried out in the chemoprevention of prostate cancer with cancer incidence as the end point. In this trial, finasteride, a 5α-reductase inhibitor, was given to the subjects for 7 years in 18, 882 men and compared with placebo. 86.3 % of participants had completed 7 years of treatment at the time of analysis. Finasteride consumption reduced 26 % of prostate cancer incidences ($P<0.001$), and it showed protection toward lower-grade tumors (Thompson et al. 2003). Concerns were raised by seeing an increase in the number of biopsy cases with higher-grade disease (1.8 % vs 1.1 %). Prostatectomy specimen analysis suggested that it was an artifact which arised as an effect of finasteride on prostate size affecting the sampling in biopsy specimens rather than being a factual increase (Lucia et al. 2007) which led the FDA not to approve finasteride for prostate cancer chemoprevention because of the initial findings. Another 5α-reductase inhibitor, dutasteride, reduced the risk of biopsy-proven prostate cancer by 23 % compared with placebo. This drug also showed better results in low-grade malignancies as showed by finasteride as per results of a successive randomized trial (Andriole et al. 2010). NSAIDs have shown a promising role in preventing colorectal cancer according to reports obtained from preclinical and epidemiological studies.

In spite of their potent efficacy, there have been no prospective trials to study the impact on colorectal cancer till date. Cardiovascular disease is the principal end point from prospective randomized controlled trials by analysis at secondary level which demonstrates a decrease in the development of colorectal cancer and death from malignancy. It was found that aspirin consumed daily at any dose decreased the risk of colorectal cancer by 24 % and mortality coupled to it by 35 % after a delay of 8–10 years from a long-term follow-up of participants in five trials (Flossman and Rothwell 2007).

Data from eight randomized trials demonstrated that consumption of aspirin daily at any dose reduced 21 % in all cancer deaths and the benefits were observed after 5 years (Rothwell et al. 2010). Another 43 randomized trials were done on aspirin, and it was found that it decreased cancer death by 15 % within 3 years at higher doses and after 5 years for lower doses. Therefore it could be inferred that the risk of sporadic colorectal adenomas can be reduced within a few years except on invasive cancer and cancer death where it requires 5 years to produce an effect (Rothwell et al. 2012). However it is reported that aspirin directly has effects on the cancer initiation and progression decreasing cancer death within 2–3 years after random follow-up. It also decreased the risk of cancer with distant metastasis as reported from five trials (Algra and Rothwell 2012).

During a successive follow-up, it was found that aspirin consumption lowered the developing malignancy. It is still debatable whether aspirin should be recommended on a daily basis for cancer prevention because of the negative results obtained from two large prospective trials, the Women's Health and Physicians' Health Studies (Harris et al. 2003), and the cardiovascular trials were done before checking up of cardiovascular system and surveillance were routine in their course. On the other hand, positive effects of aspirin on cancer were also obtained. But the

ratios of benefit to risk will give a clear picture which will be obtained from the results of two ongoing trials in the USA.

A randomized study was done on the individuals having hereditary colon cancer (Lynch syndrome) which significantly benefited from aspirin which resulted in a second potential screening through optimal dose to be used that will be elucidated (Burn et al. 2011). To reduce gastrointestinal toxicity symptoms, selective COX2 inhibitors were tested in clinical trials as a substitute to standard NSAIDs. Aspirin reduced 28 % of adenoma burden when given to 83 patients having FAP (Steinbach et al. 2000) which resulted in the approval of celecoxib for the treatment of FAP. The preventative effects of celecoxib against recurrent adenomas were confirmed from two consecutive trials except that there was two- to threefold rise in severe cardiovascular events (Solomon et al. 2005). Patients with FAP were given fish oil extract, eicosapentaenoic acid, deciphering comparable reduction in adenoma burden as that given celecoxib treatment and negligible toxicity (West et al. 2010), and subsequent studies are carried out on this.

