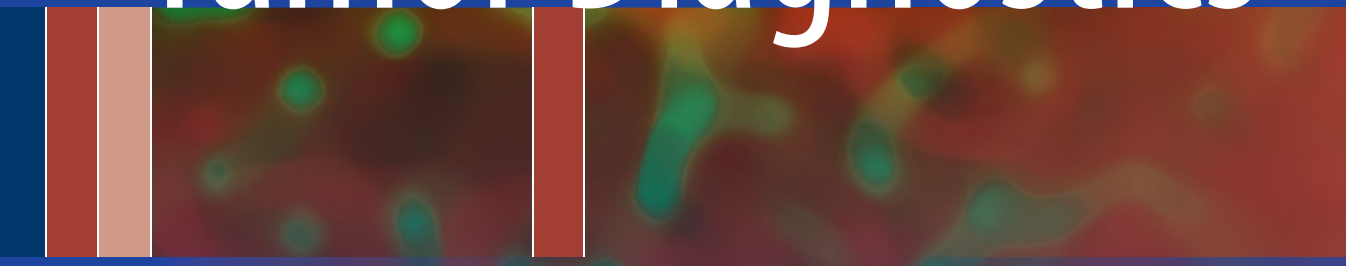
Immunohisto-chemistry in Tumor Diagnostics



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This book is dedicated to the memory of my father Sami and to the two great women in my life, my mother Haya and my wife Ayah.

Muin S.A. Tuffaha

Dedicated to my wife Maren, and daughters Maren and Silja who all are involved in human medicine.

Hans Guski

Dedicated to my wife Ilka and to our children Charlotte, Clara and Karl for their support and patience.

Glen Kristiansen

Contents

Introduction

Immunohistochemistry in Tumor Diagnostics

In recent years, classical histopathology has rapidly developed with a number of additional high sensitive diagnostic tools including immunohistochemistry, cytogenetics, and molecular pathology aside from conventional microscopy and electron microscopy. These methods provide further objective and reproducible criteria for diagnosis, classification, and follow-up of tumors.

In modern diagnostic histopathology, immunohistochemistry plays a central role as a very informative tool for tumor diagnosis and management of oncologic patients. This method has been used since the 1940s and was primarily published by Coons et al [1]. In the last 20 years, immunohistochemistry was dramatically developed into a highly specialized molecular technique combining the principles of immunology, biochemistry, and histology and became a very powerful tool in the daily diagnostic histopathology. Nowadays, we have several thousands of monoclonal and polyclonal antibodies specific of cellular and extracellular structures. Immunohistochemistry is essential to determine the histogenetic origin of tumors required for tumor classification by the detection of specific cellular antigens on tissue sections prepared from frozen tissue or formalin-fixed paraffin-embedded tissue blocks or even from cytology specimens. It is also one of the most efficient methods to detect minimal residual tumor cells in different locations such as surgical margins, lymph nodes, and bone marrow, which is very important for tumor staging and the planning of therapeutic strategies.

Immunohistochemistry is also helpful to determine the sensitivity of different tumors to several types of therapeutic agents such as steroid-receptor-antagonists, humanized monoclonal antibodies, and enzyme antagonists including tyrosine-kinase inhibitors. To merge proteomics or epitomics into a morphological context is an invaluable asset to the discerning and knowledgeable pathologist. Furthermore, immunohistochemistry offers a number of significant prognostic and etiopathological markers interesting for tumor follow-up and research. However, it must also be said that quantitative immu-

nohistochemistry is still evolving, and it is highly unlikely that cutoff-based prognostic immunohistochemistry, as it is practiced today in many research papers, will be largely contributory in future precision medicine.

Reference

1. Coons AH. The development of immunohistochemistry. Ann N Y Acad Sci. 1971;177:5–9.

Immunohistochemistry in Tumor Diagnostics

1

Contents

1.1 Expression Pattern and Diagnostic Pitfalls

The following chapters provide an overview of the most common immunohistochemical markers used for tumor diagnosis in addition to the immunoprofile of the most common tumors. The expression pattern of targeted antigens is also listed as an important factor to consider in the interpretation of the immunohistochemical stains and includes the following expression (stain) patterns:

1. Nuclear staining pattern: characteristic for antigens expressed in cellular nuclei or on the nuclear membrane. Good examples for this expression pattern are transcription factors and steroid hormone receptors.
2. Cytoplasmic staining pattern: characteristic for antigens located in the cytoplasm. Common examples are the cellular skeletal proteins such as vimentin, actin, desmin, and cytokeratins. Some antigens display a further restricted cytoplasmic staining pattern and stain-specific organelles, as, e.g., mitochondria (leading to a granular cytoplasmic staining) or the Golgi apparatus (unilateral perinuclear pattern).
3. Membrane staining pattern: characteristic for antigens located within the cell membrane, typical examples are the majority of CD antigens.

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4. Extracellular staining pattern: this pattern is characteristic for extracellular and tissue matrix antigens in addition to the cell secretion products such as collagens and CEA.

It is noteworthy to mention that some antigens have different expression patterns depending on cell cycle phase or on differentiation stage such as the immunoglobulin expression in lymphoid tissue. Other antigens have a unique expression pattern characteristic for some tumors.

Finally, it is important to remember that the interpretation of immunohistochemical results is not the description of positive or negative stains. The conventional H&E morphology of the tumor in addition to the characteristics of each antibody and the expression pattern of targeted antigens must be considered as well as the results of internal positive and negative controls, which may be present in examined tissue sections.

1.2 Immunohistochemical Pathways for the Diagnosis Primary Tumors and of Metastasis of Unknown Primary Tumors

Because of the large number of available antibodies for immunohistochemical antigen profiling of tumors, it is important to choose an initial informative screening antibody panel. For the choice of such initial diagnostic panel, the histomorphology of the examined tumor, the tumor location and clinical data, as well as the specificity and the sensitivity of the available antibodies must be considered.

For tumors with an ambiguous morphology or tumors with undetermined histogenic differentiation, we found that the most informative, time-, and money-saving primary panel consists of antibodies reacting with epithelial, mesenchymal, neural, and hematopoietic cell lines (Algorithm 1.1) [1–4].

The following panel is an example for an initial screening panel:

1. Pan-cytokeratin (cytokeratin cocktail)
2. LCA (leukocyte common antigen)
3. S100 and HMB45 (or melanoma cocktail)
4. Oct4/SALL-4
5. Vimentin

Other tissue-specific markers can be added if the morphology of the tumors favors any differentiation line.

If tumors reveal the small round blue cell morphology, another screening antibody panel is necessary and can include the following antibodies (Algorithm 1.2):

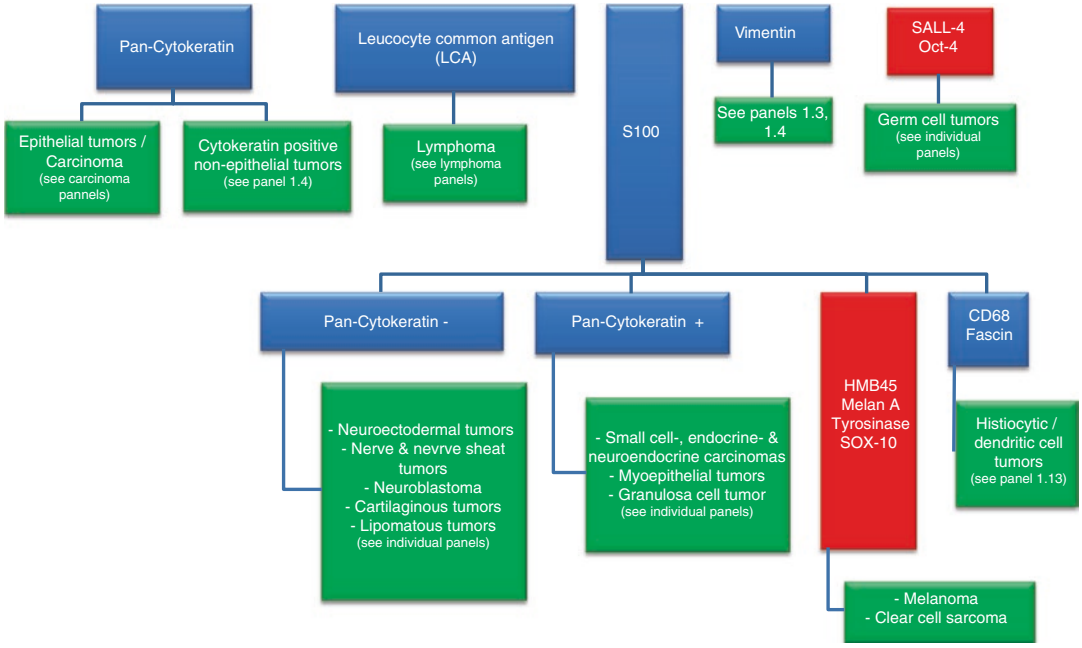
1. S100
2. Pan-cytokeratin (cytokeratin cocktail)
3. Desmin and/or myogenic transcription factors
4. LCA
5. CD99
6. CD56

This panel can be modified according to the age of the patient, tumor location, and clinical history. Adding one or more of tissue- or organ-specific markers to the initial diagnostic panel can give additional valuable diagnostic information.

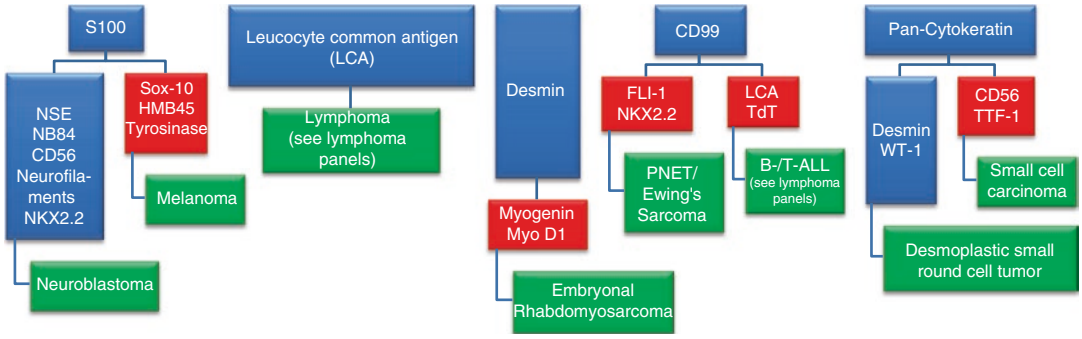
For orientation, we suggest a group of diagnostic algorithms to ease solving the most common diagnostic problems (Algorithms 1.1–1.13). According to the results obtained from the initial algorithm, a second panel with more selective antibodies can be assembled using tissue and/or tumor-specific markers for the final histopathologic diagnosis. The immunohistochemical conclusion must be made considering the histomorphology of the tumor and the expression profile of all antibodies in the used panel and always to remember that there is no antibody exclusively specific for a certain tissue type or particular tumor entity.

In the following 13 algorithms, general screening antibodies are placed in blue boxes,

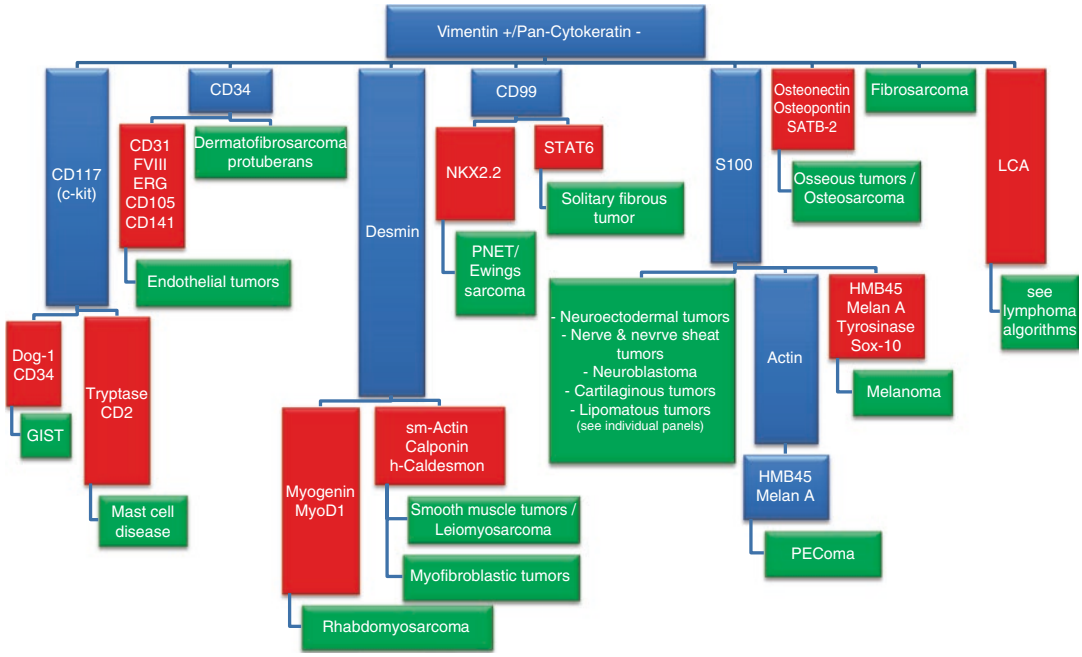
more specific antibodies in red boxes, and the most probable diagnosis in green ones. It is important to remember that the immunoprofile of tumors may be a subject of exceptions or aberrant expression of different antigens, which may cause misdiagnosis. Finally, all immunohistochemical markers have to be interpreted in the appropriate morphological context.



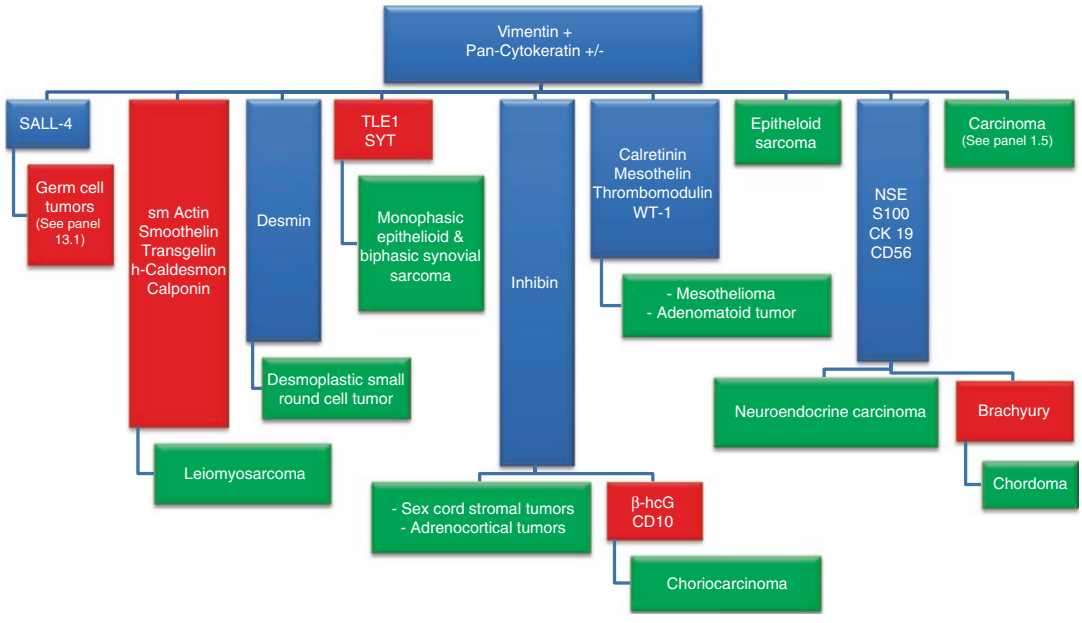
Algorithm 1.1 Primary Screening Antibody Panel



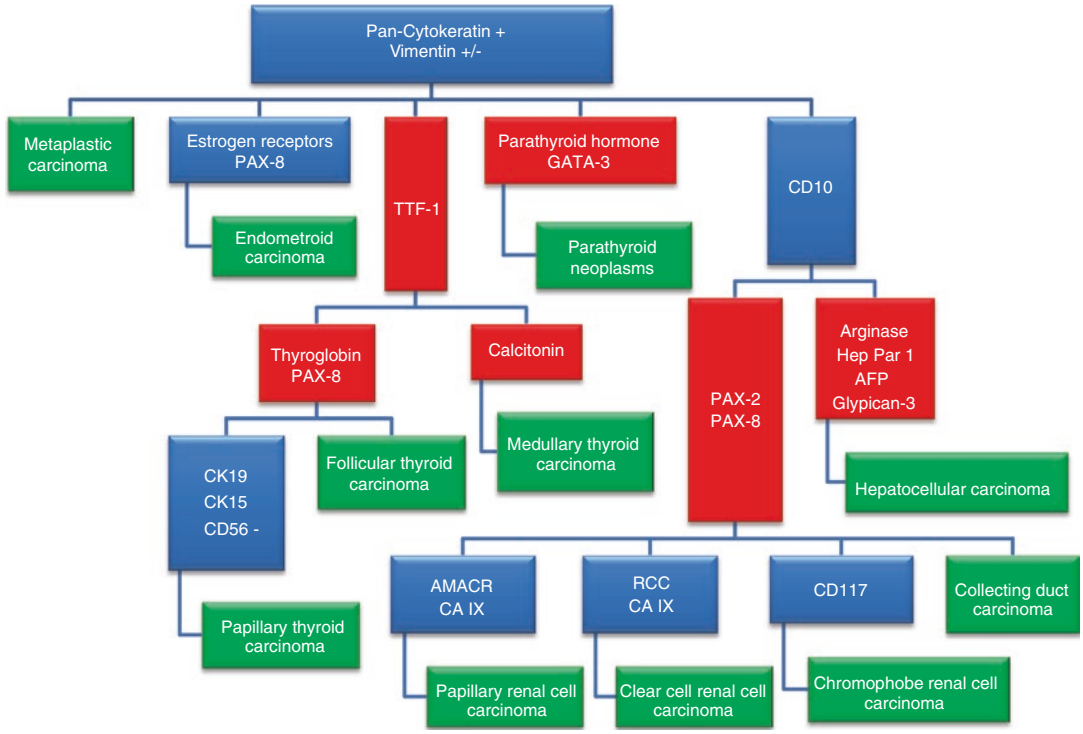
Algorithm 1.2 Antibody Panel for Tumors with Small Round Blue Cell Morphology



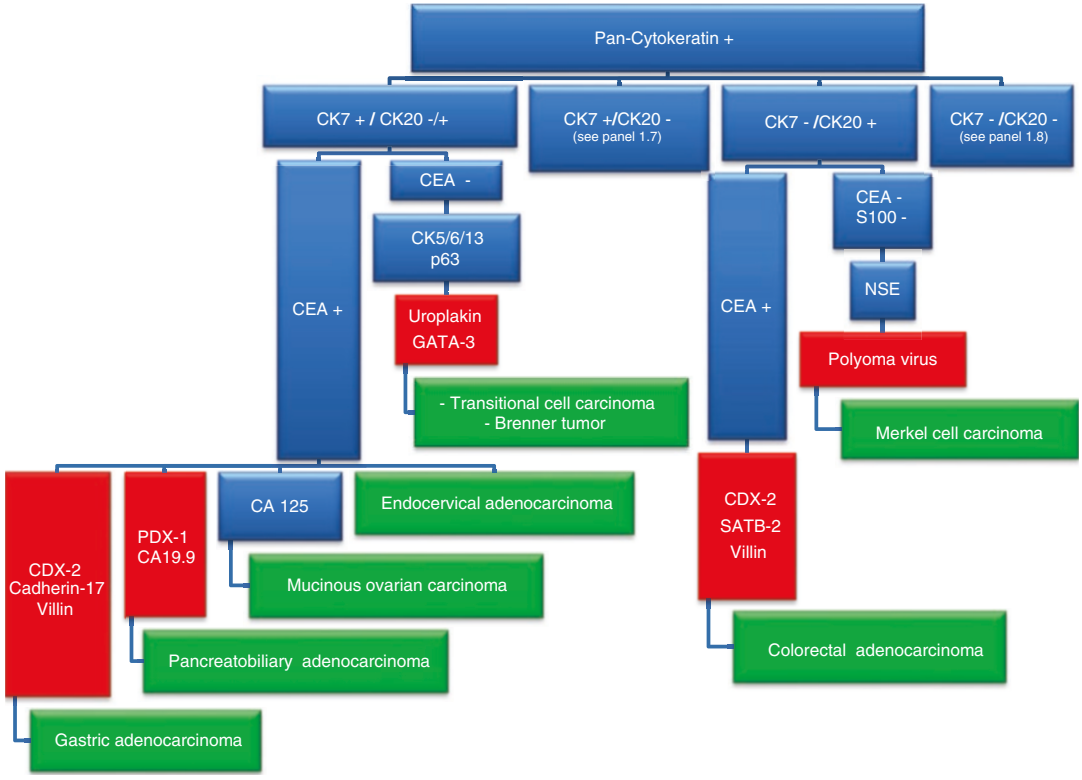
Algorithm 1.3 Cytokeratin-Negative Tumors



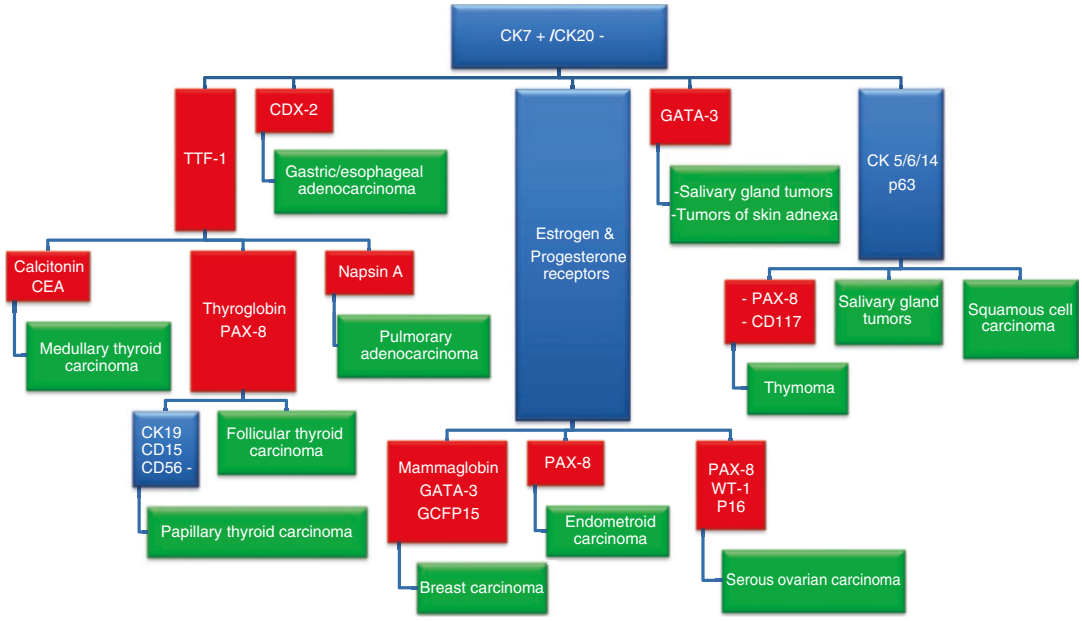
Algorithm 1.4 Tumors with Cytokeratin/Vimentin Co-expression