Important Negative Trials

Intake of carotenoids was found to be associated with lowering cancer risk as deduced from the results of the two earliest chemoprevention trials. This study enrolled 29,133 smokers of male gender which were given α-tocopherol, β-carotene, and combination of both randomly with placebo and lung cancer occurrence as the end point. The results were frightening which depicted enhancement in cardiovascular disease and 18 % increase in the incidence of lung cancers, and overall mortality rate was enhanced by 8 % for those on β-carotene (the α-Tocopherol, β-Carotene Cancer Prevention Study Group 1994). However incidences and death rates of prostate cancer were reduced in vitamin E supplementation groups (by 32 % and 41 %, respectively).

The β-Carotene and Retinol Efficacy Trial that was randomized to β-carotene and retinyl palmitate or placebo constituted 18,314 participants who included men and women, who were currently or formerly cigarette smokers, and who had asbestos exposure at their workplace. Due to elevated lung cancer death rates of 17 % and 28 % as well as cardiovascular disease mortality, this trial was closed early (Omenn et al. 1996) which ultimately resulted in discontinuation of β-carotene for cancer prevention. Selenium and Vitamin E Cancer Prevention Trial (Lippman et al. 2009) is one of the largest prevention studies. 35, 534 men received α-tocopherol, selenium, or combination of both agents and a placebo randomly. This trial was also closed unfortunately because of initial analysis that revealed negligible positive results which was further carried in a study which reported that vitamin E supplementation increased by 17 % the risk of prostate cancer (Klein et al. 2011).

Two previous studies on the role of selenium were again analyzed which suggested the protective effect of selenium. Selenium did show beneficial effects which were limited by the lowest baseline blood selenium levels (Duffield-Lillico et al. 2002). Therefore one must elucidate that the beneficial effects and risks

associated with nutritional administration depend on preceding exposure, i.e., those groups who have sufficient or high intake may be harmed while that group who has deficiency of nutrients may be benefited which has been summarized from the prostate and lung prevention trials suggesting that chemoprevention also depends upon the dose of the experimental agent chosen.

Selenium and β-carotene are imperative in normal human physiology for the normal functioning of the body and occur naturally in diet. A U-shaped dose–response curve is formed in case of deficiency or supraphysiological doses which may be harmful in both conditions. The negative results from these large expensive trials have led many to reassess the design of clinical chemoprevention studies and to move toward smaller studies focusing on higher-risk individuals and to rely on more detailed prior preclinical mechanistic evaluation to provide information that may better guide dose selection and efficacy (Steward and Brown 2013).

References

Alagra AM, Rothwell PM (2012) Effects of regular aspirin on long-term cancer incidence and metastasis, a systematic comparison of evidence from observational studies versus randomised trials. Lancet Oncol 13:518–527

Andriole GL, Bostwick DG, Brawley OW, Rittmaster RS, REDUCE Study Group et al (2010) Effect of dutasteride on the risk of prostate cancer. N Engl J Med 362:1192–1202

Burn J, Gerdes A-M, Macrae F, CAPP2 Investigators et al (2011) Long-term effect of aspirin on cancer risk in carriers of hereditary colorectal cancer, an analysis from the CAPP2 randomised controlled trial. Lancet 378:2081–2087

Cuzick J, Sestak I, Cawthorn S et al (2015) Tamoxifen for prevention of breast cancer, extended long-term follow-up of the IBIS-I breast cancer prevention trial. Lancet Oncol 161:67

Duffield-Lillico AJ, Reid ME, Turnbull BW et al (2002) Baseline characteristics and the effect of selenium supplementation on cancer incidence in a randomized clinical trial, a summary report of the Nutritional Prevention of Cancer Trial. Cancer Epidemiol Biomarkers Prev 11:630–639

Fisher B et al (1998) Tamoxifen for prevention of breast cancer, report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. J Natl Cancer Inst 90:1371–1388

Flossman E, Rothwell PM (2007) Effect of aspirin on long-term risk of colorectal cancer, consistent evidence from randomised and observational studies. Lancet 369:1603–1613

Goss PE, Ingle JN, Alés-Martínez JE et al (2011) Exemestane for breast-cancer prevention in postmenopausal women. N Engl J Med 36425:2381–2391