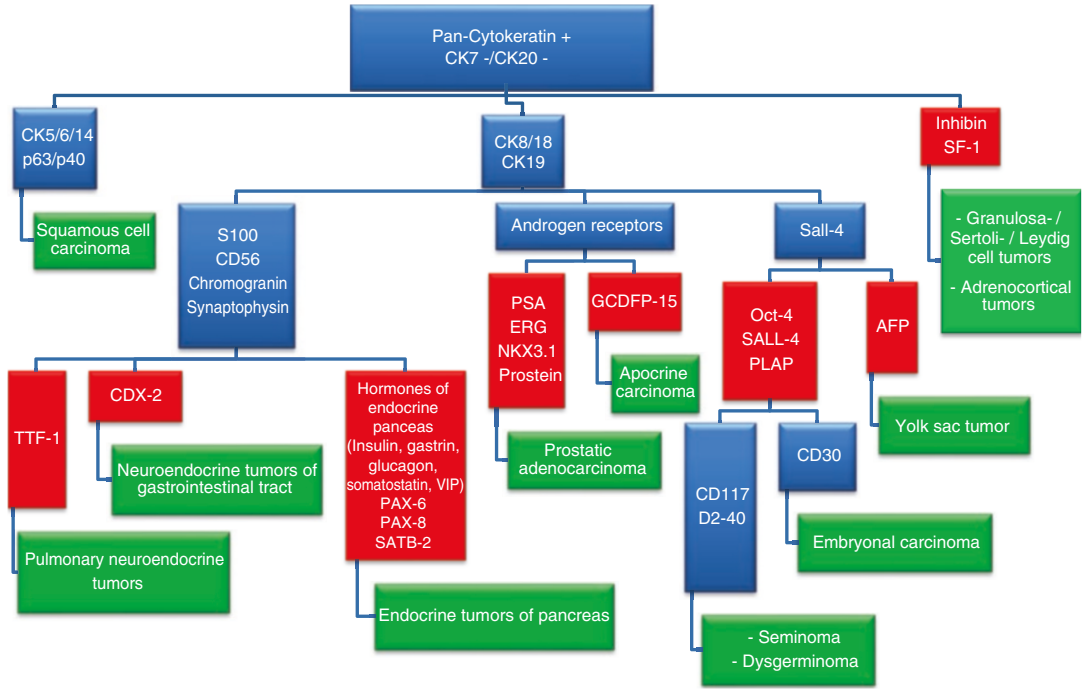
Algorithm 1.5 Carcinomas with Cytokeratin/Vimentin Co-expression



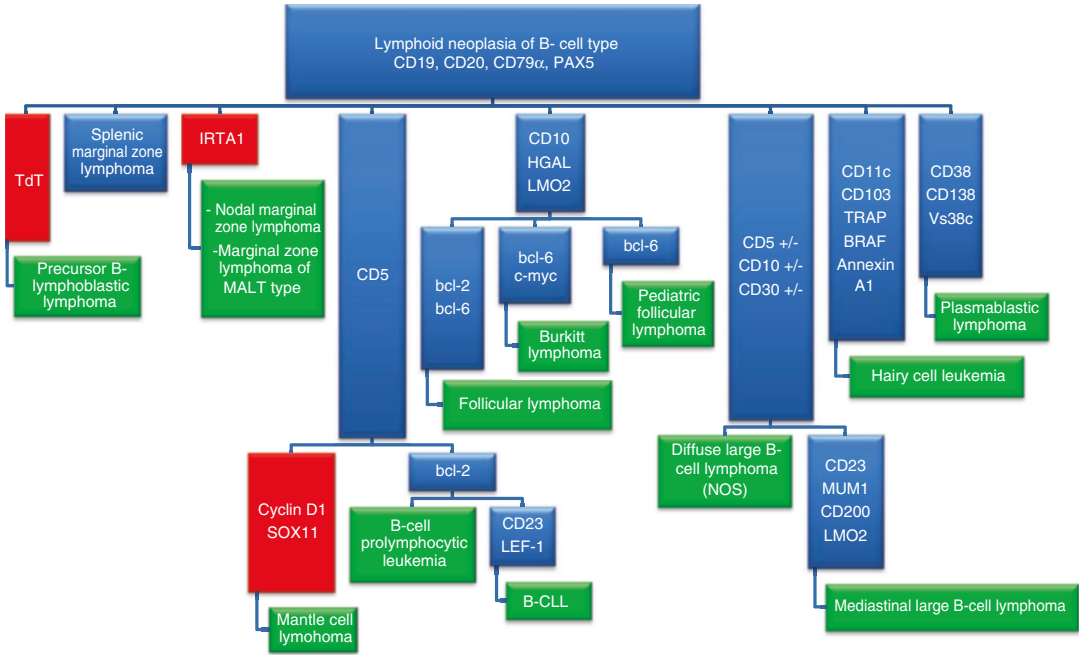
Algorithm 1.6 CK7/CK20 Expression Pattern in Carcinomas



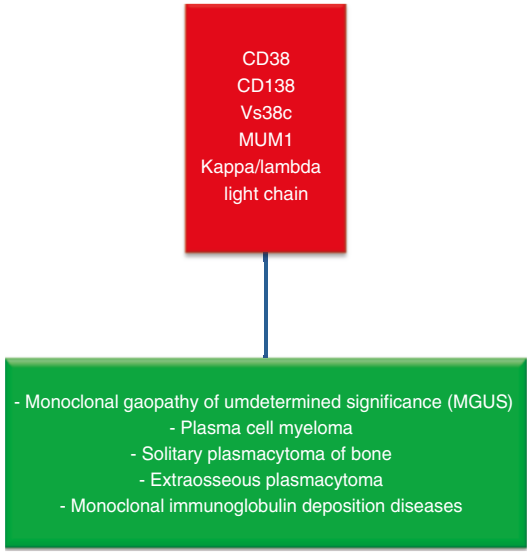
Algorithm 1.7 Cytokeratin CK7+/CK20− Carcinoma



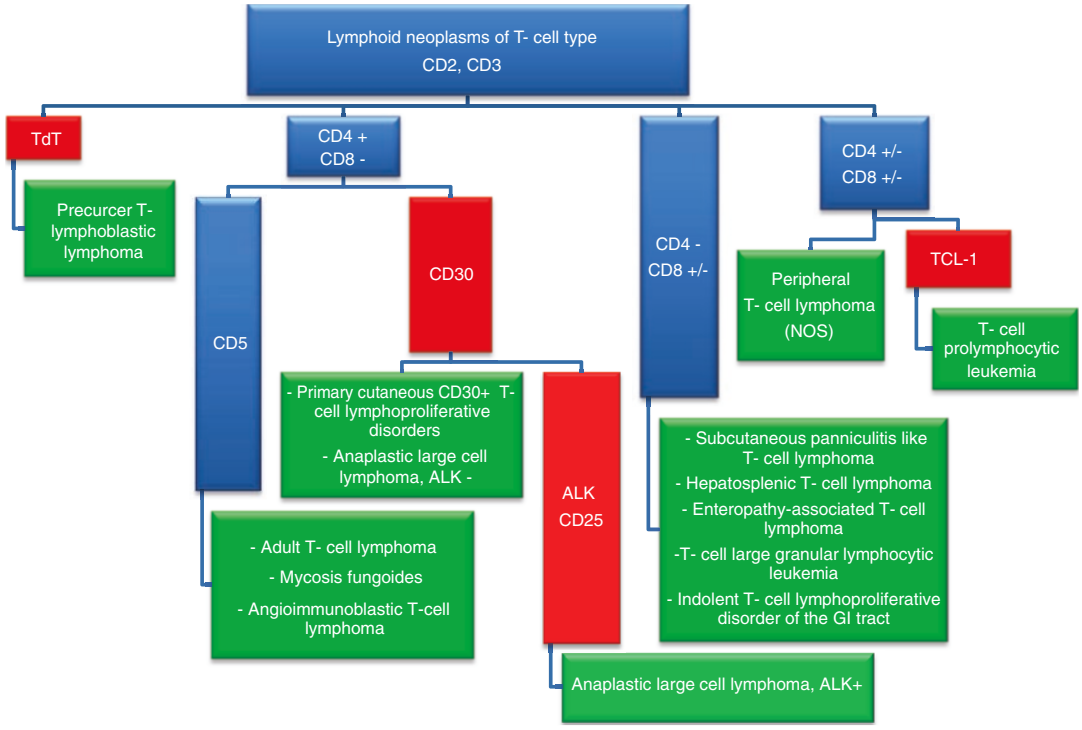
Algorithm 1.8 Cytokeratin CK 7−/CK 20− Carcinoma



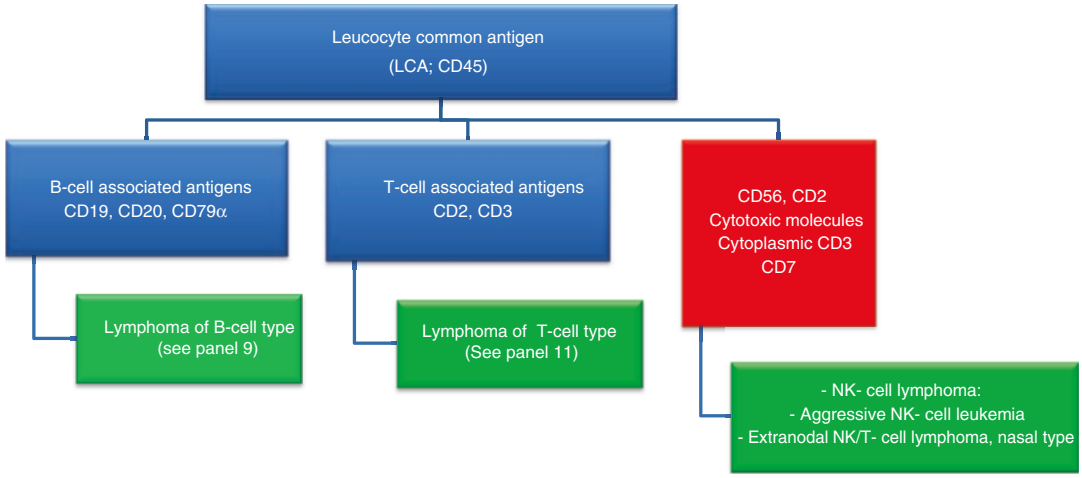
Algorithm 1.9 B-cell Neoplasms



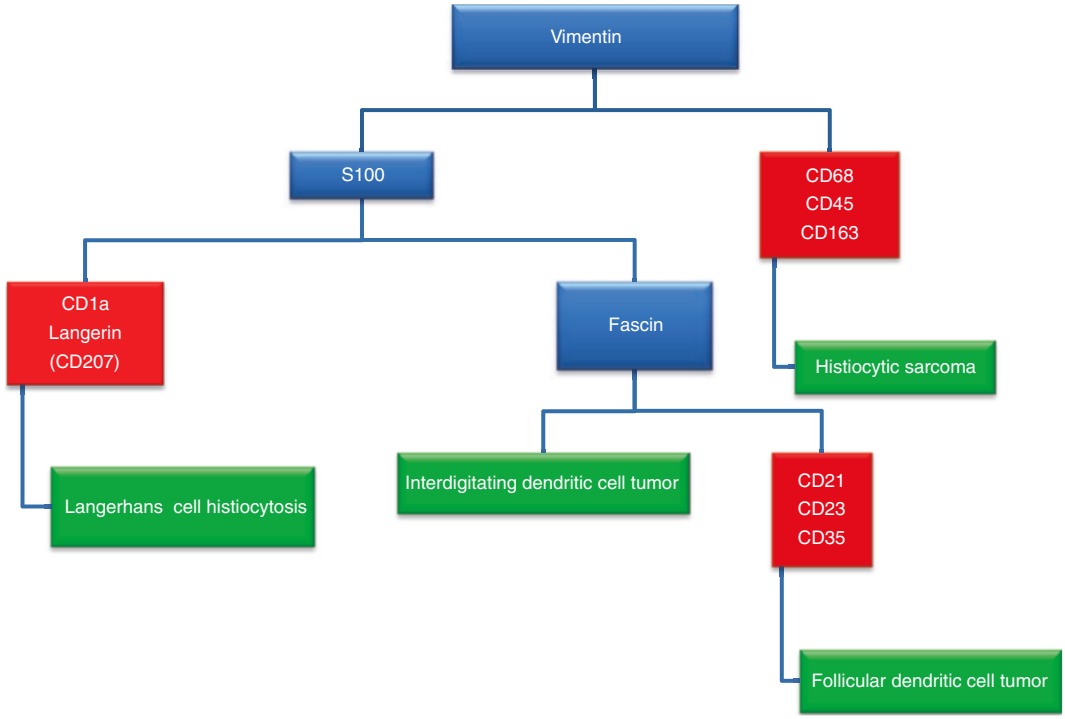
Algorithm 1.10 Plasma Cell Neoplasms



Algorithm 1.11 T-cell Neoplasms



Algorithm 1.12 T/NK-cell Neoplasms



Algorithm 1.13 Histiocytic and Dendritic Cell Tumors

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Common Immunohistochemical Markers, Diagnostic approach, Pitfalls and Immunoprofiles of Most Common Tumors

In modern immunohistochemistry, a large number of monoclonal and polyclonal antibodies directed to different cellular and extracellular antigens, covering a huge number of cell and tissue types at different stages of differentiation are used. Many of the available antibodies are highly specific to a cell type or organ, good examples are CD3, CD20, Thyroglobulin and PSA but a large number of the available antibodies have a wide expression spectrum. CD15, CD10, CD30, CD34, Desmin and S100 are typical antibodies with a multilineage expression pattern. On the other hand, there are many tumors exhibiting a bilineage or atypical expression of different antigens. This phenomenon is described in various tissue and tumor types causing serious diagnostic pitfalls in the differential diagnosis between these tumors, especially tumors with ambiguous morphology such as spindle cell tumors and tumors with epithelioid differentiation. Good examples are synovial sarcoma exhibiting the expression of CD99, CD34 and Cytokeratins, leiomyosarcoma with the aberrant expression of Cytokeratins and epithelial membrane antigen as well as epithelioid sarcoma, metaplastic carcinoma and desmoplastic small round cell tumor.

In the following chapters, the most common antigens targeted in routine immunohistochemistry are discussed according to their diagnostic value and expression profile. In the end of each chapter, the immunoprofiles of the most common tumors are listed in details. These immunoprofiles are to use as general guidelines for histopathologic tumor diagnosis and differential diagnosis.

Immunohistochemical Markers for the Diagnosis of Epithelial Tumors

2

Contents

2.1 Cytokeratins

Cytokeratins are the most important markers used for the diagnosis of epithelial neoplasms. Cytokeratins are intermediate filament proteins building an intracytoplasmic network between the nucleus and cell membrane of epithelial cells. Cytokeratins are a complex family composed of more than 20 isotypes and divided into 2 types [1, 2].

- Type I (acidic group) including cytokeratins 9–20
- Type II (basic group) including cytokeratins 1–8

Different cytokeratins are expressed in different epithelial types and at different stages of differentiation; consequently, different epithelial types have different specific cytokeratin expression profiles, which usually remains constant after neoplastic transformation [3–5].

Often cytokeratins from the acidic group are paired with their basic counterpart such as CK8 and CK18 that frequently go together. In immunohistochemical sections, cytokeratins reveal typically a diffuse cytoplasmic expression pattern; nevertheless, abnormal staining patterns such as perinuclear and dot-like expression patterns are characteristic for different neuroendocrine tumors. The following examples demonstrate this phenomenon, which is also of diagnostic value:

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1. Merkel cell carcinoma with perinuclear cytokeratin deposits (mainly cytokeratin 20)
2. Small cell carcinoma (mainly cytokeratin 19)
3. Carcinoid tumors and pancreatic endocrine tumors
4. Renal oncocytoma (with low molecular weight cytokeratins)
5. Medullary thyroid carcinoma
6. Seminoma (with low molecular weight cytokeratins)
7. Granulosa cell tumor
8. Rhabdoid tumor
9. Few mesenchymal tumors including desmoplastic small round cell tumor, leiomyosarcoma, and monophasic synovial sarcoma

The most commonly used cytokeratins in routine histopathology are listed in this chapter in addition to other frequently used epithelial markers such as epithelial membrane antigen, epithelial specific antigen, carcinoembryonic antigen, p63, p40, claudin, and different mucins.

Pan-cytokeratin and cytokeratin cocktails		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Screening for epithelial neoplasms	See diagnostic pitfalls below	Epithelial and myoepithelial cells
Positive control: appendix, tonsil		

Diagnostic Approach Before the interpretation of a pan-cytokeratin stain, it is always to consider that there is no pan-cytokeratin that reacts absolutely with all cytokeratins; nevertheless, cytokeratin cocktails are very effective in screening for epithelial differentiation or epithelial neoplasms [6]. The following cytokeratin cocktails and clones are the most commonly used markers in routine immunohistochemistry:

- *AE1/AE3* is a mixture of both AE1 and AE3, whereas AE1 reacts with type I cytokeratins and AE3 with type II cytokeratins. AE1/AE3 is a widely used as pan-cytokeratin marker but lacks the reactivity with cytokeratin 18. Few epithelial tumors are negative or weakly positive for this cocktail such as hepatocellular and renal cell carcinoma, adrenal cortical carcinoma, prostatic adenocarcinomas, and neuroendocrine tumors. Cross-reactivity of this cocktail with glial fibrillary acidic protein (GFAP) is reported and can be a source of interpretation error [7].
- *KL1* is a broad-spectrum cytokeratin clone that reacts with the cytokeratins 1/2/5/6/7/8/11/14/16/17/18, which makes it one of the best broad-spectrum epithelial markers. Similarly, the AE1/AE3 cocktail KL1 shows also cross-reactivity with GFAP.
- *MNF116* is a cytokeratin clone that reacts with the cytokeratins 5/6/8/17/19.
- *CAM 5.2* is a cytokeratin clone that reacts with the cytokeratins 8/18/19.
- *MAK-6* is a cytokeratin clone that reacts with the cytokeratins 14/15/16/18/19.
- *Cytokeratin OSCAR* is a broad-spectrum cytokeratin that reacts with the majority of epithelial cell types and carcinomas derived from these cells. Cytokeratin OSCAR reacts with the cytokeratins 7, 8, 18, and 19. Cytokeratin OSCAR does not show cross-reactivity with GFAP, but it reacts with follicular dendritic cells in lymphatic tissue.

Diagnostic Pitfalls Different cytokeratins are also expressed in various non-epithelial tissue types and neoplasms or in tumors with features of epithelial differentiation. The following list represents the most popular examples:

- Mesothelial cells and mesothelioma
- Smooth muscle and smooth muscle tumors
- Meningioma and chordoma
- Epithelioid sarcomas
- Synovial sarcoma
- Desmoplastic small round cell tumor
- Angiosarcoma
- A small subset of alveolar rhabdomyosarcoma
- Clear cell sarcoma
- Subset of germ cell tumors
- Nerve sheath tumors
- Rhabdoid tumor
- Malignant melanoma
- Undifferentiated pleomorphic sarcoma
- Proliferating myofibroblasts
- Anaplastic and diffuse large cell lymphomas [8]
- Plasma cell neoplasms

The aberrant expression of cytokeratin in mesenchymal tumors is usually patchy and may show dot-like expression pattern. The diagnosis of carcinoma based only on a positive pan-cytokeratin reaction is one of the sources of serious mistakes in tumor diagnosis. For appropriate diagnosis, it is always advisable to determine the cytokeratin profile of the tumor and then to search for other tissue-specific markers. Ectopic benign epithelial structures in lymph nodes such as heterotopic ducts and glands in cervical, thoracic, and abdominal lymph nodes in addition to Müllerian epithelial inclusions and endometriosis in pelvic lymph nodes must be kept in mind in screening lymph nodes for metastatic carcinoma or disseminated tumor cells (Fig. 2.1).

Cytokeratin 5		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Squamous cell carcinoma, mesothelioma, myoepithelial tumors	Myoepithelial cells in prostatic and mammary glands, basal-like phenotype breast carcinoma, adrenocortical tumors	Squamous epithelium, basal-type epithelial cells, myoepithelial cells, transitional epithelium, mesothelial cells, cornea
Positive control: tonsil		

Diagnostic Approach Cytokeratin 5 is a type II cytokeratin and a main component of the cytoskeleton of basal cells of stratified epithelium. Cytokeratins 5, 6, and 14 are related cytokeratins expressed in stratified squamous epithelium, myoepithelium, and mesothelium. This expression spectrum makes these cytokeratins valuable markers for the diagnosis of squamous cell carcinoma. They also clearly label normal myoepithelial cells, myoepithelial cell components in some tumors such as salivary gland tumors and myoepithelial tumors. Highlighting the myoepithelial cells using this group of cytokeratins is essential for the interpretation of prostatic biopsies, as basal cells are absent in neoplastic prostatic glands. An identical approach is also important to distinguish between simple hyperplasia, atypical ductal hyperplasia, and ductal carcinoma in situ (DCIS) in breast biopsies highlighting the myoepithelial and luminal cells with the cytokeratins 5/6/14 and 8/18, respectively. Cytokeratins 5/6/14 are highly expressed in mesothelial cells and are not suitable for discriminating between squamous cell carcinoma and mesothelioma in pleural or peritoneal biopsies or cytology (Fig. 2.2). This group of cytokeratins is usually absent in gastrointestinal adenocarcinomas, germ cell tumors, prostatic carcinoma, thyroid tumors, and hepatocellular and renal cell carcinomas.

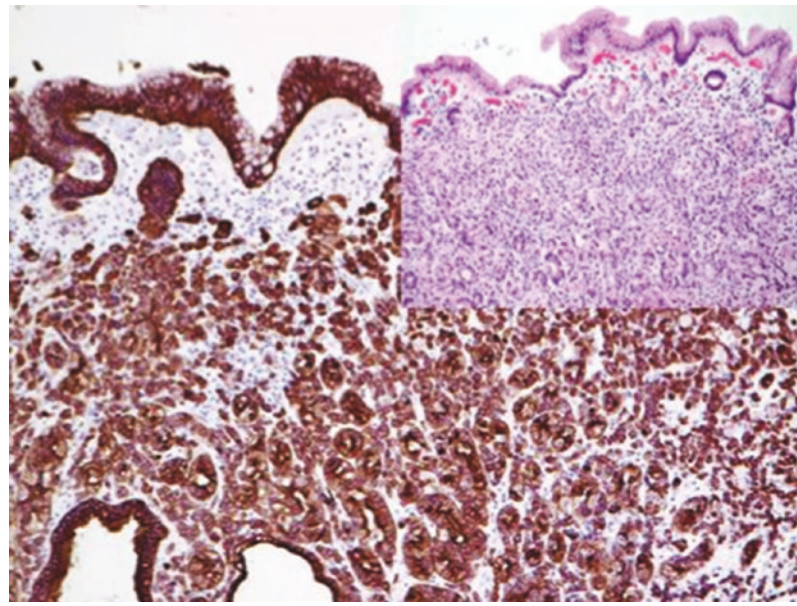

Fig. 2.1 Pan-cytokeratin (CK MNF116) highlighting the neoplastic cells in diffuse gastric adenocarcinoma

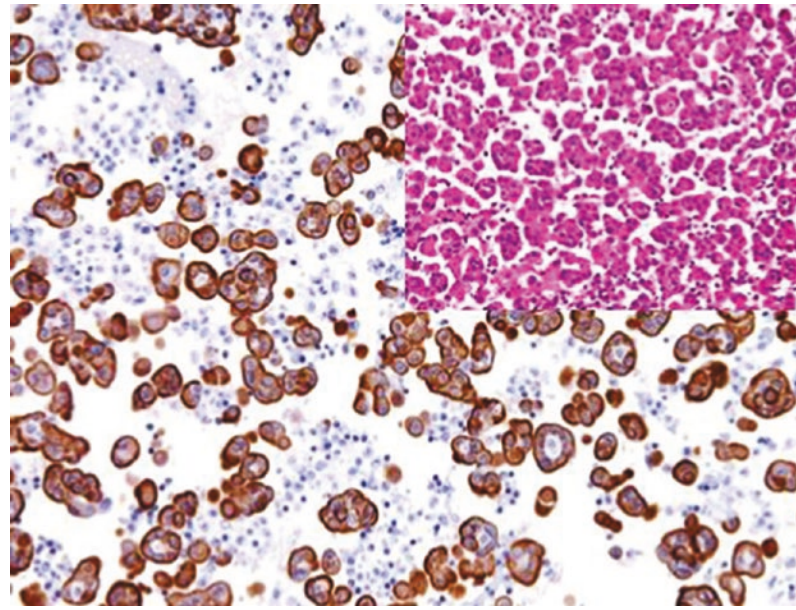

Fig. 2.2 Mesothelioma cells labeled by cytokeratin 5 in pleural effusion

Recently, CK5/14 is frequently replaced by p63 and p40 that highlights the nuclei of myoepithelial and basal cells of the glands as well as the basal and intermediate cells of squamous epithelium and urothelium [1]. Both markers are discussed below.

Cytokeratin 6		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Squamous cell carcinoma	Poorly differentiated breast carcinoma (basal-like phenotype breast carcinoma)	Suprabasal cells, hair shaft, nail
Positive control: Tonsil		

Diagnostic Approach Cytokeratin 6 is a type I cytokeratin with the same tissue distribution as cytokeratin 5 and is usually used in routine immunohistochemistry as cocktail with cytokeratin 5.

Cytokeratin 7		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Adenocarcinomas of the lung, salivary glands, upper gastrointestinal tract, pancreas, biliary tract, breast, endometrium, transitional cell carcinoma, ovarian serous tumors	Thyroid carcinoma, papillary and chromophobe renal cell carcinoma, mesothelioma, synovial sarcoma, Merkel cell carcinoma	Epithelium of the upper gastrointestinal tract, salivary glands, biliary tract, pancreas, lung, female genital tract, renal collecting ducts, transitional epithelium, mesothelial cells, thyroid follicle cells, endothelia
Positive control: appendix		

Diagnostic Approach Cytokeratin 7 is a type II cytokeratin expressed in the majority of ductal and glandular epithelium in addition to transitional epithelium of the urinary tract. Cytokeratin 7 is one of the main markers for the diagnosis of adenocarcinoma of different origin; hence, it cannot be used alone to differentiate between primary and metastatic adenocarcinoma. An important diagnostic criterion is the co-expression of cytokeratin 7 and cytokeratin 20 (see diagnostic algorithms 1.6, 1.7, and 1.8) [2]. Cytokeratin 7 is strongly expressed by mesothelial cells and not suitable for discriminating between adenocarcinoma and mesothelioma.

Diagnostic Pitfalls In the differential diagnosis between adenocarcinoma and squamous cell carcinoma, it is important to keep in mind that a minor component of cytokeratin 7-positive cells can be found in squamous cell carcinoma of different locations including carcinoma of the head and neck, lung, esophagus, and uterine cervix, mainly in poorly differentiated carcinoma. Cytokeratin 7 can also be expressed in non-epithelial tumors such as the epithelioid component of synovial sarcoma. Cytokeratin 7 is usually absent in seminoma and yolk sac tumors, epidermal squamous cell carcinoma, prostatic carcinoma, and pituitary tumors.

Cytokeratin 8 (tissue polypeptide antigen, TPA)		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Adenocarcinoma of the lung, GIT, pancreas, biliary tract, breast, endometrium and transitional cell carcinoma, hepatocellular carcinoma, renal cell carcinoma, prostatic carcinoma, neuroendocrine carcinoma	Ameloblastoma, leiomyosarcoma, malignant rhabdoid tumor	Epithelium of the gastrointestinal tract, salivary glands, biliary tract, pancreas, lung, female genital tract, hepatocytes, proximal renal tubules, transitional epithelium, mesothelial cells, smooth muscle cells, myofibroblasts, arachnoid cells
Positive control: appendix		

Diagnostic Approach Cytokeratin 8 is a type II cytokeratin usually building heterodimer with cytokeratin 18. Both cytokeratins 8 and 18 are intermediate filament proteins expressed in the early embryonal stages and persist in adult simple epithelium. Cytokeratin 8 is usually positive in non-squamous carcinomas and accordingly cannot be used to discriminate between adenocarcinoma types. Cytokeratin 8b stains also few mesenchymal tumors such smooth muscle tumors and malignant rhabdoid tumor.

Diagnostic Pitfalls Cytokeratin 8 reacts with several non-epithelial tissues and tumors such as smooth muscle cells and leiomyosarcoma.

Cytokeratin 10		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Squamous cell carcinoma	Breast ductal carcinoma	Keratinizing epithelium (suprabasal cells)
Positive control: Tonsil		

Diagnostic Approach Cytokeratin 10 is type I cytokeratin and intermediate filament usually associated with cytokeratin 1. Cytokeratin 10 is expressed in keratinizing and nonkeratinizing squamous epithelium. In routine immunohistochemistry, cytokeratin 10 is used in a cocktail with cytokeratins 13 and 14 as marker for squamous cell carcinoma.

Cytokeratin 13		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Squamous cell carcinoma		Mature nonkeratinizing squamous epithelium, basal and intermediate cells of transitional epithelium
Positive control: Tonsil		

Diagnostic Approach Cytokeratin 13 is a type I Cytokeratin expressed in suprabasal and intermediate layers of stratified epithelium. Cytokeratin 13 is usually used in cocktails with Cytokeratin 10 or Cytokeratin 14 as marker for squamous cell carcinoma.

Cytokeratin 14		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Squamous cell carcinoma, basal cell carcinoma, Hürthle cell tumors	Myoepithelial cells in prostatic carcinoma, basal-like phenotype breast carcinoma	Keratinizing and nonkeratinizing squamous epithelium, hair shaft cells, basal and myoepithelial cells in salivary glands, breast, prostate and uterus, Hürthle thyroid cells
Positive control: tonsil		

Diagnostic Approach Cytokeratin 14 is a type I cytokeratin usually building heterodimer with

cytokeratin 5. Cytokeratin 14 is a good marker for the diagnosis of squamous cell carcinoma (see cytokeratin 5). In combination with cytokeratin 5, it is an excellent marker to stain the myoepithelial cells in breast and prostatic biopsies. The frequently used cytokeratin 34βE12 to stain myoepithelial cells reacts with the cytokeratins 1, 5, 10, and 14.

Cytokeratin 18		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Adenocarcinoma of the lung, gastrointestinal tract, pancreas, biliary tract, breast, endometrium, transitional cell carcinoma, hepatocellular carcinoma, renal cell carcinoma, neuroendocrine carcinoma	Leiomyosarcoma, chordoma	Epithelium of the salivary glands, gastrointestinal and biliary tract, pancreas, lung, female genital tract, hepatocytes, proximal renal tubules, transitional epithelium, mesothelial cells, smooth muscle cells, myofibroblasts, endothelial cells, arachnoid cells
Positive control: appendix		

Diagnostic Approach Cytokeratin 18 is a type I cytokeratin, an intermediate filament expressed in simple epithelial cells and found in the majority of non-squamous carcinomas including adenocarcinoma of unknown origin and neuroendocrine carcinoma in addition to hepatocellular and renal cell carcinoma.

Diagnostic Pitfalls It is important to consider that endothelial cells of lymphatic and small venous vessels are positive for cytokeratin 18—which can also be a component of different cytokeratin cocktails—that might mimic the intravascular tumor spread. Cytokeratin 18 is also expressed in smooth muscle cells and smooth muscle tumors.

Cytokeratin 19		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Adenocarcinoma of the lung, gastrointestinal tract, pancreas and biliary tract, breast, endometrium; transitional cell carcinoma	Neuroendocrine tumors, papillary thyroid carcinoma, mesothelioma	Epithelium of the gastrointestinal tract, salivary glands, biliary tract, pancreas, lung, female genital tract, transitional epithelium, mesothelial cells, thyroid follicle cells, basal squamous epithelium
Positive control: appendix		

Diagnostic Approach Cytokeratin 19 is a type I cytokeratin and the smallest human cytokeratin found in both simple and complex epithelium. It is positive in the majority of carcinomas and has a limited use in differentiating between carcinoma types. Cytokeratin 19 strongly labels papillary thyroid carcinoma and can be used in combination with other markers such as CD56 and p63 to differentiate between papillary and follicular thyroid carcinomas, as the latter is usually negative or very weak positive for cytokeratin 19 (see related chapter) [9].

Cytokeratin 20		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Adenocarcinoma of the gastrointestinal tract, pancreas, and extrahepatic bile duct system, mucinous ovarian tumors	Merkel cell carcinoma, mucinous pulmonary adenocarcinoma, hepatocellular carcinoma, transitional cell carcinoma	Gastric and colorectal epithelium, umbrella cells of transitional epithelium
Positive control: appendix		

Diagnostic Approach Cytokeratin 20 is a type I cytokeratin, an intermediate filament and the

main protein of mature enterocytes and goblet cells in gastrointestinal mucosa (Fig. 2.3). Cytokeratin 20 is constantly expressed by colorectal adenocarcinomas, mucinous ovarian carcinoma, and less frequently transitional cell carcinoma (Fig. 2.4). Also characteristic, is the dot-like perinuclear staining pattern in Merkel cell carcinoma (Fig. 2.3). Cytokeratin 20 is a useful marker to discriminate between reactive atypia and dysplasia of transitional epithelium of the urinary tract. In normal and reactive transitional epithelium, the expression of cytokeratin 20 is restricted to the umbrella cells, whereas carcinoma in situ shows a transepithelial expression

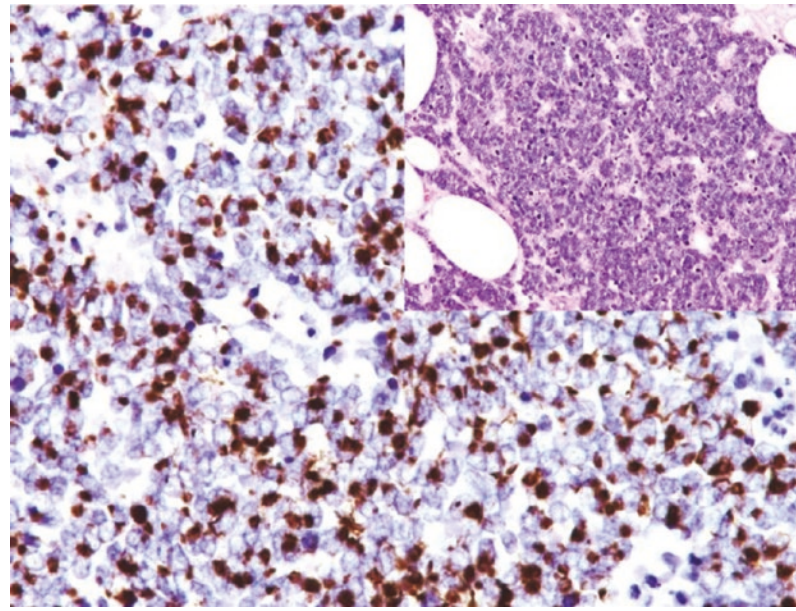

Fig. 2.3 Characteristic dot-like perinuclear expression of CK20 in Merkel cell carcinoma

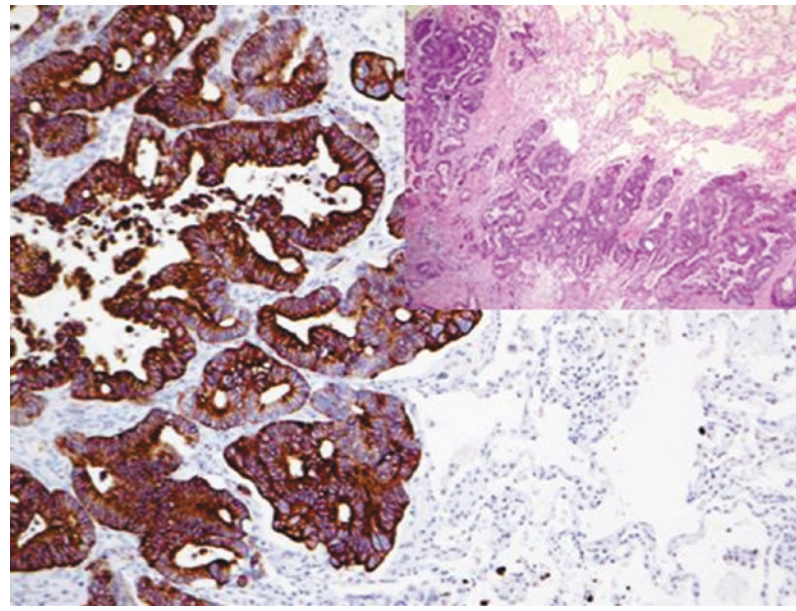

Fig. 2.4 Metastatic colorectal adenocarcinoma with strong CK20 expression

of cytokeratin 20. Cytokeratin 20 is consistently negative in squamous cell, breast, prostatic, and thyroid carcinomas and in endometrial adenocarcinoma and mesothelioma. As the expression of cytokeratin 20 is restricted to a limited number of carcinomas, it is a helpful marker to differentiate between different carcinoma types. The co-expression with cytokeratin 7 is also an important diagnostic criterion for the differential diagnosis between different carcinoma types (see diagnostic algorithms 6–8) [2].

2.2 Mucins

Mucins are a family of high molecular hyperglycosylated proteins (mucoproteins), mainly synthesized by epithelial cells, composed of 75% carbohydrates and 25% amino acids able to form gel-like substances [10]. Mucins function as lubricants or form chemical barriers that protect the surface of epithelial cells in addition to their role in cell signaling processes. Some mucins are also an important component of glandular secretion products such as saliva. In humans, more than 15 mucins are identified and divided into two main groups and encoded by different genes. The first group includes the gel-forming and secreted mucins such as MUC-2, MUC-5 AC, MUC-5B, and MUC-6. The second group comprises of the membrane-bound mucins such as MUC-1, MUC-3A, MUC-3B, MUC-4, MUC-12, MUC-13, and MUC-17. In routine histopathology, the combination of PAS and alcian blue is a very useful pan-mucin stain. The expression pattern of mucins is characteristic for some tumors and tissue types and can be useful for the classification of tumors derived from these cell types, and many specific antibodies are now available for characterization of mucins. Below, the most important mucins used in routine immunohistochemistry are listed.

Epithelial membrane antigen (Mucin-1, CD227, Ca15.3, episialin)		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Adenocarcinoma of different origin, anaplastic large cell lymphoma, lymphocyte-predominant Hodgkin's lymphoma	Epithelioid sarcoma, epithelioid meningioma, choroid plexus tumors, ependymoma, chordoma and parachordoma, plasmacytoma	Apical surface of glandular and ductal epithelial cells, activated T cells, plasma cells, monocytes, follicular dendritic cells
Positive control: Appendix, tonsil		

Diagnostic Approach Epithelial membrane antigen (EMA) also known as MUC-1 is a transmembrane glycoprotein composed of cytoplasmic and extracellular domains. EMA is also one of the major components of the mucosal layer protecting gastric mucosa. EMA is highly expressed in different types of epithelial cells mainly glandular epithelium and neoplasms originating from these epithelial types, whereas very low expression level is found in squamous and transitional cell carcinomas. EMA is also frequently expressed in the L&H cells of nodular lymphocyte-predominant Hodgkin's lymphoma, making the EMA positivity a helpful criterion for the diagnosis since L&H cells in this Hodgkin's lymphoma type are CD30, CD15, and fascin negative. EMA is constantly negative in basal cell carcinoma, adrenocortical tumors, melanoma, hepatocellular carcinoma, and germ cell tumors, i.e., seminoma, embryonal carcinoma, and yolk sac tumor.

Diagnostic Pitfalls EMA is not a specific epithelial marker and is widely expressed in other non-epithelial tumor and cell types such as anaplastic large cell lymphoma [11], plasma cell neoplasms, meningioma, epithelioid mesothelioma, perineuroma, and synovial, epithelioid, and neurogenic sarcomas (Figs. 2.5 and 2.6). Since EMA is highly glycosylated and some antibodies detect carbohydrate domains, the stain results may show marked differences using different antibodies. Overexpression of EMA in carcinomas has been associated with worse prognosis.

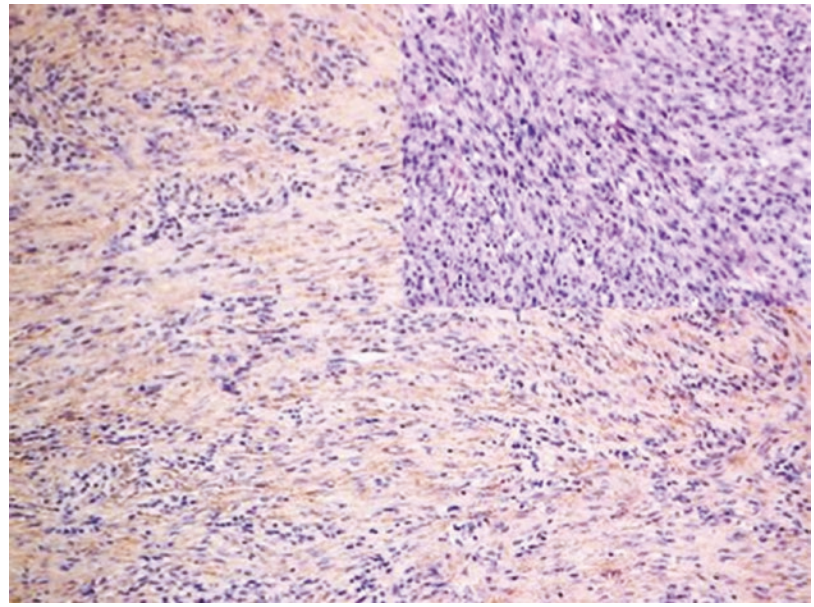

Fig. 2.5 EMA expression in atypical meningioma

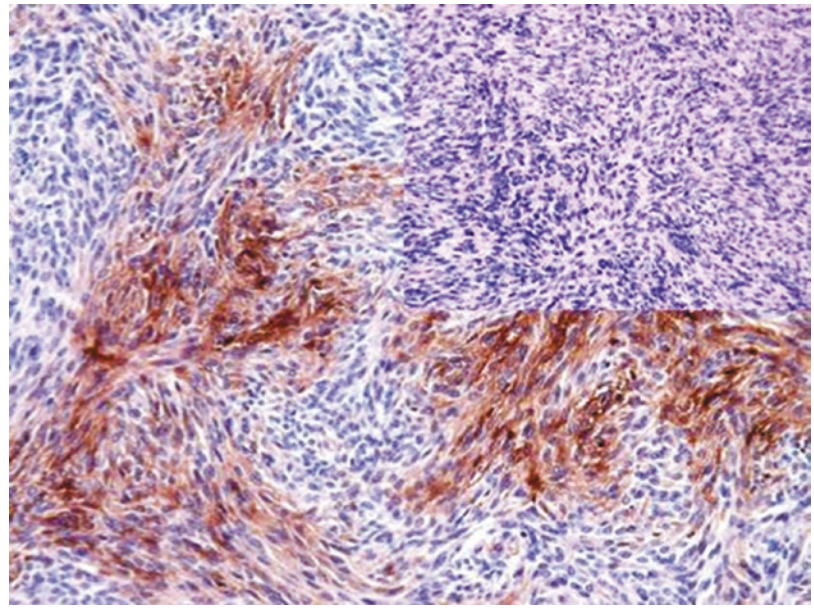

Fig. 2.6 Focal EMA expression in neurogenic sarcoma

Mucin-2: is a gel-forming mucin mainly synthesized in the goblet cells of gastric and small intestinal mucosa in addition to the bronchial mucosa and salivary glands providing a protective lubricating mucin membrane against mechanical and infectious agents. MUC-2 is a marker for of colonic, gastric, pancreatic, breast, and ovarian mucinous adenocarcinomas (Fig. 2.7).