Harris RE, Chelbowski RT, Jackson RD, Women's health initiative et al (2003) Breast cancer and nonsteroidal anti-inflammatory drugs, prospective results from the women's health initiative. Cancer Res 63:6096–6101

Klein EA, Thompson IM Jr, Tangen CM et al (2011) Vitamin E and the risk of prostate cancer, the Selenium and Vitamin E Cancer Prevention Trial SELECT. JAMA 306:1549–1556

Lippman SM, Klein EA, Goodman PJ et al (2009) Effect of selenium and vitamin E on risk of prostate cancer and other cancers, the Selenium and Vitamin E Cancer Prevention Trial SELECT. J Amer Med Ass 301:39–51

Lucia MS, Epstein JI, Goodman PJ et al (2007) Finasteride and high-grade prostate cancer in the Prostate Cancer Prevention Trial. J Natl Cancer Inst 99:1375–1383

Omenn GS, Goodman GE, Thornquist MD et al (1996) Effects of a combination of β carotene and vitamin A on lung cancer and cardiovascular disease. N Engl J Med 334:1150–1155

Rothwell PM, Wilson M, Elwin CE et al (2010) Long-term effect of aspirin on colorectal cancer incidence and mortality, 20-year follow-up of five randomised trials. Lancet 376:1741–1750

Rothwell PM, Price JF, Fowkes GR et al (2012) Short-term effects of daily aspirin on cancer incidence, mortality, and non-vascular death, analysis of the time course of risks and benefits in 51 randomised controlled trials. Lancet 379:1602–1612

Solomon SD, McMurray JJ, Pfeffer MA et al (2005) Cardiovascular risk associated with celecoxib in a clinical trial for colorectal adenoma prevention. N Engl J Med 352:1071–1080

Steinbach G, Lynch PM, Phillips RK et al (2000) The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. N Engl J Med 342:1946–1952

Steward WP, Brown K (2013) Cancer chemoprevention, a rapidly evolving field. Br J Cancer 109:1–7

Thompson IM, Goodman PJ, Tangen CM et al (2003) The influence of finasteride on the development of prostate cancer. N Engl J Med 349:215–224

Vogel VG, Costantino JP, Wickerham DL et al (2010) National Surgical Adjuvant Breast and Bowel Project Update of the National Surgical Adjuvant breast and Bowel Project Study of Tamoxifen and Raloxifene STAR. P-2 Trial, preventing breast cancer. Cancer Prev Res 3:696–706

West NJ, Clark SK, Phillips RKS et al (2010) Eicosapentaenoic acid reduces rectal polyp number and size in familial adenomatous polyposis. Gut 59:918–925

Future of Chemoprevention

25

Chemoprevention is a comparatively innovative field for research. Nowadays agents are progressively selected for further development depending on their mechanisms of action and not on the basis of their historical epidemiological observations but from preclinical and clinical testing results. New targets like Nrf2, NFκB, and STAT family members of transcription factors have been identified. Cyclin family of cell cycle regulators which include cyclin D1, D2, and D3 are also being targeted since they are unusually expressed in the state of preneoplasia. The strategy of short-term intermittent therapy to eliminate premalignancy (SITEP) (Wu and Lippman 2011) which is based on the hypothesis that discontinuous therapy may remove premalignant cells in the course of activating apoptosis selectively induced by synthetic lethal interactions is being studied. It is being tested in breast cancer chemoprevention depending upon the synthetic lethality between the mutated tumor suppressor genes BRCA1 or BRCA2 and PARP1 which has resulted in effectiveness in mouse models of carcinogenesis (Fong et al. 2009).

Currently individual agent chemoprevention is shifting the paradigm to the use of various chemopreventive agents in combination against a particular disease. Combination of difluoromethylornithine and sulindac was given to 375 patients, which revealed 60 % decline in recurrence rates in those having a history of resected adenoma in an important trial (Meyskens et al. 2008).

The combinational therapy of chemopreventive agents with chemotherapeutic drugs is expected that it may produce a synergistic or additive effect and may facilitate to choose a low dose of chemotherapeutics so as to reduce untoward toxicity. Much work is needed to be done on cardiovascular medicine in spite of the major success in chemoprevention. It is quite disappointing that aspirin which has deciphered excellent results against colorectal cancer has not been appropriately evaluated in prospective randomized trials. Some significant results might be obtained from the lately completed AspECT trial in Barrett's esophagus upon its safety and nontoxicity in large populations which will be an important achievement in the field of chemoprevention.