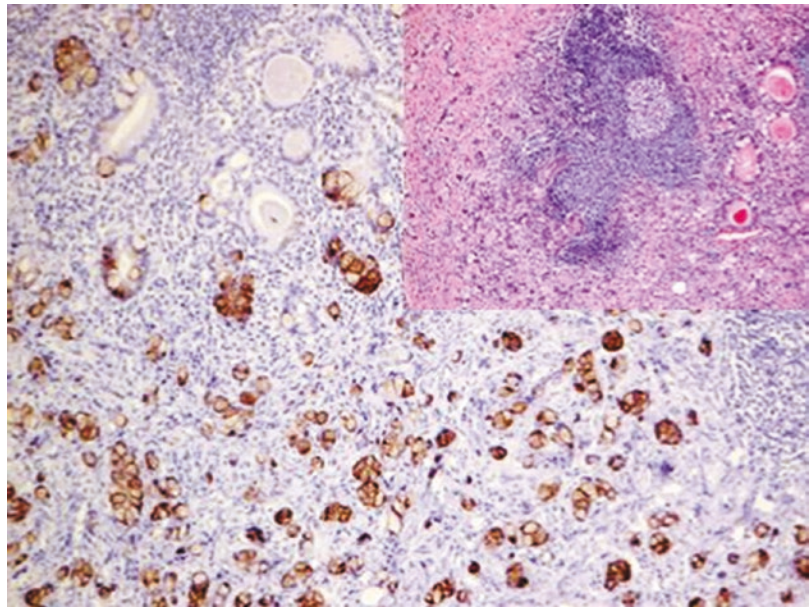

Fig. 2.7 MUC-2 highlighting tumor cells of appendicular mucinous carcinoma

Mucin-3: Two closely related subtypes of this mucoprotein have been identified in humans A and B primarily expressed in intestinal mucosa as membrane-bound mucin. MUC-3 is a marker for invasive breast carcinoma and gastric carcinoma. The overexpression of MUC-3 is associated with poor prognosis.

Mucin-4: is a transmembrane mucoprotein composed of alpha and beta chains and found in on the apical surface of many types of epithelial cells. MUC-4 is involved in the regulation of cellular adhesion and in cell surface signaling. MUC-4 is highly expressed in pulmonary, gastric, and pancreatic adenocarcinomas in addition to pancreatic intraepithelial neoplasia (PanIN). MUC-4 is also a sensitive and specific marker for low-grade fibromyxoid sarcoma and sclerosing epithelioid fibrosarcoma.

Mucin-5 AC: is a gel-forming mucoprotein initially recognized as two different proteins A and C encoded by the same gene. Mucin-5 AC is primarily found on the surface of gastric mucosa and in the respiratory tract. MUC-5 AC is a marker for many carcinoma types such as esophageal, gastric, colonic, pancreatic, cholangiocellular, endometrial carcinomas, endocervical adenocarcinomas, and mucinous ovarian carcinoma.

Mucin-5B: is a gel-forming mucoprotein predominantly expressed by the sublingual salivary gland and mucosal glands of the airway system.

Mucin-6: is a gel-forming mucoprotein and one of the major mucins protecting gastric mucosa. MUC-6 is synthesized by gastric and pyloric glands and mucosa of the gall bladder, bile, and pancreatic ducts in addition to colonic and endocervical mucosa. MUC-6 is a marker for invasive ductal carcinoma of breast and gastric adenocarcinomas.

Mucin-16 (also known as CA125): is a characteristic marker for serous, endometrioid, and

clear cell ovarian carcinomas. It is also expressed in pancreatic carcinoma. This marker is listed in details in a later section.

2.3 Claudins

Claudins is a family of integral transmembrane proteins that includes 23 members. These integral transmembrane tight junction-associated proteins are found in all types of thigh junction-bearing cells including epithelial and endothelial cells. Claudins form paracellular barrier and pores and regulate the transport of molecules through the intercellular space. In routine immunohistochemistry, Caludin-4 is mostly used as a marker to discriminate between reactive mesothelial cells and carcinoma cells in pleural and peritoneal effusion (Fig. 2.8). Caludin-4 is normally expressed in most types of epithelial cells and related carcinomas including colorectal adenocarcinoma, ovarian carcinoma, and breast and prostatic carcinomas but constantly negative in mesothelial cells. The expression of Caludin-4 is also found in endothelial cells and cells of submucosal and myenteric plexus [12, 13].

2.4 Miscellaneous Epithelial Markers

Epithelial specific antigen (EPCAM, CD326)		
Expression pattern: basolateral surface/cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Most adenocarcinoma types, neuroendocrine tumors, small cell carcinoma	Basal cell carcinoma, trichoepithelioma, Merkel cell carcinoma, squamous cell carcinoma, renal cell carcinoma, olfactory neuroblastoma, synovial sarcoma, desmoplastic small round cell tumor	Most normal epithelial cells
Positive control: appendix, basal cell carcinoma		

Diagnostic Approach Epithelial specific antigen (CD326) also known as human epithelial antigen or epithelial cell adhesion molecule (EPCAM) is a transmembrane glycoprotein mediating calcium-independent cell-cell adhesion and involved in cell signaling, migration, proliferation, and differentiation [14]. In routine

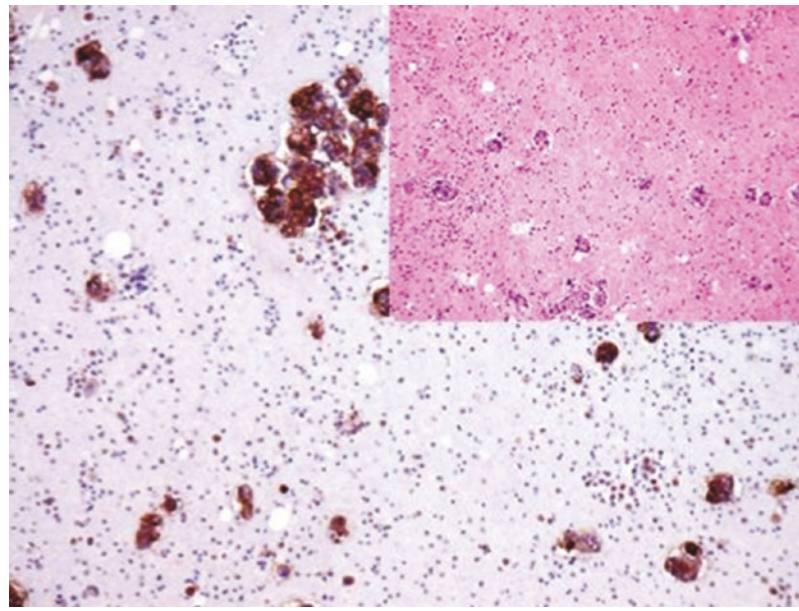

Fig. 2.8 Claudin-4 highlighting tumor cells of ovarian carcinoma in ascitic fluid

immunohistochemistry, Ber-EP4 is the most commonly used clone. EPCAM is expressed on most normal epithelial cells with the exception of superficial layers of squamous epithelium and epidermal keratinocytes, thymic cortical epithelium, myoepithelial cells, gastric parietal cells, hepatocytes, and renal proximal tubular cells. EPCAM is usually negative in the mesothelium; accordingly it is helpful to distinguish between pulmonary adenocarcinoma (EPCAM positive) and mesothelioma (EPCAM negative) and between basal cell carcinoma (EPCAM and bcl-2 positive, EMA negative) and squamous cell carcinoma (EPCAM and bcl-2 negative, EMA positive) (Fig. 2.9). Furthermore, it is a useful marker to differentiate between various types of hepatoid carcinomas positive for EPCAM and hepatocellular carcinoma usually lacking the EPCAM expression.

Diagnostic Pitfalls Up to 20% of mesothelial cells and malignant mesotheliomas may express the EPCAM antigen (usually as focal weak stain), which must considered in the differential diagnosis in pleural and peritoneal effusions.

Epithelial-related antigen: is a transmembrane glycoprotein expressed on normal and neoplastic glandular epithelium. The MOC31 clone is the most used clone in diagnostic immunohistochemistry and has the similar features of the above-mentioned EPCAM antigen. It is usually used to label epithelial tumors of different origin and to discriminate between metastatic carcinoma and atypical mesothelial proliferation. MOC31 stains also chromophobe renal cell carcinoma but negative in clear cell renal cell carcinoma.

p63/p40		
Expression pattern: nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Squamous cell carcinoma, basal (myoepithelial) cell marker in prostatic and mammary glands	Thymoma, myoepithelial tumors, transitional cell carcinomas, Brenner tumor, papillary thyroid carcinoma, a subset of non-Hodgkin's lymphoma	Stratified epithelium, transitional epithelium, myoepithelial basal cells
Positive control: prostate		

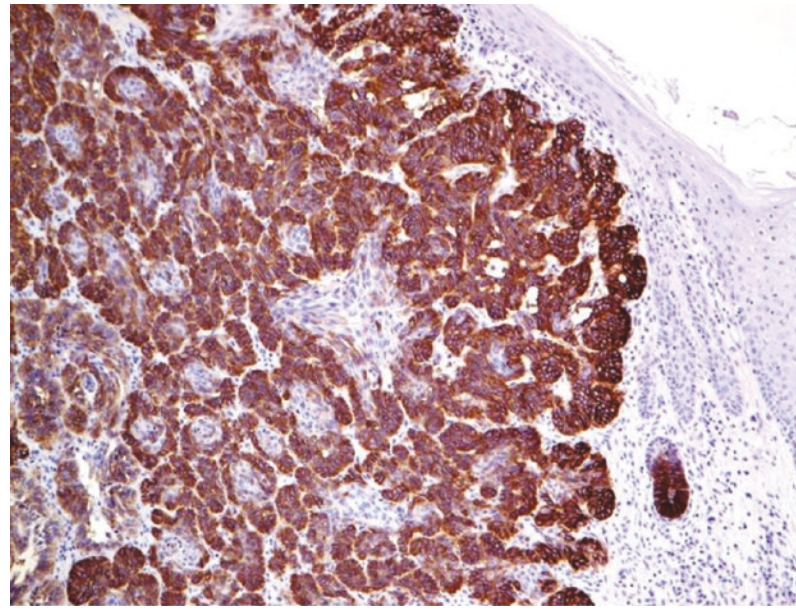

Fig. 2.9 Basal cell carcinoma with strong EPCAM (clone Ber-EP4) expression

Diagnostic Approach p63 (also called KET or p73L) is a member of the p53 gene family. p63 plays an important role in the differentiation of stratified epithelia and regulation of cell cycle progression. The p63 gene encodes two protein isoforms with different N-termini TA and ΔN. The ΔN isoform is highly expressed in squamous and basal cells. This isoform can be labeled by the p63 antibody (clone 4A4) or by the p40 antibody directed to the ΔNp63-a isoform; however, the latter seems to be more specific for squamous and basal cells [15, 16]. Both antibodies are excellent markers for squamous cell carcinoma and basal myoepithelial cells and related tumors. The high expression of p63 in myoepithelial basal cells makes both p63 and p40 antibodies very helpful markers to discriminate between benign and malignant prostatic and breast lesions (Fig. 2.10). p63 is also a useful marker to discriminate between follicular variant of papillary thyroid carcinoma and other benign follicular lesions of the thyroid gland as follicular structures in non-papillary carcinoma lack the p63 expression [9].

Diagnostic Pitfalls p63 has been detected in about 30% of pulmonary adenocarcinoma specifically poorly differentiated adenocarcinomas, which also might lack the expression of TTF-1 and/or Napsin A and can be misinterpreted as squamous cell carcinoma. Since p40 is more specific for squamous cells and squamous cell carcinomas than p63, it is highly recommended to replace p63 by p40 for the immunohistochemical classification of pulmonary carcinomas. It is remarkable that p63 but not p40 expression was found in a subset of soft tissue tumors including Ewing's sarcoma/PNET, neurothecoma, perineuroma, giant cell tumor, synovial sarcoma, rhabdomyosarcoma, MPNST, and extraskeletal myxoid chondrosarcoma [17]. The expression of p63 in different soft tissue is to consider in the interpretation of tumors with epithelioid appearance.

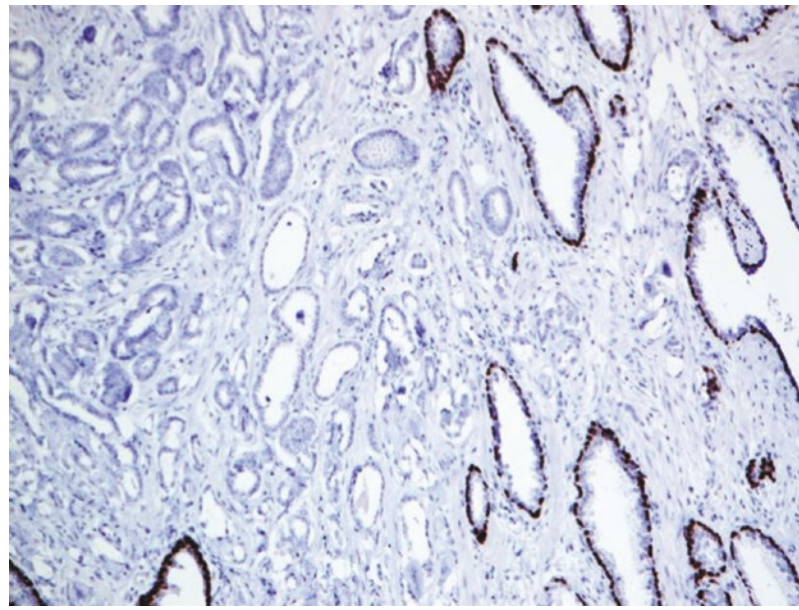

Fig. 2.10 p63 highlighting basal cells in normal prostatic glands; note neoplastic glands lacking the basal cell layer

Carcinoembryonic antigen (CEA; CD66e)		
Expression pattern: cytoplasmic/extracellular		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Gastrointestinal and pancreatic adenocarcinoma, pulmonary adenocarcinoma, cholangio-carcinoma, and hepatocellular carcinoma	Breast carcinoma, nonkeratinizing lung squamous cell carcinoma, cervical adenocarcinoma, ovarian mucinous carcinoma, medullary thyroid carcinoma, adenocarcinoma of sweat glands, secretory meningioma	Gastrointestinal mucosa, hepatocytes, thyroid C cells, granulocytes
Positive control: colonic adenocarcinoma		

Diagnostic Approach Carcinoembryonic antigen (CEA) is a cell surface glycoprotein normally expressed by colonic mucosa of fetal colon and to a lesser degree in adult colonic mucosa. CEA is highly expressed in different carcinoma types of various origins. CEA-negative tumors are of importance in the differential diagnosis. Prostatic carcinoma, endometrioid carcinoma, renal cell carcinoma, ovarian serous tumors, adrenal tumors, and follicular and papillary thyroid carcinoma in addition to mesothelioma are constantly CEA negative. CEA is helpful in the differential diagnosis between mesothelioma and carcinoma, endocervical and endometrioid carcinoma, medullary carcinoma, and other types of thyroid carcinoma.

Epidermal growth factor receptor-1 (EGFR)		
Expression pattern: membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Squamous cell carcinoma, embryonal rhabdomyosarcoma, endometrial stromal sarcoma, choriocarcinoma	Glioblastoma, triple-negative breast carcinoma, malignant Müllerian mixed tumor	Placenta (trophoblasts), endometrial stromal cells, squamous epithelium hepatocytes, urothelial cells, Leydig cells, melanocytes, myocytes
Positive control: placenta		

Diagnostic Approach Epidermal growth factor receptor-1 (EGFR, Erb1) is a member of type I receptor tyrosine kinase family, a transmembrane glycoprotein normally expressed on the membrane of various types of normal epithelial and non-epithelial cells. The EGFR molecule consists of an extracellular ligand-binding domain, a transmembrane lipophilic region, and an intracellular domain with tyrosine kinase activity. EGFR is activated by the epidermal growth factor and transforming growth factor alpha and is involved in the development of many cell types.

The expression/overexpression of EGFR has been observed in various tumors of different origin, mostly carcinomas including carcinoma of the breast, head and neck, renal, colonic, pancreatic, ovarian, and bladder. The expression of EGFR is also characteristic for many other non-epithelial tumors such as embryonal rhabdomyosarcoma and endometrial stromal sarcoma in addition to glioblastoma.

The EGFR molecule is the therapeutic target for specific monoclonal antibodies approved and used for the therapy of EGFR-positive tumors including lung, colorectal, and head and neck carcinomas. Colorectal adenocarcinomas sensitive for the specific immunotherapy must have a wild RAS gene. Semiquantitative evaluation of the EGFR expression on tumor cells might be required to estimate the response to the specific immunotherapy; in these cases, the three-point scoring system used for HER-2 can be used. Additionally, pulmonary carcinomas associated with driver mutations within the EGFR gene show a good therapeutic response to different EGFR tyrosine kinase inhibitors.

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Markers and Immunoprofile of the Upper Respiratory Tract and Pulmonary Tumors

3

Contents

3.1 Diagnostic Antibody Panel for Tumors of the Upper Respiratory Tract

Cytokeratin profile, CD56, synaptophysin, chromogranin, EBV, NUT, p16

3.2 Diagnostic Antibody Panel for Epithelial Pulmonary Tumors

Cytokeratin profile, TTF-1, napsin A, p63, p40, CD56, and surfactant proteins [1]

3.3 Diagnostic Antibody Panel for Mesenchymal Pulmonary Tumors

CD1a, langerin (CD207), HMB45, STAT6, CD31, CD34, CD99

M.S.A. Tuffaha et al., *Immunohistochemistry in Tumor Diagnostics*,
DOI 10.1007/978-3-319-53577-7_3

Thyroid transcription factor-1 (TTF-1)		
Expression pattern: nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Pulmonary carcinoma (adenocarcinoma, bronchioloalveolar carcinoma, and small cell carcinoma), carcinoma of thyroid gland (papillary, follicular, and medullary)	Non-pulmonary small cell carcinoma of different locations, subset of extrahepatic cholangiocarcinomas, glial-ependymal and choroid plexus tumors, pituicytoma, granular cell tumor of the sellar region, meningeal tumors	Type II pneumocytes and Clara cells of the lung, thyroid follicular and parafollicular C cells, parathyroid, pituitary gland, diencephalon
Positive control: thyroid tissue		

Diagnostic Approach Thyroid transcription factor (TTF-1 also known as NKX2-1 or thyroid-specific enhancer-binding protein) is a homeobox-containing transcription factor that regulates the development, differentiation, and gene expression of the thyroid gland (follicular and parafollicular C cells). TTF-1 plays also an active role in the regulation of development and transcriptional activity of the lung and central nervous system (diencephalon). In adult thyroid gland, TTF-1 is expressed in both follicular and parafollicular cells and controls the synthesis of different thyroid hormones and thyrotropin receptor. In normal lung, TTF-1 is strongly expressed in type II alveolar cells, Clara bronchiolar cells, and, in a lesser degree, in the epithelial cells of tracheal mucosa. In lung tissue, TTF-1 regulates the expression of different surfactant proteins, Clara cell secretory protein, and ATP-binding cassette transporter A3 and other active factors [2].

In routine immunohistochemistry, TTF-1 is widely used as a specific and sensitive marker for the majority of bronchopulmonary adenocarcinomas and pulmonary small cell carcinoma in addition to follicular, papillary, and medullary thyroid carcinomas (Figs. 3.1 and 3.2). A lesser degree of expression is found in large cell carcinoma of the lung and undifferentiated thyroid carcinoma [3, 4]. Pulmonary squamous cell carcinoma

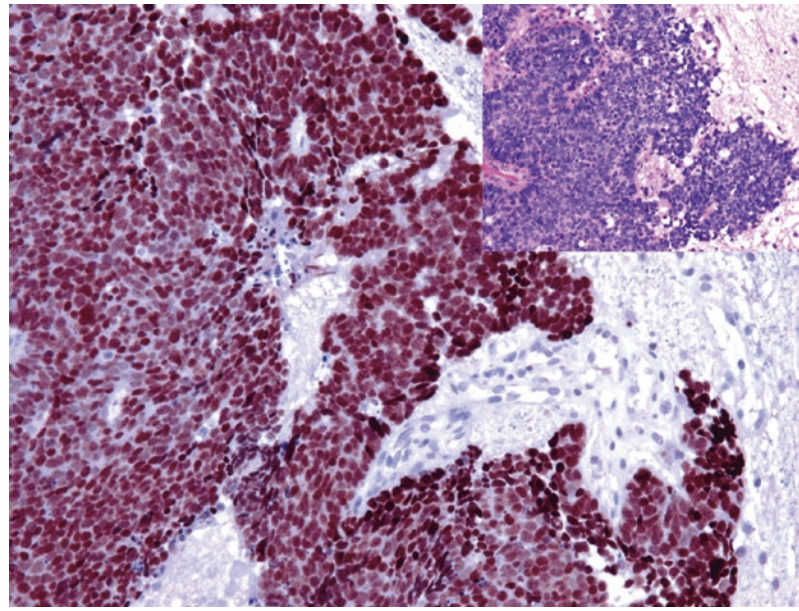

Fig. 3.1 Strong nuclear TTF-1 expression in pulmonary small cell carcinoma

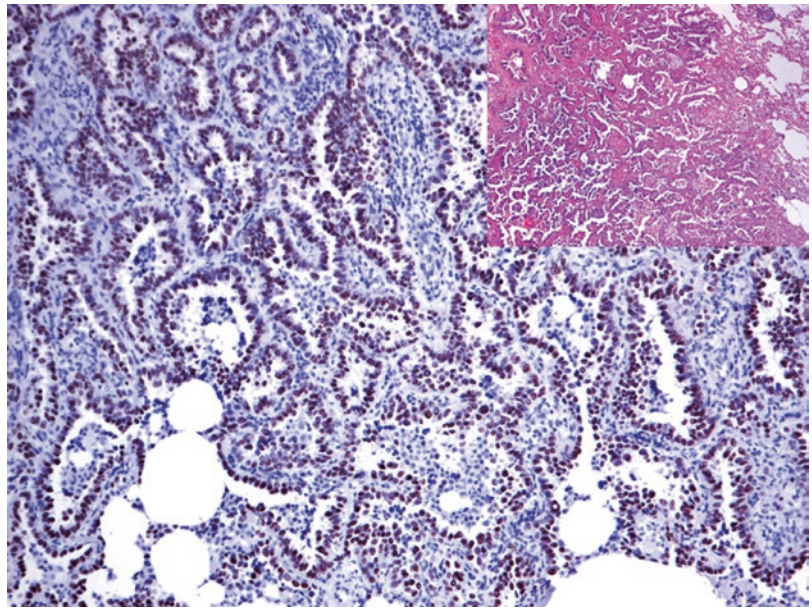

Fig. 3.2 Strong nuclear TTF-1 expression in pulmonary adenocarcinoma

is usually negative for TTF-1, but low expression levels in a small percentage of pulmonary squamous cell carcinoma are reported using the TTF-1 clone SPT24.213.

Diagnostic Pitfalls Despite the known specificity of TTF-1 to lung and thyroid tumors, TTF-1 positivity is also reported in different extrapulmonary tumors such as small cell carcinomas of the urinary bladder and ovaries in addition to Merkel cell carcinoma. The aberrant expression of TTF-1 is also reported in about one half of extrahepatic cholangiocarcinomas including gallbladder adenocarcinoma, while nonneoplastic biliary epithelium lacks the TTF-1 expression [5]. TTF-1 positivity is also found in rare uterine and ovarian tumors such as mixed Müllerian tumor.

The TTF-1 expression in various types of CNS tumors especially those in the third ventricle region is also to take in consideration when searching for the primary of brain metastases [6]. Remarkable is the nuclear TTF-1 expression in tumors of the neurohypophysis including pituicytoma and granular cell tumor of the sellar region [7]. A further interesting observation is the strong cytoplasmic stain found in hepatocytes and hepatocellular carcinoma using the 8G7G3/1 clone, probably due to a cross-reaction with 150–160 KDa mitochondrial protein, which can be used as a diagnostic marker [8].

Napsin A		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Pulmonary adenocarcinoma	Papillary renal cell carcinoma, endometrial and ovarian clear cell carcinoma, subset of cholangiocarcinomas	Type 2 pneumocytes, respiratory epithelium of bronchioles, alveolar macrophages, Clara cells, proximal renal tubules, pancreatic acini and ducts, plasma cells and small subset of lymphocytes
Positive control: lung tissue		

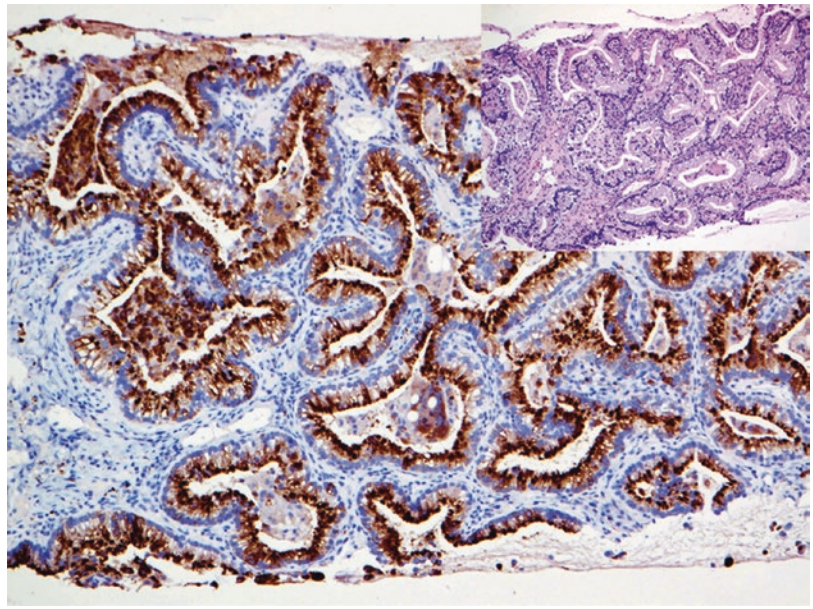

Fig. 3.3 Metastatic pulmonary adenocarcinoma with strong cytoplasmic napsin A expression

Diagnostic Approach Napsin A is pepsin like aspartic proteinase, a member of the novel aspartic proteinase of the pepsin family taking part in the proteolytic processing of surfactant precursors. Napsin A is expressed in the majority of pulmonary adenocarcinomas and is used as a specific marker for pulmonary adenocarcinoma, whereas all other primary pulmonary carcinoma types lack the expression of napsin A (Fig. 3.3). Generally, the expression of napsin A correlates with the expression of TTF-1, and only a small percentage of pulmonary adenocarcinomas are napsin positive but TTF-1 negative. All mesothelioma types constantly lack the expression of napsin.

Diagnostic Pitfalls The expression of napsin A may be found in other non-pulmonary tumors. Low expression level of napsin A is observed in papillary renal cell carcinoma and in a small subset of clear renal cell carcinoma. The expression of napsin A is also reported in about 90% of endometrial and ovarian clear cell carcinoma, in about one third of extrahepatic cholangiocarcinomas, and later may be also positive for TTF-1 [5, 9, 10]. Weak napsin expression is also reported in a small subset of colorectal and esophageal and pancreatic adenocarcinomas. As the morphology of the mentioned adenocarcinoma types may be similar to that of pulmonary adenocarcinoma especially in metastatic tumors, a complete diagnostic antibody panel must be used for accurate diagnosis.

Surfactant proteins		
Expression pattern: cytoplasmic/membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Pulmonary adenocarcinoma		Type 2 pneumocytes, bronchiolar cells
Positive control: lung tissue		

Diagnostic Approach Surfactant proteins including A, B, C, and D in addition to surfactant precursors are lipoproteins synthesized by type II pneumocytes and Clara bronchiolar epithelial cells. Antibodies to surfactant proteins are good markers for pulmonary adenocarcinoma.

Pulmonary squamous cell carcinoma, large cell carcinoma, and non-pulmonary adenocarcinomas beside mesothelioma are usually negative for surfactants.

Diagnostic Pitfalls The expression of some surfactants is described in a small subset of breast carcinoma types. Macrophages in pleural effusion may be also positive to surfactant. The diagnosis of primary or metastatic pulmonary adenocarcinoma must be based on clinical data, microscopic appearance, cytokeratin profile, and TTF-1 expression. The expression of surfactant and the absence of CDX-2, GATA-3 and steroid receptor are helpful to support the diagnosis of primary pulmonary carcinoma.

Nuclear Protein in Testis (NUT): NUT is the product of the NUT gene located on chromosome 15 and normally expressed in testicular tissue. Midline carcinoma is a rare highly malignant carcinoma that accrues in the thorax, head, and neck region and characterized by the t(15;19) translocation causing the expression of the NUT protein. Antibodies to NUT are specific markers for midline carcinoma [11–13]. Weak NUT expression is also reported in primary and metastatic seminomas.

Immunoprofile of lung and respiratory tract tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (±)	+ in 10–50% (∓)	+ in <10% (−)
A. Tumors of upper respiratory tract				
Sinonasal undifferentiated carcinoma	CK8, *p16*	CK7	CK19, EMA	CK4, CK5, CK6, CK10, CK13, CK14, CK20, NUT
Olfactory neuroblastoma (esthesioneuroblastoma)	*CD56*, CD57, NSE, PGP9.5, GATA-3, neurofilaments	Calretinin, bombesin, synaptophysin, chromogranin, S100	Fli-1, Pan-CK	EMA, WT-1, CD99, NUT
Nasopharyngeal (undifferentiated) carcinoma	*CK5/6*, CK8, CK13, CK19, EMA	bcl-2, *EBV*, HLA-DR		CK4, CK7, CK10, CK14, *p16*, NUT
Squamous cell carcinoma	*CK5/6*, *p63*, *p40*, CK8, CK13, CK19, EMA			CK4, CK7, CK10, CK14, *p16*, bcl-2, EBV, HLA-DR
Midline carcinoma	Pan-CK, *NUT*[a]	CK7, CD56, p63	CD34	*p16*, EBV, chromogranin, synaptophysin
B. Lung tumors				
Squamous cell carcinoma	*CK5/6/10/13/14*, CK8/18, CK19, *p40*	*p63*	CK7[b], calretinin	TTF-1, CK4, CK20, p16[c]
Pulmonary adenocarcinoma • Lepidic adenocarcinoma • Acinar adenocarcinoma • Papillary adenocarcinoma • Micropapillary adenocarcinoma • Solid adenocarcinoma	*CK7*, CK8, CK18, CK19, *TTF-1*[d], CEA	*Napsin A*, surfactant proteins, CK12	p63, CK5/6/14[e], mesothelin, villin	CD141, calretinin, p40, CK20, CDX-2
Pulmonary adenocarcinoma mucinous type	*CK20*	CK7	CDX-2	TTF-1, napsin A

Pulmonary adenocarcinoma colloid type	*CK20, MUC2*	CDX-2	CK7, TTF-1, napsin A	CK5/6/14, p40
Pulmonary adenocarcinoma fetal type	*CK7, TTF-1*, β-catenin[f]	Chromogranin, ER-β	AFP[g], SALL4[f]	CK5/6/14, p40
Pulmonary adenocarcinoma enteric type	*CK7, CK20*	CDX-2, villin		CK5/6/14, p40
Large cell carcinoma	*CK7*, CK8, CK14, CK18, CK19, EMA		CK5/6/14	*TTF-1, napsin A*, CK20
Typical and atypical carcinoid tumor (NET G1 & G2)	CK-MNF[h], CK8, CK18, *CD56*, NSE, *chromogranin, synaptophysin,* PGP 9.5 *Proliferation index (Ki-67) in typical carcinoid (NET G1): <5% Proliferation index (Ki-67) in atypical carcinoid (NET G2): <20%*	S100, E-cadherin, EMA, CEA	CD99, CD117, TTF-1	CK5/6/14, p40, CK20
Small cell carcinoma	CK-MNF[h], CK8, CK18, CK19, *CD56*, NSE, synaptophysin, chromogranin, S100 *Proliferation index (Ki-67): > 90%*	Neurofilaments, *TTF-1,* CD99, PAX-5, CD117, CK7, vimentin	TdT	CK5/6/14, CK20
Large cell neuroendocrine carcinoma	CK7, CK8, CK18, CK19 *CD56, chromogranin Proliferation index (Ki-67): 40–80%*	TTF-1, *synaptophysin*, CD117		CK5/6/14, p40, CK20
Pleomorphic, spindle cell, and giant cell carcinoma	Pan-CK, vimentin	CK5/6/14, CK7, fascin	TTF-1	
Salivary gland-type tumors	See salivary gland tumors			
Pulmonary blastoma	CK7, TTF-1, CEA	Chromogranin	Synaptophysin	
Clear cell tumor (sugar tumor)	*HMB45*, HMB50, cathepsin B, CD63		S100, CD57 (leu7), synaptophysin, NSE, CD34	Pan-CK, EMA, chromogranin, CD56
Pulmonary sclerosing hemangioma (inverting alveolar pneumocytoma)	*Stromal clear cells in solid portions*: EMA, TTF-1 *Surface-lining cells*: CK 7, EMA, *TTF-1*, surfactant	Vimentin, estrogen and progesterone receptors Napsin A, CD15	CK7, Ki-67 (MIB-1 clone)[i] vimentin	CK5/6, CK20, CD31, CD34, surfactant, calretinin CK5/6, CK20, calretinin, ER and PgR
Epithelioid hemangioendothelioma (intravascular bronchoalveolar tumor)	*CD31*, CD34, vimentin			Pan-CK, calretinin
Pulmonary blastoma	*Epithelial component*: pan-CK, EMA, CEA	TTF-1	Chromogranin	

Inflammatory pseudotumor (pulmonary inflammatory myofibroblastic tumor)	Actin (in spindle cells), vimentin	Cyclin D1, *ALK (p80)*	Desmin, bcl-2	Pan-CK, EMA, CD56
Pulmonary histiocytosis X	*CD1a, CD207 (langerin),* S100, HLA-DR	CD11c, CD68 CD31		Pan-CK
Pulmonary lymphangiomyomatosis	Smooth muscle component: actin, caldesmon, *HMB45*	Estrogen and progesterone receptors		
Solitary fibrous tumor of the pleura	CD34, *STAT6*, vimentin	CD99, bcl-2	Actin, TLE1, CD10, β-catenin	Desmin, S100, pan-CK, EMA, CD56, CD68, CD117

[a]Consider molecular detection of t(15;19)(q13;p13.1)-specific translocation
[b]CK7 found in up to 30% of pulmonary squamous cell carcinoma. In addition to the cytokeratin profile, a complete panel including TTF-1, napsin, and p40 is required to classify pulmonary carcinomas
[c]p16 can be useful to distinguish between primary pulmonary squamous cell carcinoma, negative for p16, and metastatic oropharyngeal squamous cell carcinoma, frequently positive for p16 due to HPV association
[d]TTF-1 can be absent in poorly differentiated pulmonary adenocarcinomas
[e]Frequently positive in poorly differentiated pulmonary adenocarcinoma
[f]Nuclear expression
[g]Usually in poorly differentiated carcinoma
[h]Often dot-like expression pattern
[i]Atypical membranous and cytoplasmic stain pattern is noted when the MIB-1 clone is used

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Markers and Immunoprofile of Thymic Epithelial Tumors

4

Contents

Diagnostic Antibody Panel for Thymic Epithelial Tumors

1. Markers for thymic epithelium: PAX-8, CD117, CD5, cytokeratin profile (high molecular weight cytokeratins), p63
2. Markers for lymphoid stroma: CD1a, CD3, TdT [1, 2]

PAX-8 and CD117 are two markers helpful to discriminate between normal thymic epithelium and neoplastic thymic epithelium, which is positive for both markers, whereas other epithelial cells or carcinoma types are negative for both markers (Figs. 4.1, 4.2, 4.3, and 4.4).

p63 stains benign and neoplastic thymic epithelial cells (including all thymoma types), whereas CD5 is a marker for malignant thymic epithelium (thymic carcinoma) but negative benign thymic epithelium.

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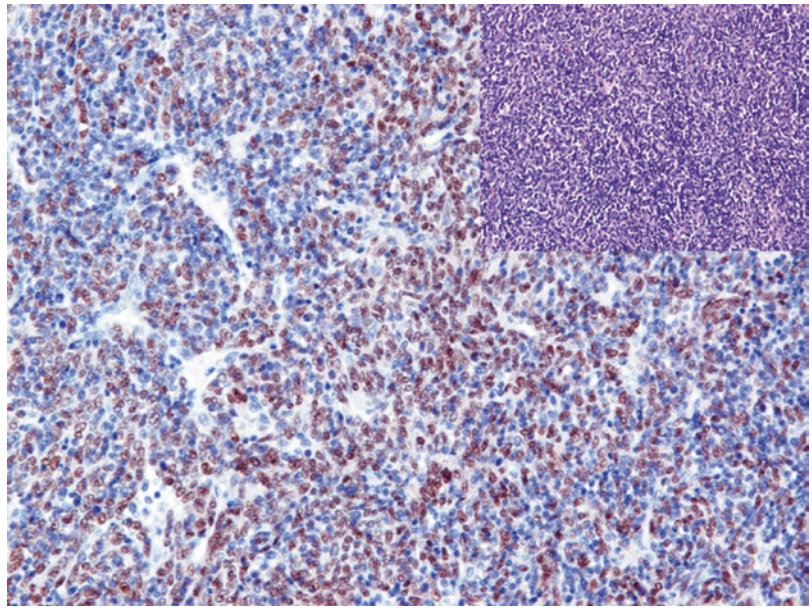

Fig. 4.1 Nuclear PAX-8 expression in neoplastic epithelial cells of AB thymoma type

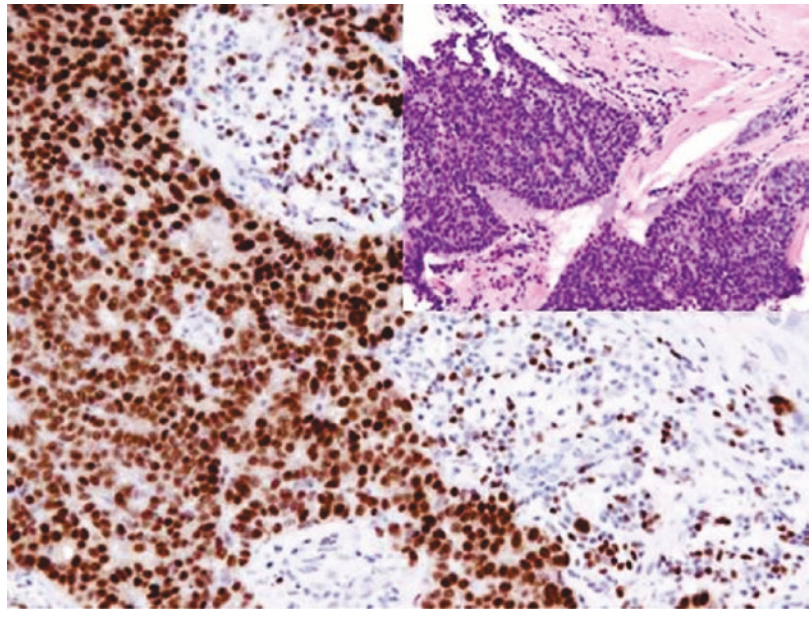

Fig. 4.2 Nuclear PAX-8 expression in malignant epithelial cells of thymic carcinoma

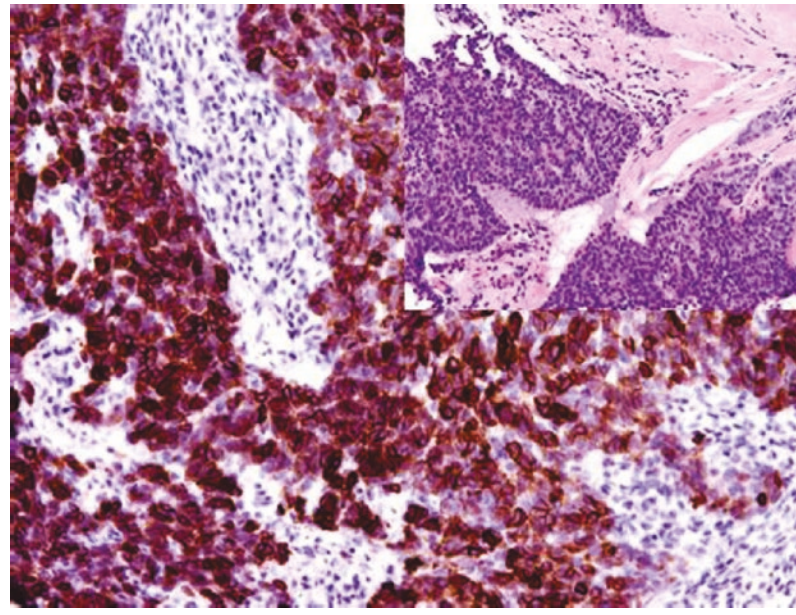

Fig. 4.3 CD117 staining malignant epithelial cells of thymic carcinoma

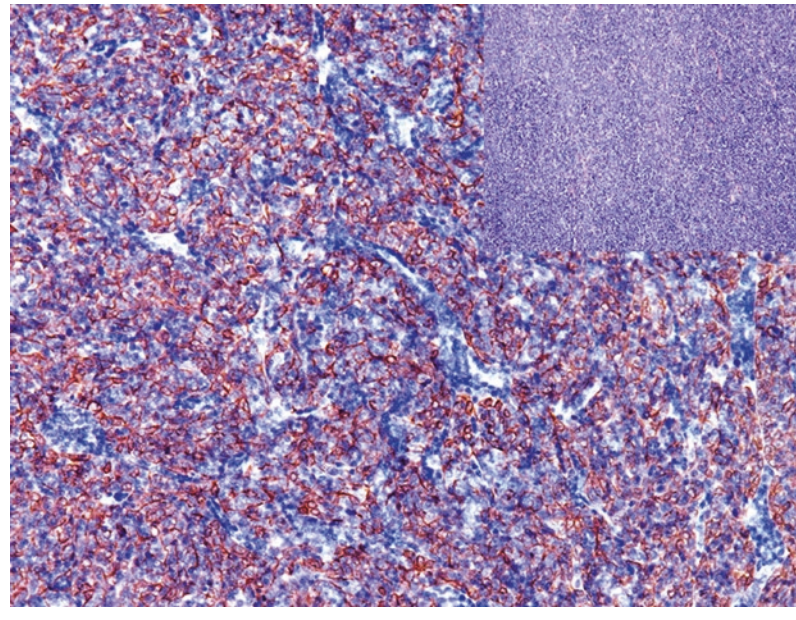

Fig. 4.4 Cytokeratin 5/14 expression in neoplastic epithelial cells of AB thymoma type

Immunoprofile of thymic epithelial tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (±)	+ in 10–50% (∓)	+ in <10% (−)
Thymoma (types A, AB, and B1, B2, and B3) • Type A (medullary thymoma) • Type AB (mixed thymoma) • Type B1 (predominantly cortical thymoma) • Type B2 (cortical thymoma) • Type B3 (well-differentiated thymic carcinoma)	*Neoplastic epithelial cells:* CK5/6, CK7, CK8, CK18, CK19, p63 *Tumor-associated lymphocytes*: predominantly immature T lymphocytes positive for TdT, CD1a[a], CD3, CD99	CD15, CD57 (leu7), PAX-8		EMA, CD20[b], *CD5*, bcl-2, CD117, HER-2
Thymic carcinoma	*Neoplastic epithelial cells:* CK5/14, p63, *CD5*[c], CD70, CD117, bcl-2, EMA *Tumor-associated lymphocytes:* predominantly mature T and B lymphocytes negative for TdT	CD15, CK6, CK8, CK18, CK19, PAX-8	CK7, synaptophysin, chromogranin, HER-2	

[a]See Fig. 4.5
[b]CD20 may be expressed in thymomas types A and AB
[c]CD5 negative in spindle cell thymic carcinoma

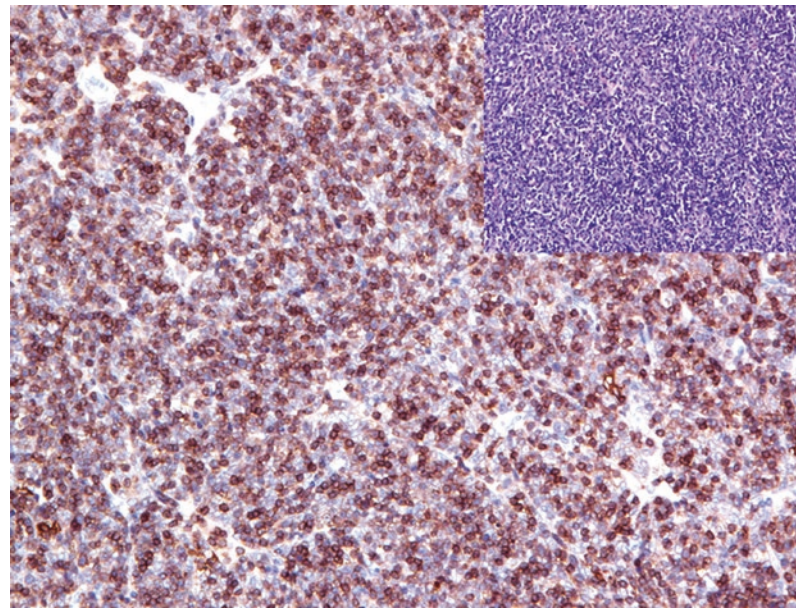

Fig. 4.5 CD1a labeling the tumor-associated T lymphocytes in AB thymoma type

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Markers and Immunoprofile of Heart and Pericardial Tumors

5

Diagnostic Antibody Panel for Heart Tumors

Tumors of the heart are heterogeneous and of different histogeneses and constellations; the immunohistochemical panel depends on the histogenesis and morphology of the tumor (Fig. 5.1).