S. Rashid, *Cancer and Chemoprevention: An Overview*,
DOI 10.1007/978-981-10-2579-2_25

Some more studies and trials need to be designed appropriately of dietary-derived agents. Even after spending $30 billion every year on dietary supplements (Cohen 2012), no chemoprevention trials have yielded steady positive results till now.

The selection of individuals who are at higher risk must be included in studies besides the aim of reducing size, cost, and duration of clinical trials enabling more agents to be investigated. Besides the predictability of a patient to have premalignancy should be done by identifying various clinicopathological variables and molecular markers.

Modeling germ line and somatic markers of risk and predictive markers of agent benefit or toxicity would be one such approach which will lead to personalizing cancer prevention. Larger numbers of trials done on high-quality preclinical research will give more positive results in the future hopefully. Therefore chemoprevention will be a practical approach to reduce the risk of cancer in society (Steward and Brown 2013).

It is quite frustrating because of paucity of obvious confirmation of positive or negative results of cancer chemoprevention in clinical trials. The situation is the same as that of early days of adjuvant chemotherapy and could be considered equivalent to the issues of chemoprevention now like substantial verification of results with utmost concern for advantage/disadvantage ratios and our lack of ability to test right agents, in right doses, in the right populations. We must not forget its successes and debate about the reasons of its apprehensive failure.

Despite of negative or neutral results from preclinical testing, many clinical trials have been performed depending on the basis of excited extrapolations. Therefore many researchers don't accept and believe that cancer chemoprevention is a complete failure and are of the opinion that if chemoprevention is rightly designed, it will propose an efficient and successful alternative approach and option for the management of cancer for high-risk groups in any case (Mukhtar 2012).

Designing of clinical trial interventions is quite intricate which essentially reflects the animal modeling data from which they were obtained and seems to be a persistent problem in cancer chemoprevention drug development. Once an agent is about to reach the clinical trial stage, it will be practical to assess it in a prudently selected animal model accurately in the manner as is intended and designed to be used in humans.

There is a likelihood of obtaining positive results from this strategy and might be helpful in deciphering potent undesirable effects which is the main reason for discarding clinical trials. The strategy of disease prevention by means of chemicals is being effectively applied against cardiovascular disease, atherosclerosis, diabetes, and other diseases. However human studies have given enough instances about chemoprevention being a practical and possible option for cancer management (Hong et al. 1990; Elmets et al. 2010; Wang et al. 2014; Havrilesky et al. 2013). A decrease with tamoxifen use was apparent in the incidence of invasive and noninvasive breast cancer in women at increased risk for the development of the disease (Fisher et al. 1998).

The prevention of colorectal adenomas could be done by NSAIDs as reported (Baron et al. 2006; Bertagnolli et al. 2006). Another report of α-difluoromethylornithine

given to patients having a history of non-melanoma skin cancer previously demonstrated excellent positive results with a significant difference in new basal cell carcinomas (Bailey et al. 2010).

Low oral doses of α-difluoromethylornithine and sulindac given in combination strikingly reduced recurrent adenomatous polyps along with a few side effects as reported by Meyskens et al. (2008).

Individuals having widespread actinic damage and at high risk for development of non-melanoma skin cancers on treatment with celecoxib deciphered significant prevention of squamous cell and basal cell carcinoma as reported by Elmets et al. (2010).

Prostate cancer chemoprevention trial is another successful trial with green tea which was not carried further because of some unknown reasons (Brausi et al. 2008), although this trial was done on a small but targeted right population designed based on prior strong preclinical studies (Gupta et al. 2001; Adhami et al. 2009) which was followed up for 2 years resulting in significant prevention of the disease (Brausi et al. 2008). There was a decrease in lethal prostate cancer and reduced angiogenesis in the tumor by the dietary intake of lycopene as reported by Zu et al. (2014).