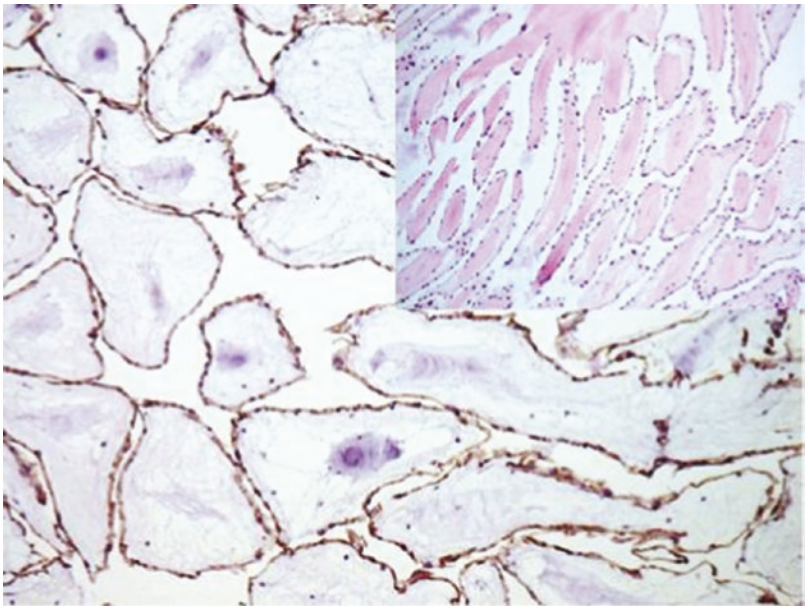

Fig. 5.1 Papillary fibroelastoma lined by CD31-positive endothelial cells

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Immunoprofile of heart and pericardium tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (±)	+ in 10–50% (∓)	+ in <10% (−)
Rhabdomyoma/ rhabdomyosarcoma	Desmin, sr-actin, myosin, myoglobin	Myo-D1		Pan-CK, sm-actin, S100
Cardiac myxoma	Calretinin[a], PGP9.5, actin, synaptophysin	Desmin		Pan-CK, CD68
Cardiac fibroma:	Vimentin	Actin		CD34
Papillary fibroelastoma	CD31[b], CD34, factor VIII			
Cystic tumor of the atrioventricular node	Pan-CK, CK5/6	CEA		Calretinin, CD31, CD34
Purkinje cell tumor	Actin, myoglobin		Desmin	CD68, pan-CK
Undifferentiated pleomorphic sarcoma	Vimentin	Actin, CD34		Calretinin
Solitary fibrous tumor	CD34, *STAT6*, vimentin	CD99, bcl-2	Actin, TLE1, CD10, β-Catenin	Desmin, S100, pan-CK, EMA, CD56, CD68, CD117
Mesothelioma	See mesothelioma chapter			

[a]See Fig. 5.2
[b]See Fig. 5.1

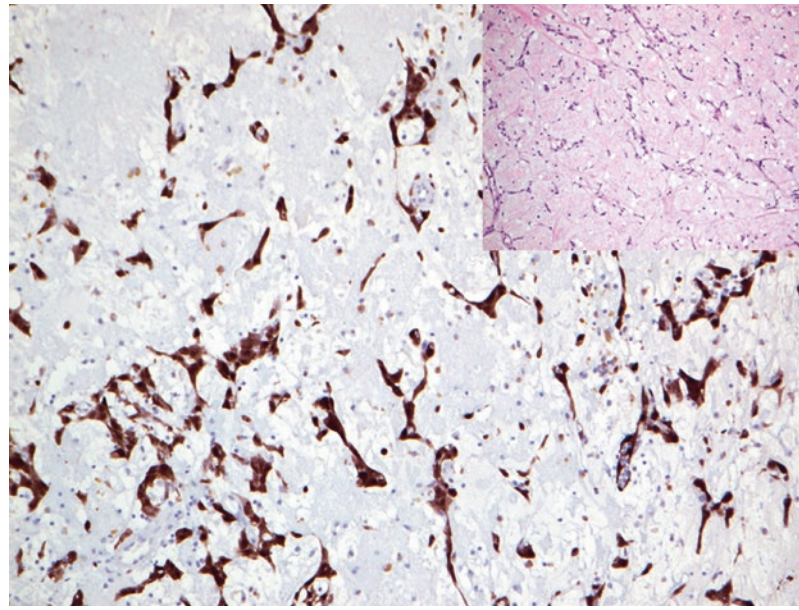

Fig. 5.2 Cardiac myxoma, cells showing strong calretinin expression

Markers and Immunoprofile of Tumors of the Oral Cavity and Salivary Gland Tumors

6

Contents

6.1 Odontogenic Tumors and Tumors of the Oral Cavity

Diagnostic Antibody Panel for Odontogenic Tumors and Tumors of the Oral Cavity Cytokeratin profile, p63, p40, EBV, p16

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DOI 10.1007/978-3-319-53577-7_6

Immunoprofile of odontogenic tumors and tumors the oral cavity				
Tumor type	+ in >90% (+)	+ in 50–90% (±)	+ in 10–50% (∓)	+ in <10% (−)
Basal cell carcinoma	*Epithelial specific antigen (BerEP4)*, bcl-2, androgen receptors			EMA, CEA
Squamous cell carcinoma	*CK5/6*, CK8, CK14, CK18, CK19, p63, *p40*		EBV	CK7, CK20
Sebaceous carcinoma	*Adipophilin*, EMA, androgen receptors, CEA			
Ectomesenchymal chondromyxoid tumor of the tongue	GFAP, Pan-CK, vimentin	S100	Actin	EMA, CK7, p63, calponin, desmin
Ameloblastoma	Pan-CK, CK5, CK14, vimentin	Calretinin		
Clear cell odontogenic carcinoma	CK8, CK 13, CK14, CK18, CK19, EMA			Vimentin, desmin, actin, S100, HMB45
Granular cell tumor	S100, SOX-10, CD56, CD68, NSE, vimentin	Inhibin		Pan-CK, actin, HMB-45

The immunoprofile of miscellaneous soft tissue tumors arising in the oral cavity are listed in related sections.

6.2 Salivary Gland Tumors

Diagnostic Antibody Panel for Salivary Gland Tumors α-Amylase, CD117, GFAP, GATA-3, DOG-1, cytokeratin profile, p63, EMA, sm-actin, h-caldesmon, calponin, S100 [1–3]

Cytokeratin Profile Salivary glands are composed of luminal cells including ductal and acinar cells in addition to the myoepithelial cells. The cytokeratin profile is an important tool to highlight the different cell types forming salivary gland units or tumors derived from these cell types. High molecular weight cytokeratins (CK5/10/14) label the myoepithelial and basal cells. p63, actin, and myosin are also additional markers that label these cells. Recently Sox-10 is also found to label the myoepithelial cells. Cytokeratin 7 is a marker for acinar and ductal cells. The atypical distribution of these cell types is clearly seen in tumors composed of both cell types (Fig. 6.1).

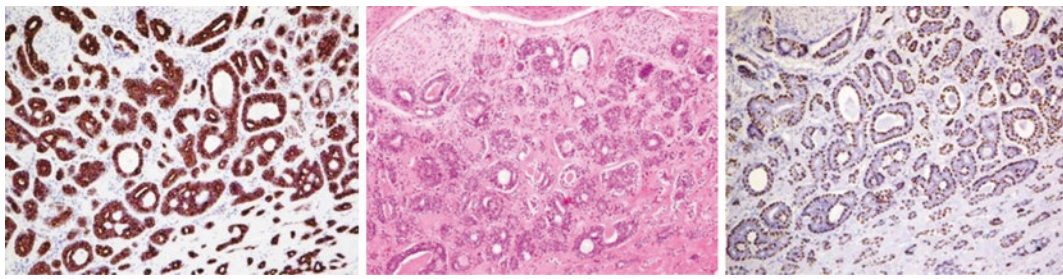

Fig. 6.1 Cytokeratin expression pattern in adenoid cystic carcinoma; CK7, HE, p63. Lumenal ductal cells positive for CK7 (*left*), basal cells labeled by p63 (*right*)

Anoctamin-1 (DOG-1): DOG-1 is a transmembrane chloride channel protein highly expressed in the cells of Cajal and in gastrointestinal stromal tumors derived from these cells. DOG-1 is also expressed on the apical surface of normal serous and mucinous acinic cells of salivary glands and pancreas (Fig. 6.2). Consequently, DOG-1 is a diagnostic immunohistochemical marker for acinic cell carcinomas of salivary glands. Weak expression levels of DOG-1 are also found in a subset of polymorphous low-grade adenocarcinoma, adenoid cystic carcinoma, and epithelial-myoepithelial carcinoma.

Alpha-Amylase: α-Amylase is an enzyme that catalyzes the cleavage of large sugar molecules into oligosaccharides. It is synthesized by acinic cells of salivary glands and pancreas. In immunohistochemistry, antibodies to amylase are used as specific markers for acinic cell carcinoma of salivary glands and pancreas. Other salivary gland tumors are usually negative for amylase.

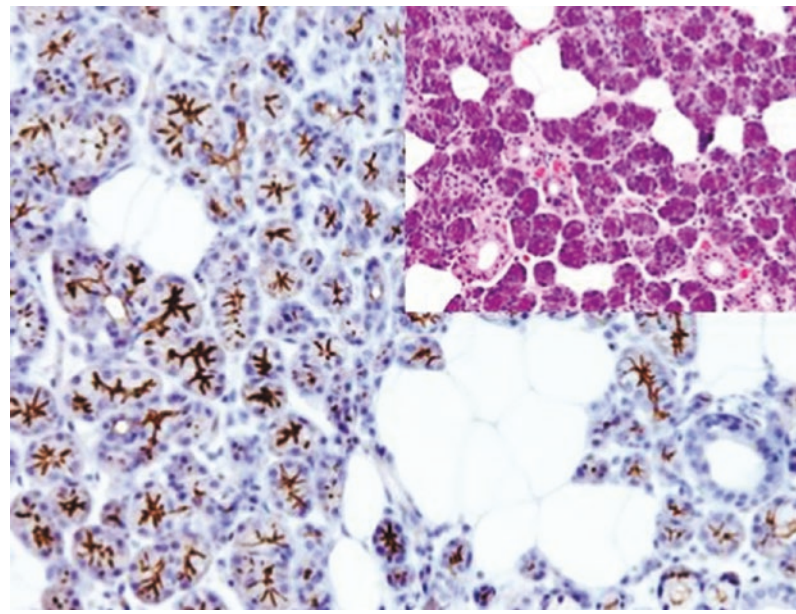

Fig. 6.2 DOG-1 highlighting the apical surface of acinar cells of the parotid gland

Immunoprofile of salivary gland tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (±)	+ in 10–50% (∓)	+ in < 10% (−)
Pleomorphic adenoma	*Luminal epithelial cells*: CK7, CK8, CD10, CK11, CK 13, CK14, CK18, CK19, EMA *Myoepithelial cells*: vimentin, S100, calponin, actin, GFAP, CK5/6/14 Proliferation index (Ki-67)[a] > 2%	CK7, CEA	GATA-3	CK14, CK20, vimentin, GFAP EMA, CEA

Immunoprofile of salivary gland tumors				
Oncocytoma/ oncocytic carcinoma	CK7, CK8, CK18, CEA, GATA-3	p63		Actin
Myoepithelial adenoma/carcinoma	S100, CK5/6/14, calponin, Sox-10, vimentin Proliferation index (Ki-67) in myoepithelial adenoma, <10%; in myoepithelial carcinoma, >10%	CK19, EMA, p63, GFAP, actin, caldesmon		CEA, EMA, CK7
Basal cell adenoma/ adenocarcinoma	*Luminal epithelial cells*: Pan-CK, CK7, CK8, CK18, EMA *Myoepithelial cells*: S100, actin, calponin, vimentin, GFAP, CK5/6/14, p63	CEA		CD43, vimentin
Mucoepidermoid carcinoma	*Mucous-secreting cells*: CK8, CK17, CK18, CK19, EMA *Epidermoid cells*: CK5/6, CK8, CK10/13/14	CK7	GATA-3	CK7
Acinic cell carcinoma	CK7, CK8, CK18, EMA, CEA, *DOG-1*, transferrin, lactoferrin	CK19, NSE, *α-amylase*, bone morphogenetic protein 6, cyclooxygenase-2	bcl-2, PgR, GATA-3	CK14, p63
Adenoid cystic carcinoma	*Myoepithelial and luminal ductal cells*: CK8, CK14, CK17, CK18, CK19, bcl-2, CD43 *Ductal luminal cells*: EMA, CK7 *Myoepithelial cells*: CK5/6, p63, p40, calponin, sm-actin, Sox-10, S100, vimentin Proliferation index (Ki-67): >20%	CEA, S100, CD117 (c-kit), DOG-1, MUC-1, p63 CEA	GATA-3, GFAP	CK20
Polymorphous low-grade adenocarcinoma (terminal duct carcinoma)	CK7, CK8, CK18, CK19, EMA, S100, galectin-3, E-cadherin, vimentin, bcl-2 Proliferation index (Ki-67): 1.5–7%	CEA, DOG-1	GFAP	CK20, CD43, calponin, actin, GATA-3
Salivary duct carcinoma	CK7, CK8, CK14, CK18, CK19, EMA Proliferation index (Ki-67): >25%	Androgen receptors, p53, CEA, GATA-3, GCDFP15, HER-2	PSA	S100, CK20
Epithelial-myoepithelial carcinoma	*Epithelial luminal cells*: CK8, CK18, EMA *Myoepithelial cells*: S100, actin, CK5/6, CK14, p63, Sox-10, vimentin		GATA-3 CEA	
Clear cell carcinoma (hyalinizing clear cell carcinoma)	CK7, CK8, CK18, EMA	p63, CEA, GATA-3	Vimentin	S100, actin, calponin, GFAP, CD10, PAX-8
Low-grade cribriform cystadenocarcinoma	*Luminal epithelial cells*: CK7 *Myoepithelial cells*: S100, actin, CK5/6, CK14, p63, vimentin			HER-2
Mammary analogue secretory carcinoma[b]	CK7, CK8, 18, EMA, GCDFP-15, GATA-3, mammaglobin, S100, CD117			CK5/14, p63, actin, calponin

Immunoprofile of salivary gland tumors				
Sebaceous carcinoma	*Adipophilin*, EMA	Perilipin, CK5/14, CK8/18, CK7, CK19, CD15		CK20, CEA, S100

[a]Atypical membranous and cytoplasmic stain pattern may be additionally noted when the MIB-1 clone is used
[b]Carcinoma associated with the t(12;15)(p13;q25) translocation

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Markers and Immunoprofile of Tumors of the Gastrointestinal Tract

7

Contents

7.1 Gastrointestinal Epithelial Tumors

7.1.1 Diagnostic Antibody Panel for Gastrointestinal Carcinoma

Cytokeratin profile, CDX-2, SATB-2, CDH-17, CEA, and villin

7.1.2 Diagnostic Antibody Panel for Gastrointestinal Neuroendocrine Carcinoma

Cytokeratin profile, CDX-2, SATB-2, synaptophysin, chromogranin, somatostatin, and Ki-67

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DOI 10.1007/978-3-319-53577-7_7

CDX-2		
Expression pattern: nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Colorectal adenocarcinoma	Gastric adenocarcinoma, carcinoids of gastrointestinal tract, islet pancreas tumors, sinonasal carcinoma, adenocarcinomas of urinary bladder, ovarian mucinous adenocarcinoma, adenocarcinoma of uterine cervix	Intestinal epithelium and intestinal metaplasia, pancreatic epithelial cell
Positive control: appendix		

Diagnostic Approach Caudal-related homeobox 2 (CDX-2) is an intestine specific transcription factor protein regulating the differentiation and proliferation of intestinal epithelial cells. The expression of CDX-2 begins normally in the post-gastric mucosa in the late stages of embryogenesis of the gastrointestinal tract and is characteristic for different types of adult intestinal mucosa including absorptive, goblet, and Paneth cells in addition to neuroendocrine cells.

The expression of CDX-2 protein is found in esophageal and gastrointestinal adenocarcinomas in addition to gastrointestinal neuroendocrine tumors in different intensities, whereas the highest frequency and intensity is characteristic for the colorectal adenocarcinomas (Fig. 7.1) [1]. CDX-2 is also an early marker for esophageal Barrett's metaplasia as the expression of CDX-2 initiates the transformation of squamous epithelium into columnar epithelium with goblet cells.

The expression of CDX-2 is usually associated with the expression of cytokeratin 20. CDX-1 is a further transcription factor and a marker for gastrointestinal tumors analogous to CDX-2.

Diagnostic Pitfalls The expression of CDX-2 is reported in many non-gastrointestinal adenocarcinomas. High expression level of CDX-2 is found in bladder adenocarcinoma derived from intestinal urachus, pancreatic adenocarcinoma, biliary adenocarcinoma, and mucinous ovarian carcinoma. CDX-2 expression is also reported in

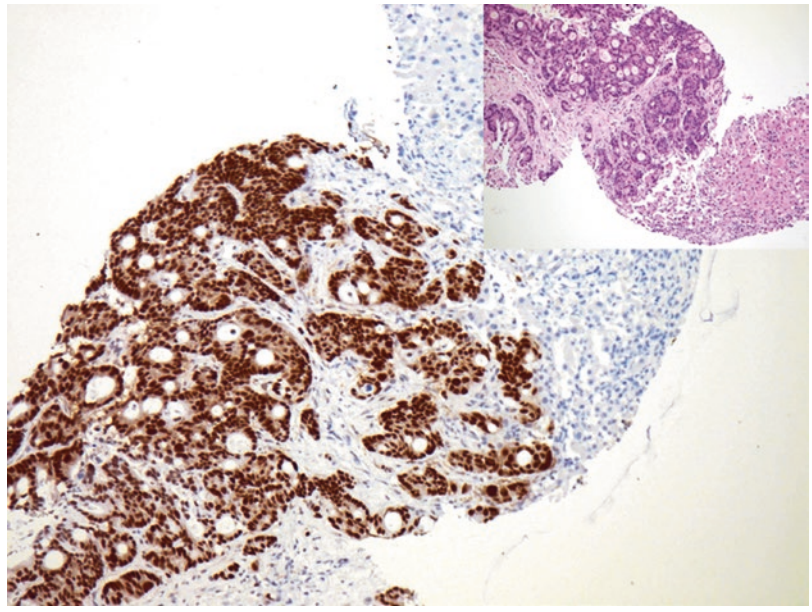

Fig. 7.1 Strong nuclear CDX-2 expression in metastatic colonic adenocarcinoma

rare cases of prostatic cancer. Pulmonary adenocarcinoma with mucinous differentiation can also be positive for CDX-2; this type of pulmonary adenocarcinoma is also positive for cytokeratin 20 and lacks the expression of TTF-1 [2, 3]. Some neuroendocrine tumors outside the GIT are also reported to be positive for CDX-2 [4]. The loss of CDX-2 expression has been noted in anaplastic high-grade gastrointestinal adenocarcinomas and in medullary adenocarcinomas.

SATB-2		
Expression pattern: nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Colorectal adenocarcinoma and medullary carcinoma, osteosarcoma	Hepatocellular carcinoma, laryngeal squamous cell carcinoma, neuroendocrine tumors of the colon and rectum	Colorectal epithelium, neuronal cells of the central nervous system, hepatocytes, kidney, epithelial cells of the epididymis and seminiferous ducts
Positive control: appendix		

Diagnostic Approach **S**pecial AT-rich sequence-**b**inding protein **2** (SATB-2) is a nuclear matrix-associated transcription factor and DNA-binding protein involved in the differentiation of osteoblasts. In the gastrointestinal tract, SATB-2 is selectively expressed in colorectal epithelium, while gastric and small intestinal mucosa and pancreatic epithelium lack the expression of SATB-2. SATB-2 is a specific marker for colorectal adenocarcinomas including medullary carcinoma (Fig. 7.2). In routine histopathology, SATB-2 is usually used in combination with cytokeratin 20. SATB-2 is also selectively expressed in neuroendocrine tumors of the left colon and rectum whereas other neuroendocrine tumors reported to be negative or weak positive for this marker [5]. Low expression level of SATB-2 is reported in a subset of pulmonary adenocarcinomas in addition to ovarian carcinomas. Adenocarcinomas of the upper gastrointestinal tract and pancreas typically lack the expression of SATB-2. SATB-2 is also an important diagnostic marker for osteosarcoma [6, 7].

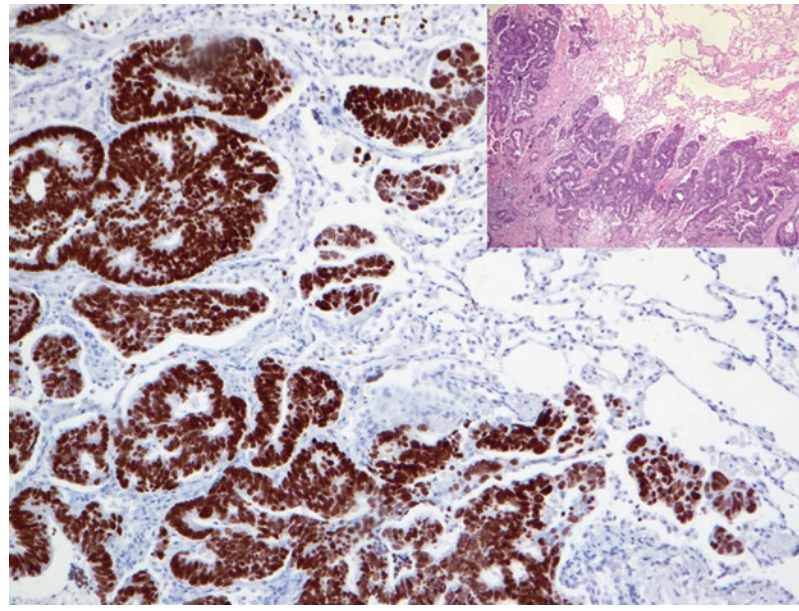

Fig. 7.2 Nuclear SATB-2 expression in metastatic rectal adenocarcinoma (lung metastases)

Cadherin-17 (CDH17)		
Expression pattern: membranous and cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Esophageal and gastrointestinal adenocarcinoma	Pancreatic ductal carcinoma, gastrointestinal and pancreatic neuroendocrine tumors, cholangiocellular carcinoma, osteosarcoma	Gastrointestinal epithelium, pancreas, gall bladder mucosa, adrenal cortex, pituitary gland
Positive control: appendix		

Diagnostic Approach **C**alcium-dependent **adhe**sion molecule **17** (CDH17) also known as liver-intestine cadherin (LI-cadherin) is a member of the cadherin family regulated by CDX-2. CDH17 is normally expressed in gastrointestinal and pancreatic epithelium and related adenocarcinomas (Fig. 7.3) [8, 9].