There was also considerable regression in the burden of rectal polyps on treatment with black raspberries in FAP patients as reported by Wang et al. (2014).

Havrilesky et al. conducted a systematic review and meta-analysis of 24 case–control and cohort studies to investigate the risk for ovarian cancer development in oral contraceptive pill users (Havrilesky et al. 2013) and found that there is a significant decrease in ovarian cancer incidence in ever users as compared to never users. He also observed that >50 % of women on oral contraceptive pills for 10 or more years reduced oral cancer incidences which was concluded from duration–response curve (Havrilesky et al. 2013).

There is an increased risk for cancer incidences in diabetic patients (Evans et al. 2005). However it was demonstrated that metformin lowered the risk of cancer mortality and incidence like that of cancers of the colorectum, liver, and lung substantially as compared to other treatments for diabetes from a systematic review and meta-analyses of worldwide reports (Noto et al. 2012). Aspirin might be helpful in lowering the risk of cancer principally colorectal cancer followed by gastric, breast, ovarian, prostate, and lung cancer (Kim 2014), and benefits are directly proportional to its period of use and higher doses showed good effects in earlier trials (Kim 2014), therefore providing clear evidence in favor of potential efficacy of cancer chemoprevention.

Cancer chemoprevention studies have been conducted on the basis of mechanisms of carcinogenesis in various cancer types which include tamoxifen for breast cancer, finasteride for prostate cancer, NSAIDs for colon cancer, and others. Epigenetics and its role in the process of carcinogenesis must be studied for chemoprevention. Consumption of higher levels of fruits and vegetables suggests that dietary habits (consumption of higher levels of fruits and vegetables) are constantly coupled with a decreased risk of cancer at most sites as shown in epidemiological reports which seems rational because cancer is a diverse disease and uses a plethora of pathways to survive. But the most important point is the collection of resources available for chemoprevention.

There are several concerns which at present amaze flourishing chemoprevention of cancer, quite the reverse to the situation in chemotherapy (Adhami et al. 2013; Mukhtar 2012). Some important issues are the lack of vigorous surrogate markers of efficacy which would examine and evaluate the success or failure of early clinical trials, deficiency of interest of the pharmaceutical industry, non-awareness and non-attentiveness of members of the health-care team, public's philosophical attitude, and finally baseless and speculative disbelief and doubt toward the prevention as a basic strategy among some basic scientists.

Another imperative issue is our exponentially positive expectation of preventive efficacy of a particular natural compound tested in any given trial. Detail-based study must be done on the volunteers or patients first undergoing chemoprevention trials about their regular dietary habits and exposure to potentially preventive agents in the drinking water. However large effects to be observed in trial results may be impractical even if such details are measured. But it may be sensible enough to identify slight to moderate beneficial effects. On the other hand, for high-risk individuals, chemoprevention could be an exceptionally practical approach (Adhami et al. 2014).

To summarize, an abrupt rush of explicit evidence should not be expected sustaining that the societal advantage of cancer chemoprevention will occur as other health-care interventions which include cardiovascular prevention, adjuvant chemotherapy, and antihypertensives that are accepted. But determination and careful estimation of proof of principles, measured progress to promote the benefit/risk ratio, and a greater diversity of effort toward cancer chemoprevention are expected to direct to success. It has been estimated currently that one in two males and one in three females will be diagnosed with cancer at any point in their lifetime and many will die from the disease. Therefore some sort of risk in chemoprevention needs to be accepted so that essential issues related to the success of chemoprevention are predicted and documented. Though being complicated biologically, the simplicity and overall reason of the concept of cancer chemoprevention are why it persists to be (Adhami et al. 2014).

References

Adhami VM et al (2013) Human cancer chemoprevention, hurdles and challenges. Top. Curr. Chem 329:203–220.