CDH17 is generally negative in pulmonary adenocarcinoma, breast carcinoma, papillary thyroid carcinoma, transitional cell carcinoma, renal cell carcinoma, hepatocellular carcinoma, and mesothelioma.

Villin: Villin is an actin-binding protein and a component of brush border of different epithelial types including cells of intestinal mucosa, mucosa of fallopian tubes, and seminiferous ducts and cells lining proximal renal tubules. Villin is a marker for gastrointestinal adenocarcinomas. Ovarian, endometrioid, and renal cell carcinomas may also be positive for villin. Villin expression is also reported in well-differentiated neuroendocrine tumors of different origin.

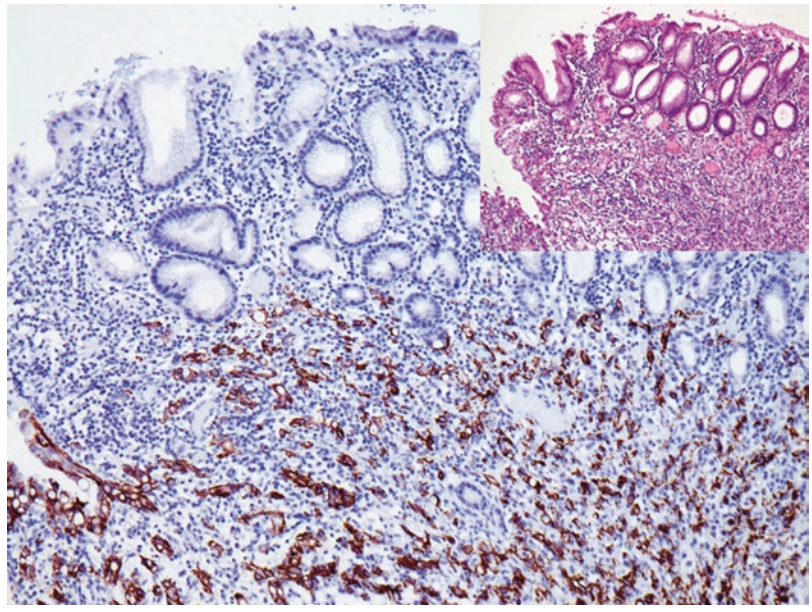

Fig. 7.3 CDH17 expression in cells of gastric adenocarcinoma

Immunoprofile of gastrointestinal tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (±)	+ in 10–50% (∓)	+ in <10% (−)
A. Esophageal and gastric tumors				
Squamous cell carcinoma of the esophagus	*CK5*/6, CK8, CK14, CK18, CK19, *p63*, *p40*	β-Catenin, cyclin D1		CK7, CK20
Adenocarcinoma of the esophagus	CK7, CK8, CK18, CK19	E-Cadherin, CDX-2, cyclin D1, villin	CK20	CK5/6, p40
Adenocarcinoma of the stomach	CK8, CK18, CK19, villin, EMA, CDH-17	CK7, CEA, CDX-2, glicentin	CK20	CK5/6, CK14, CK17, CA125, *SATB-2*
B. Intestinal tumors				
Adenocarcinoma of the duodenum and small bowel	CK8, CK18, CK19, *CDX-2*[a], villin	*CK7*, CK20, *PDX-1*, AMACR	Hep Par-1	*SATB-2*
Adenocarcinoma of the ampullary region	CK8, CK18, CK19, CK7, *PDX-1*		CK20,CDX-2	
Colorectal adenocarcinoma	CK8, CK18, CK19, *CK20, CDX-2, SATB-2*, CEA, villin, MUC-2	β-Catenin[b], CD10	CK7	CA125, CK5/6, CK14, AMACR, GATA-3, thrombomodulin
Colorectal mucinous adenocarcinoma	*CK20, CDX-2, SATB-2*, villin, β-catenin[b]		CK7, PDX-1	
Basaloid (cloacogenic) carcinoma	CK1, *CK5/6*, CK8, CK15, CK17, CK18, CK19	CK10	CK7	CK20
Anorectal squamous cell carcinoma	CK5/6, CK10, CK17, CK18, CK19			CK7, CK20
Anal Paget's disease	CK7, CK8, CK18, EMA, MUC-2	CEA	CK20, GCDFP-15	MUC-1
C. Gastrointestinal neuroendocrine tumors				
Broad-spectrum markers for gastrointestinal neuroendocrine tumors/ carcinoma: NET[c] G1 NET[d] G2 NEC[e] G3 (small and large cell type)	*Synaptophysin, chromogranin,* NSE, S100, *CD56* Epithelial markers: CK8/18, CK19, CK-MNF *Proliferation index (Ki-67) in NET G1: <2% NET G2: 3–20% NEC G3: >20%*	CDX-2, villin		CK20
Gastric ECL[f] cell NET	Broad-spectrum neuroendocrine markers		Histamine, gastrin	
Gastric EC cell NET	Broad-spectrum neuroendocrine markers		Serotonin	

Immunoprofile of gastrointestinal tumors				
Gastrinoma NET	Broad-spectrum neuroendocrine markers, gastrin			
NET of small bowel and colon	Broad-spectrum neuroendocrine markers, serotonin, CEA	CD56, CDX-2, villin, somatostatin	Pancreatic polypeptide, CK7, CK20	E-Cadherin, β-catenin
Mixed adenoneuroendocrine carcinoma (MANEC)	Broad-spectrum neuroendocrine markers, E-cadherin, β-catenin	CEA	Somatostatin, pancreatic polypeptide, serotonin	
L-cell NET	Broad-spectrum neuroendocrine markers	Pancreatic polypeptide, glucagon-like peptides		
Tubular carcinoid	Broad-spectrum neuroendocrine markers	Glucagon, serotonin		S100
NEC G3; small and large cell type	Broad-spectrum neuroendocrine markers, pan-CK, CK8/18, CK19	Vimentin, CDX-2	TTF-1, CK7	CK20

[a]Usually negative in medullary-type adenocarcinoma
[b]Nuclear stain
[c]Well-differentiated neuroendocrine tumor (carcinoid)
[d]Well-differentiated neuroendocrine carcinoma (atypical carcinoid)
[e]Poorly differentiated neuroendocrine carcinoma
[f]Enterochromaffin like cells

7.2 Gastrointestinal Mesenchymal Tumors

7.2.1 Diagnostic Antibody Panel for Gastrointestinal Stromal Tumors (GIST)

CD34, CD117 (c-Kit), PDGFR-α, DOG-1

7.2.2 Diagnostic Antibody Panel for Miscellaneous Mesenchymal Gastrointestinal Tumors

sm-Actin, h-Caldesmon, Calponin, Smoothelin, SOX-10, CDE34, β-Catenin

CD117 (c-kit; mast cell growth factor receptor; steel factor receptor)		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
GIST, seminoma, mast cell disease, melanoma, CML, AML, adenoid cystic carcinoma, thymoma and thymic carcinoma	Clear cell sarcoma, small cell lung carcinoma, pulmonary large cell carcinoma, Ewing sarcoma/PNET, follicular and papillary thyroid carcinoma, renal oncocytoma, renal chromophobe carcinoma, hepatocellular carcinoma, Merkel cell carcinoma, synovial sarcoma, osteosarcoma, chondrosarcoma, angiosarcoma, neuroblastoma, glioma	Interstitial cells of Cajal, hematopoietic progenitor cells, mast cells, melanocytes, germ cells, glial and Purkinje cells, basal cells of the epidermis, secretory cells of the breast, thymic epithelial cells, endothelial cells, renal tubular cells, ovarian stroma, and corpus luteum
Positive control: brain tissue		

Diagnostic Approach CD117 (c-kit) is a member of tyrosine kinase growth factor receptor type III family. This family includes c-Kit, platelet-derived growth factor receptor (PDGFR-α), macrophage colony-stimulating factor, and FMA-like tyrosine kinase 3 and is composed of extracellular domain, transmembrane domain, and intracellular kinase domain. Normally, the activation of CD117 takes place after the binding to the stem cell factor. CD117 is involved in the differentiation of hematopoietic cells, mast cells, germ cells, melanocytes, and intestinal cells of Cajal.

In routine immunohistochemistry, CD117 has a very wide expression spectrum and is usually used as a guide marker for the diagnosis of many tumors. The expression of CD117 is found in more than 90% of gastrointestinal stroma tumors (GISTs), whereas single or multiple activating mutations of the c-Kit gene are found in about 80% of GISTs, mainly in exon 11 and less frequently in exons 9, 13, and 17. The co-expression of CD34 and DOG-1 is a characteristic profile for the diagnosis of GIST (Fig. 7.4). CD117 is also a very helpful marker for the diagnosis of other tumors such as seminoma, mast cell tumors, chronic and acute myelogenous leukemia, thymoma, adenoid cystic carcinoma, a subset of T-ALL, and multiple myeloma [10].

Diagnostic Pitfalls 5–8% of the GISTs are associated mutations within the PDGFR-α gene (mainly in exon 18) and are usually negative for CD117. These tumors show frequently epithelioid morphology and are commonly positive for PDGFR-α and/or DOG-1 [11, 12].

Platelet-Derived Growth Factor Receptor α: PDGFR-α is a tyrosine kinase receptor, a member of the type III tyrosine kinase receptor family involved in embryonic development of different tissue types and immune response. PDGFR-α is an important marker for CD117-negative GISTs as activating mutations within the PDGFR-α gene–mainly in exons 12, 14, and 18–are found in CD117-negative GISTs. CD117-positive GISTs usually lack the expression of PDGFR-α. In the interpretation of the PDGFR-α immunostain, it is important to consider that a subset of desmoid tumors is positive for this marker. Normally, PDGFR-α stains ganglion and Schwann cells, thyroid follicular cells, and spermatogonia [13, 14].

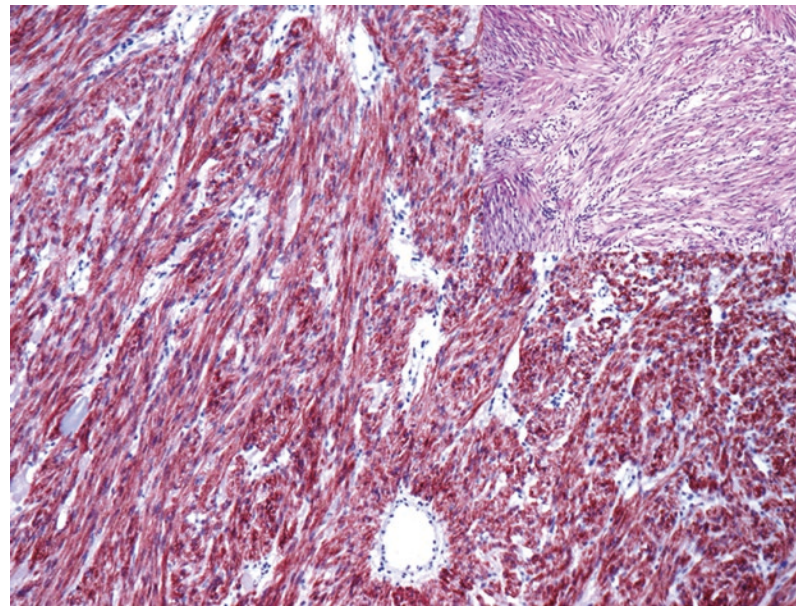

Fig. 7.4 GIST showing strong CD117 expression

DOG-1		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
GIST	Acinic cell carcinoma of salivary glands, uterine leiomyoma, synovial sarcoma, chromophobe renal cell carcinoma, renal oncocytoma, esophageal squamous cell carcinoma, hepatocellular carcinoma, biliopancreatic and acinar adenocarcinoma	Cajal cells, gastric surface epithelium, salivary gland and pancreatic acini, gallbladder epithelium, myoepithelial cells
Positive control: GIST		

Diagnostic Approach DOG-1 (anoctamin-1) is a transmembrane chloride channel protein highly expressed in the cells of Cajal of the gastrointestinal tract. DOG-1 is a highly specific marker to gastrointestinal stroma tumors (GISTs) and reacts with more than 90% of this tumor identity (Fig. 7.5). The expression spectrum of DOG-1 is different than that of CD117, but there is a high concordance between the expressions of both markers in GISTs [15–17]. Unlike CD117, DOG-1 is constantly negative in seminoma, myeloid, and mast cell tumors. DOG-1 is also an interesting marker that discriminates acinic cell carcinomas of salivary glands from other adenocarcinomas with the similar morphology as long as biliopancreatic adenocarcinomas are not in the differential diagnosis.

Diagnostic Pitfalls Low DOG-1 expression is found in up to 50% of intramural gastrointestinal leiomyoma. These are usually strongly positive for actin and h-caldesmon.

CD34: CD34 is a cell surface adhesion glycoprotein listed with the endothelial markers. CD34 labels the majority of GISTs but lacks the specificity consequently must be used in a panel with DOG-1 and CD117. In gastrointestinal mesenchymal tumors, CD34 labels also the stromal cells of inflammatory fibroid polyp of the gastrointestinal tract (Fig. 7.6).

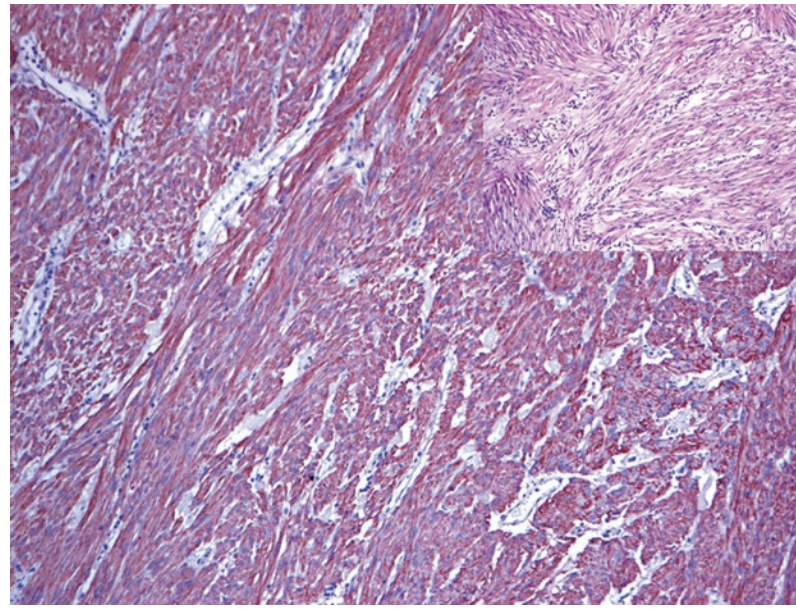

Fig. 7.5 Strong DOG-1 expression in GIST

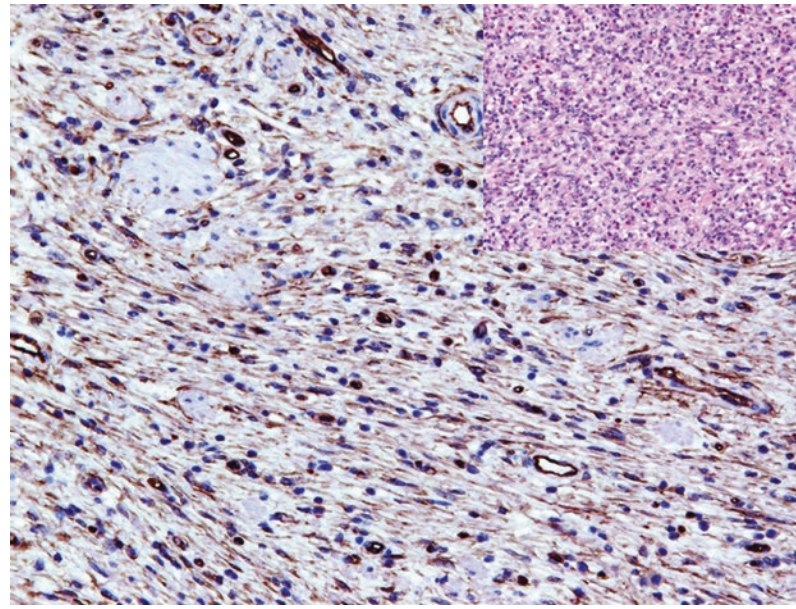

Fig. 7.6 CD34 labels stroma cells of inflammatory fibroid polyp of the gastrointestinal tract

Immunophenotype of mesenchymal gastrointestinal tumors

Tumor type	+ in >90% (+)	+ in 50–90% (±)	+ in 10–50% (∓)	+ in <10% (−)
Gastrointestinal stromal tumor (GIST)	*CD117* (c-Kit)[a], *DOG-1,* vimentin	*CD34*, CD99, nestin, bcl-2, D2–40, tau, h-caldesmon	sm-Actin, S100, CK8, CK18, PDGFR-α[b]	Synaptophysin, chromogranin, desmin, PGP9.5, calponin, β-catenin
Gastrointestinal autonomic nerve tumor (plexosarcoma) (GANT as subtype of GIST)	*CD117*, vimentin	CD34, NSE, synaptophysin, β-catenin, PGP9.5	Chromogranin, S100, neurofilaments, h-caldesmon	Desmin, actin, calponin
Inflammatory fibroid polyp of the gastrointestinal tract	*Stromal cells* CD34, fascin, cyclin D1	Calponin, CD35	Sm-Actin	CD117, S100, desmin, h-caldesmon, bcl-2
Granular cell tumor	S100, *Sox-10*, CD56, NSE, laminin, nestin	CD68, inhibin, PGP 9.5, calretinin		GFAP, neurofilaments, EMA, pan-CK
Plexiform fibromyxoma	Actin, CD10		Desmin	CD117, DOG-1
Calcifying fibrous tumor	Vimentin			Actin, desmin, h-caldesmon, CD34, CD117, pan-CK
Mesenteric fibromatosis	Vimentin, β-catenin[c]	sm-Actin	Desmin, CD117	calponin, pan-CK, S100

[a]GISTs with epithelioid morphology are frequently CD117 negative
[b]PDGFR-α positive in CD117-negative GISTs
[c]Nuclear and cytoplasmic stain (Fig. 7.7)

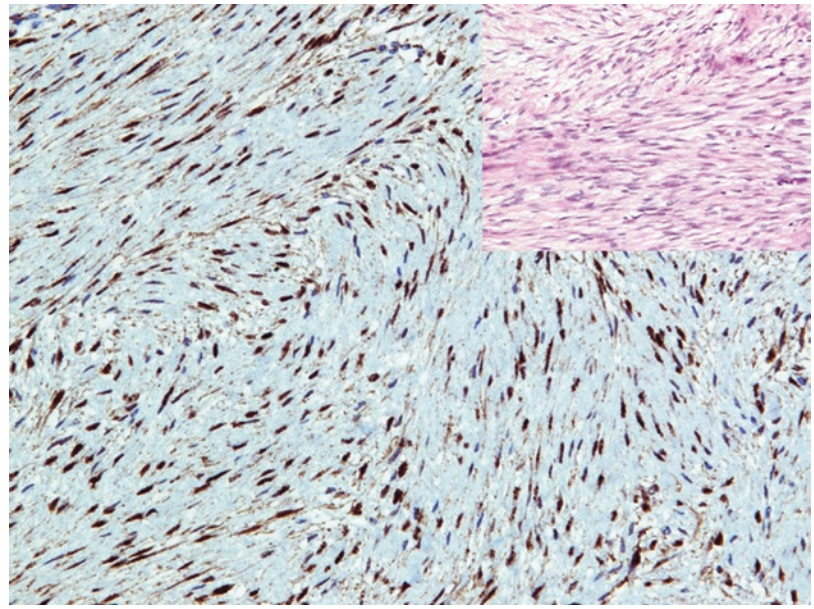

Fig. 7.7 Mesenteric fibromatosis with strong nuclear β-catenin expression

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Markers and Immunoprofile of Exocrine and Endocrine Pancreatic Tumors

8

Contents

8.1 Diagnostic Antibody Panel for Exocrine Pancreatic Tumors

Cytokeratin profile, PDX-1, S100P, CA19.9, CEA, DPG-1, IMP3, and DpC4 [1–3]

8.2 Diagnostic Antibody Panel for Endocrine Pancreatic Tumors

Cytokeratin profile, chromogranin, synaptophysin, PDX-1, CD56, PAX-6, somatostatin, insulin, gastrin, glucagon, vasoactive intestinal polypeptide (VIP), human pancreatic polypeptide (hPP), and proliferation index (Ki-67) (see also Chap. 14, Endocrine and Neuroendocrine Tumors)

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DOI 10.1007/978-3-319-53577-7_8

CA19-9		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Pancreatic and gastrointestinal carcinoma	Ovarian and lung adenocarcinoma, renal cell carcinoma, transitional cell carcinoma, mucoepidermoid carcinoma	Epithelium of the breast ducts, salivary and sweat glands, lung, gastrointestinal tract, hepatobiliary system
Positive control: Pancreatic tissue		

PDX-1		
Expression pattern: nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Pancreatobiliary adenocarcinoma, pancreatic and duodenal neuroendocrine tumors	Gastric and colorectal adenocarcinoma, pancreatic acinar cell carcinoma, prostatic carcinoma	Endocrine cells of the pancreas, pancreatic ductal epithelium and centroacinar cells, pyloroduodenal mucosa, Brunner's glands, enteroendocrine cells
Positive control: pancreatic tissue		

Diagnostic Approach CA19-9 is a glycoprotein epitope on the sialyl Lewis a structure functioning as a ligand for the adhesion molecule E-selectin. CA19-9 is normally present on the apical surface of the ductal epithelium of the breast, salivary, and sweat glands beside the glands of gastrointestinal mucosa.

CA19-9 strongly stains pancreatic, hepatobiliary, and gastrointestinal adenocarcinomas but lacks the specificity for these carcinoma types. CA19-9 has a very wide expression spectrum as it is found in many other carcinomas of different origin. Consequently, the diagnosis of primary pancreatic carcinoma must be supported by a complete immunohistochemical panel.