Adhami VM, Bailey HH, Mukhtar H (2014) Cancer chemoprevention is not a failure. Carcinogenesis 359:2154–2155

Adhami VM, Siddiqui IA, Sarfaraz S et al (2009) Effective prostate cancer chemopreventive intervention with green tea polyphenols in the TRAMP model depends on the stage of the disease. Clin Cancer Res 15:1947–1953

Bailey HH, Kim K, Verma AK et al (2010) A randomized, double-blind, placebo-controlled phase 3 skin cancer prevention study of {alpha}-difluoromethylornithine in subjects with previous history of skin cancer. Cancer Prev. Res. Phila. 3:35–47

Baron RS et al (2006) A randomized trial of rofecoxib for the chemoprevention of colorectal adenomas. Gastroenterol 131:1674–1682

Bertagnolli MM, Eagle CJ, Zauber AG, APC Study Investigators et al (2006) Celecoxib for the prevention of sporadic colorectal adenomas. N. Engl. J. Med 355:873–884

Brausi M, Rizzi F, Bettuzzi S (2008) Chemoprevention of human prostate cancer by green tea catechins, two years later. A follow-up update. Eur. Urol 54:472–473

Cohen PA (2012) Assessing supplement safety, the FDA's controversial proposal. N Engl J Med. 366:389–391

Elmets CA, Viner JL, Pentland AP et al (2010) Chemoprevention of nonmelanoma skin cancer with celecoxib, a randomized, double-blind, placebo-controlled trial. J. Natl Cancer Inst 102:1835–1844

Evans JM, Donnelly LA, Emslie-Smith AM et al (2005) Metformin and reduced risk of cancer in diabetic patients. BMJ 330:1304–1305

Fisher B et al (1998) Tamoxifen for prevention of breast cancer, report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. J. Natl Cancer Inst 90:1371–1388

Fong PC, Boss DS, Yap TA et al (2009) Inhibition of polyADP-ribose. polymerase in tumors from BRCA mutation carriers. N Engl J Med. 361:123–134

Gupta S, Hastak K, Ahmad N et al (2001) Inhibition of prostate carcinogenesis in TRAMP mice by oral infusion of green tea polyphenols. Proc. Natl Acad. Sci. USA 98:10350–10355

Havrilesky LJ, Moorman PG, Lowery WJ et al (2013) Oral contraceptive pills as primary prevention for ovarian cancer, a systematic review and meta-analysis. Obstet. Gynecol 122:139–147

Hong WK, Lippman SM, Itri LM et al (1990) Prevention of second primary tumors with isotretinoin in squamous-cell carcinoma of the head and neck. N. Engl. J. Med 323:795–801

Kim SE (2014) The benefit-risk consideration in long-term use of alternate- day, low dose aspirin, focus on colorectal cancer prevention. Ann. Gastroenterol 27:87–88

Meyskens FL Jr, McLaren CE, Pelot D et al (2008) Difluoromethylornithine plus sulindac for the prevention of sporadic colorectal adenomas, a randomized placebo-controlled, double-blind trial. Cancer Prev Res. 1:32–38

Mukhtar H (2012) Chemoprevention, making it a success story for controlling human cancer. Cancer Lett 326:123–127

Noto H, Goto A, Tsujimoto T et al (2012) Cancer risk in diabetic patients treated with metformin, a systematic review and meta-analysis. PLoS One 7:e33411

Paavonen J, Naud P, Salmerón J et al (2009) Efficacy of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): final analysis of a double-blind, randomised study in young women. Lancet. 374:301–314

Rothwell PM, Wilson M, Elwin CE et al (2010) Long-term effect of aspirin on colorectal cancer incidence and mortality, 20-year follow-up of five randomised trials. Lancet 376(9754): 1741–1750

Steward WP, Brown K (2013) Cancer chemoprevention, a rapidly evolving field. Br. J. Cancer 109:1–7

Wang LS, Burke CA, Hasson H et al (2014) A phase Ib study of the effects of black raspberries on rectal polyps in patients with familial adenomatous polyposis. Cancer Prev Res (Phila). 7:666–674

Wu X, Lippman SM (2011) An intermittent approach for cancer chemoprevention. Nat Rev Cancer. 11:879–885

Zu K, Mucci L, Rosner BA et al (2014) Dietary lycopene, angiogenesis, and prostate cancer, a prospective study in the prostate-specific antigen era. J. Natl Cancer Inst 106:djt430