PDX-1 (**p**ancreatic and **d**uodenal homeobo**x** **1**) [2] also known as insulin promoter factor 1 is a transcription factor involved in the pancreatic development and maturation of the endocrine β-cells in addition to Brunner's glands, duodenal papilla, and bile ducts. In adult tissue, PDX-1 is intensely expressed in endocrine cells of the upper gastrointestinal tract and pancreas in addition to pyloroduodenal and pancreatic duct mucosa (Fig. 8.1). PDX-1 strongly labels pancreatic endocrine tumors and pancreatobiliary adenocarcinomas including adenocarcinoma of the gallbladder and cholangiocarcinoma. Weak

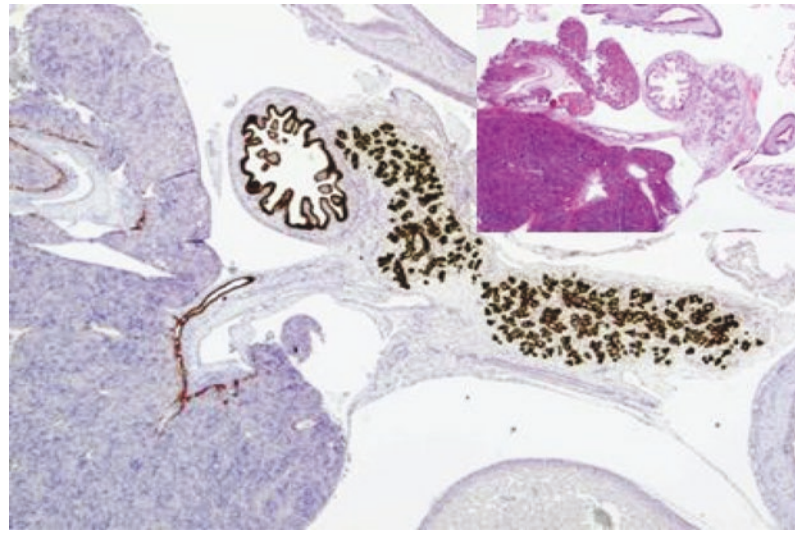

Fig. 8.1 Section through a 12-week embryo showing PDX-1 highlighting pancreatic ducts, duodenal mucosa, and mucosa of the bile ducts

expression of PDX-1 is also found in a subset of colorectal adenocarcinomas. Focal weak PDX-1 expression may be also found in the prostatic glands, lung and breast epithelium, thyroid, liver, spleen, kidney, and skin.

S100P		
Expression pattern: cytoplasmic/nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Pancreatic ductal adenocarcinoma, breast carcinoma	Non-small-cell lung carcinoma, gastrointestinal adenocarcinomas, transitional cell carcinoma, ovarian carcinoma, and melanoma	Myocardium and skeletal muscle, epithelial cells of gastrointestinal and prostatic glands, kidney, bladder, and leukocytes
Positive control: pancreatic carcinoma		

Diagnostic Approach S100P protein is one of the members of the S100 protein family consists of 95 amino acids primarily isolated from human placenta [4]. Besides the placenta, S100P is also expressed in many other types of normal tissue including the myocardium and skeletal muscle and epithelial cells of gastrointestinal tract and prostatic gland as well as the kidney, bladder, and leukocytes. S100P is also expressed in various tumor types such as non-small-cell lung carcinoma, breast carcinoma, pancreatic carcinoma including pancreatic ductal adenocarcinoma, pancreatic intraductal papillary mucinous neoplasm and preneoplastic cells, gastric and colorectal adenocarcinoma, transitional cell carcinoma, ovarian carcinoma, and melanoma [5–8]. Normal breast tissue and normal and inflamed pancreatic tissue lack the expression of S100P. This wide expression profile makes S100P a useful marker for the diagnosis of pancreatic and breast adenocarcinomas especially on small biopsies and FNP. S100P is negative in pancreatic endocrine tumors and acinar cell carcinoma. Prostatic carcinoma and renal cell carcinoma are usually negative for S100P. The expression of S100P is usually associated with a poor prognosis.

PAX-6: *PAX-6* (also known as aniridia type 2 protein, AN2) is a member of the paired box family of transcription factors. PAX-6 is a master transcription factor involved in the development of the central nervous system, endocrine glands, and sensory organs including the eye and olfactory tissue. Antibodies to PAX-6 stain neuroendocrine cells of different origin mainly those of endocrine pancreas and tumors derived from these cells (Fig. 8.2). PAX-8 is also a further marker for pancreatic neuroendocrine tumors but less specific than PAX-6 [9].

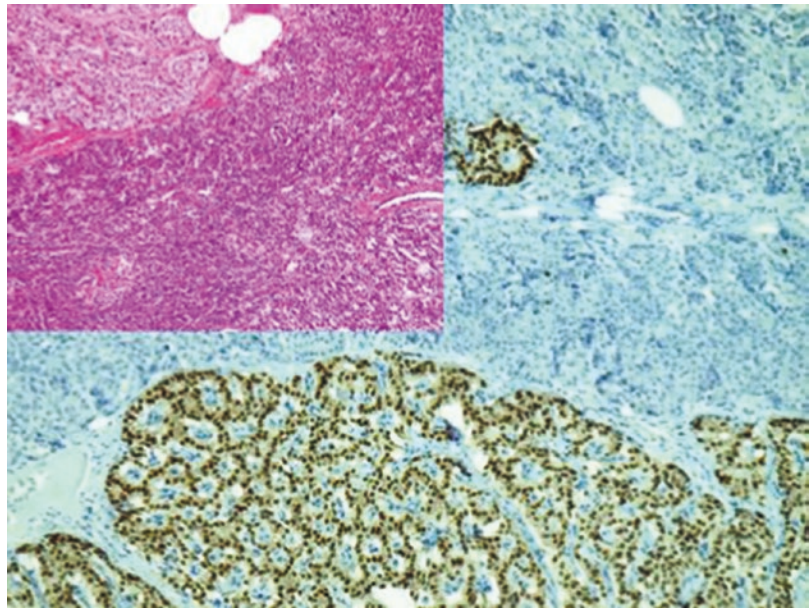

Fig. 8.2 Neuroendocrine tumor of the pancreas (NET G1). PAX-6 highlights the tumor cells and the endocrine cells of the pancreatic islets

Immunoprofile of pancreatic tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (±)	+ in 10–50% (∓)	+ in <10% (−)
A. Immunophenotype of exocrine pancreatic tumors				
Serous cystadenoma and serous cystadenocarcinoma	CK7, CK8, CK18, CK19, EMA	CK20, CA19.9		CEA, trypsin, S100
Mucinous cystic neoplasms (with low-, intermediate-, and high-grade dysplasia) and mucinous cystadenocarcinoma	CK7, CK8, CK18, CK19, EMA, S100P, CEA, CA19.9 *Ovarian-type stroma*: ER, PgR, CD10	CK20		CDX-2
Intraductal papillary mucinous neoplasm (IPMN)	CK7, CK8, CK18, S100P	CK19, CA19.9, CK20, CEA		
Oncocytic-type IPMN	CEA, CA19.9	Mesothelin	CDX-2	
Intraductal tubulopapillary neoplasm	CK7		CEA	CK20
Ductal adenocarcinoma	CK7, CK8, CK13, CK18, CK19, CEA, *S100P*, MUC1, MUC3, MUC5/MUC6, CA19.9, CA125, CEA, EMA, claudin-4	*PDX-1*, maspin, CK4, CK17, mesothelin, CDX-2, fascin, IMP3, HER-2, E-cadherin	DPC4, GATA-3	CK20, MUC2, lipase, trypsin, calretinin, thrombomodulin (CD141), vimentin

Immunoprofile of pancreatic tumors				
Acinar cell carcinoma	*Trypsin*, chymotrypsin, CK8, CK18, bcl-10	CD56, glypican-3, EMA, amylase, lipase, CEA, vimentin	CK7, CK19, AFP, PDX-1, DOG-1, chromogranin, synaptophysin	CK20, S100P, MUC1, MUC2
Solid pseudopapillary neoplasm	CD10, α-1 antitrypsin, PgR, β-catenin, NSE, vimentin	Pan-CK, CD56, CD99, galectin-3, cyclin-D1	S100, synaptophysin, CK7, CK19	Chromogranin, CA19.9, ER, CEA, AFP
Pancreatoblastoma	*Acinar cells*: CK7, CK8, CK18, CK19, EMA, trypsin, lipase *Squamoid nests*: CK8/CK18, EMA, NSE *Ductal component*: CK7, CK8, CK18, CK19, EMA, CEA *Solid component*: CK7, EMA		AFP synaptophysin, chromogranin, CEA	NSE, CEA CK5/CK6/CK14, CK7 trypsin
B. Immunophenotype of endocrine pancreatic tumors				
General screening markers for neuroendocrine pancreas tumors • NET[a] G1 • NET[b] G2 • NEC[c] G3 (small and large cell type)	CK8, CK18, CK19, CD56, chromogranin, synaptophysin, somatostatin, NSE, PGP9.5, Leu7 *proliferation index (Ki-67):* *NET G1*, *<2%* *NET G2*, *3–20%* *NEC G3*, *>20%*	*PDX-1, islet-1*, PAX-6, S100		CK5/CK6, CK7, CK20, S100P
EC[d]-cell NET	*Serotonin*			
Beta-cell NET (insulinoma)	*Insulin*, *proinsulin*	hPP[e]		
G-cell NET (gastrinoma)	*Gastrin*			
Alpha-cell NET (glucagonoma)	*Glucagon*	Glicentin		
Delta-cell NET (somatostatinoma)	*Somatostatin*		Calcitonin, ACTH	
D1-cell NET (VIPoma)	*VIP*[f]			
PP-cell NET	*hPP*			

[a]Well-differentiated neuroendocrine tumor (carcinoid)
[b]Well-differentiated neuroendocrine carcinoma (atypical carcinoid)
[c]Poorly differentiated neuroendocrine carcinoma
[d]Enterochromaffin cells
[e]Human pancreatic polypeptide
[f]Vasoactive intestinal polypeptide

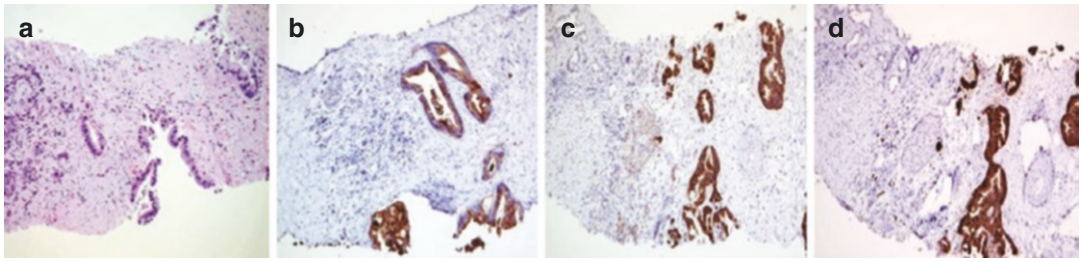

Fig. 8.3 (**a**) Pancreas core biopsy with ductal adenocarcinoma. (**b**) CEA highlighting malignant glands. (**c**) IMP3 highlighting malignant glands, whereas islet cells show also low expression intensity. (**d**) S100p highlighting malignant glands

Immunohistochemical differentiation of pancreatic ductal adenocarcinoma vs. chronic pancreatitis (Fig. 8.3).

	Ductal adenocarcinoma	Chronic pancreatitis
IMP-3	+	−
Maspin	+	−
pVHL	−	+
S100P	+	−/+
CEA	+	−/+

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Markers and Immunoprofile of Hepatobiliary Tumors

9

Contents

9.1 Hepatocellular Tumors

Diagnostic Antibody Panel for Hepatocellular Tumors Hep Par-1, arginase-1, AFP, BSEP, MDR-3, CD10, glypican-3, HSP70, CD34, and cytokeratin profile [1, 2].

Hepatocyte-specific antigen (Hep Par-1)		
Expression pattern: cytoplasmic (granular)		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Hepatocellular carcinoma, hepatoblastoma	Adrenal gland tumors, mucosal intestinal metaplasia and small intestinal adenocarcinoma, signet ring cell carcinoma, tumors with hepatoid differentiation, yolk sac tumor	Hepatocytes, intestinal enterocytes
Positive control: liver tissue		

Diagnostic Approach Hepatocyte paraffin-1 (Hep Par-1) reacts with a urea cycle enzyme located on the mitochondrial membrane of hepatocytes that is also found in the mitochondria of intestinal epithelium and cells of renal tubules. Hep Par-1 is a specific marker for liver tissue and hepatocellular tumors; however, it also labels

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small intestinal mucosa and small intestinal adenocarcinomas in addition to gastric and esophageal intestinal metaplasia including Barrett's mucosa [3–7].

Diagnostic Pitfalls Generally, extrahepatic tumors with hepatoid differentiation have the same immunoprofile as hepatocellular tumors and can be positive for Hep Par-1, AFP, and CD10 [8]. The expression of Hep Par-1 is also reported in tumors of adrenal cortex and adenocarcinomas of the stomach and small intestine, but these tumors are negative for arginase [9].

False-positive results in the immunostaining of liver tissue can be caused by the high biotin activity of the hepatocytes; thus, the inactivation of endogenous biotin is recommended to eliminate the biotin background. The use of a polymer detection system is also effective.

Arginase-1: Arginase-1 is a manganese urea cycle metalloenzyme that catalyzes the conversion of arginine to ornithine and urea. In gastrointestinal system, the expression of arginase-1 is limited to hepatocytes, whereas bile duct epithelial, sinusoidal, and endothelial cells lack the expression of this enzyme. Arginase-1 is more specific for hepatocytes and hepatocellular carcinomas than Hep Par-1 and found in 85–100% of primary and metastatic hepatocellular carcinoma, whereas the expression intensity correlates with differentiation grade of the tumor [10]. BSEP, HSP70, glypican-3, and CD34 (Fig. 9.1) can be used in a panel to support the diagnosis of hepatocellular carcinomas [11].

Various expression levels of Arginase-1 are also found in myeloid cells and macrophages.

Alpha-fetoprotein (AFP)		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Hepatocellular carcinoma, yolk sac tumor	Tumors with hepatoid differentiation, pancreatic acinar cell carcinoma, pancreatoblastoma	Fetal liver
Positive control: fetal liver		

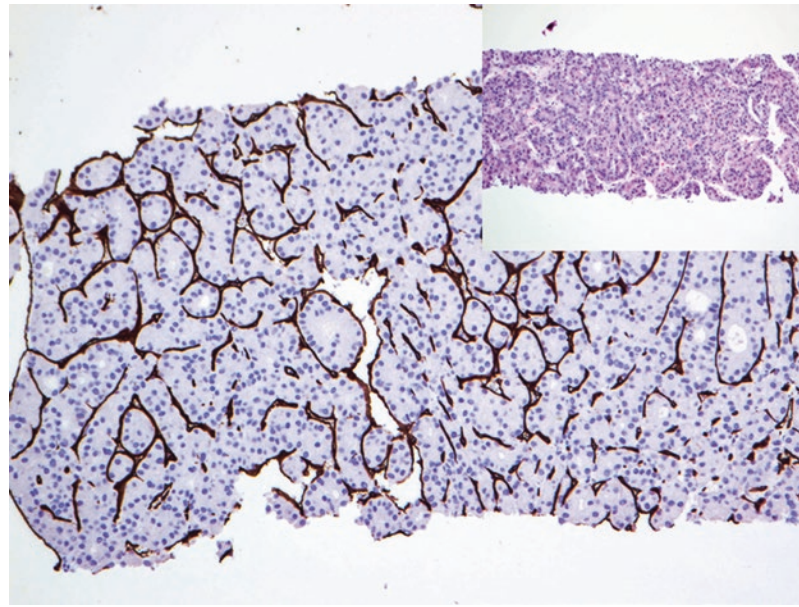

Fig. 9.1 CD34 highlighting sinusoidal cells in hepatocellular carcinoma

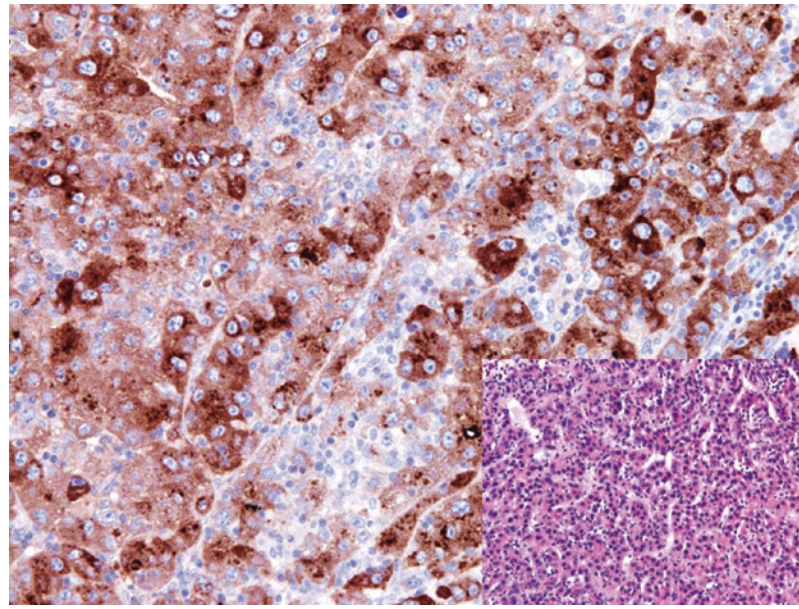

Fig. 9.2 Neoplastic cells of hepatocellular carcinoma with strong AFP expression

Diagnostic Approach Alpha-fetoprotein (AFP) is an oncofetal protein found in fetal liver, fetal gastrointestinal track, yolk sac, and fetal plasma. AFP is also present in a very low concentration in adult plasma. In the majority of cases, hepatocellular carcinoma reveals a high expression level of AFP, and a lesser expression degree is found in germ cell tumors, i.e., yolk sac tumor (Fig. 9.2).

Diagnostic Pitfalls It is important to consider that about 5% of hepatocellular carcinoma is negative for AFP. Low expression level of AFP is reported in pancreatic acinar cell carcinoma, pancreatoblastoma, and renal cell carcinoma.

Bile Salt Export Pump (BSEP) and Multidrug Resistance Protein 3 (MDR-3): BSEP is a member of the adenosine triphosphate-binding cassette transporter family encoded by the ABCB11 gene. BSEP is a membrane-associated ATP-dependent bile salt transporter protein localized on the canilicular microvilli and subcanilicular vesicles of hepatocytes and responsible for the transport of bile-conjugated salts out of hepatocytes into the canaliculus system [12].

The multidrug resistance protein 3 (MDR-3) is another member of the same transporter family and a transmembrane protein also involved in the transport of bile salts from hepatocytes. Both BSEP and MDR-3 are expressed exclusively on the membrane of hepatocytes and used as sensitive and specific markers for hepatocytes and hepatocellular tumors. These markers can be also used to differentiate between hepatocellular and bile duct tumors [13].

Glypican-3: Glypican-3 is a membrane and extracellular heparan sulfate glycoprotein that regulates signaling during embryogenesis. Glypican-3 is normally expressed in fetal tissue and trophoblasts. In adult tissue, the expression of glypican-3 is restricted to few tissue types, namely, gastric glands and renal tubules. Glypican-3 is also expressed in a wide range of epithelial and mesenchymal tumors including pulmonary squamous cell carcinoma and small

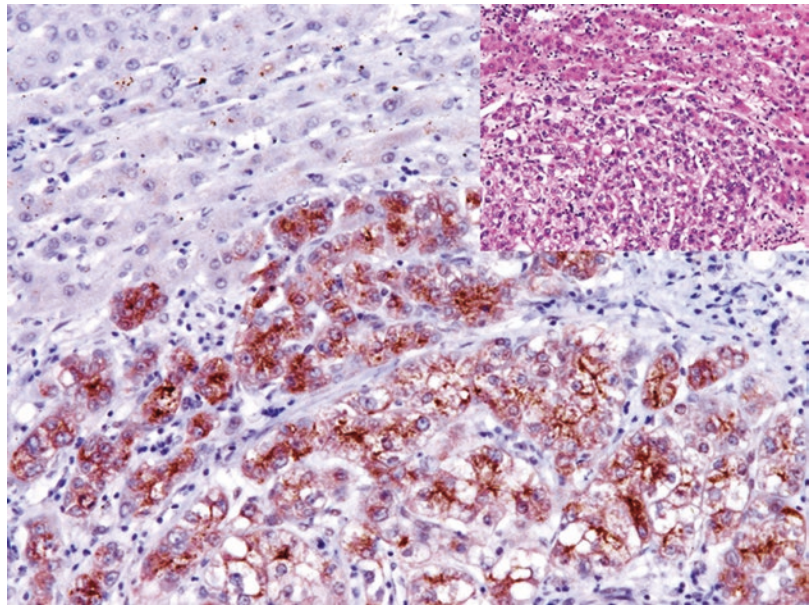

Fig. 9.3 Glypican-3 expression in hepatocellular carcinoma. Note negative reaction in nonneoplastic liver tissue

cell carcinoma, hepatocellular carcinoma and hepatoblastoma, acinar carcinoma of the pancreas, neuroblastoma, Wilms' tumor, yolk sac tumor and choriocarcinoma, liposarcoma, and rhabdomyosarcoma. Glypican-3 is a helpful marker to distinguish between hepatocellular carcinoma and benign liver tissue, but it is important to consider that it could be focally positive in cirrhotic liver tissue, active chronic hepatitis C, and dysplastic liver nodules (Fig. 9.3). Embryonal carcinoma and seminoma lack the expression of glypican-3.

Heat-Shock Protein-70 (HSP70): HSP70 is an anti-apoptotic regulator expressed in different malignant tumors. In routine immunohistochemistry, HSP70 can be used as a marker to discriminate between hepatocellular carcinoma positive for HSP70 (nuclear/cytoplasmic staining pattern) and dysplastic nodules or hepatocellular adenoma negative for HSP70. Since HSP70 is expressed in different malignant tumors, it cannot be used to discriminate between hepatocellular carcinoma and metastatic carcinoma [14, 15].

9.2 Cholangiocarcinoma

Diagnostic Antibody Panel for Cholangiocarcinoma and Gallbladder Carcinoma Cytokeratin profile, hepatocellular markers, CEA, PDX-1, and TTF-1.

All these markers are listed in details in other sections. PDX-1 is also a specific marker for primary cholangiocarcinoma. Despite the fact that TTF-1 is a specific marker for pulmonary and thyroid carcinomas, a weak to moderate nuclear expression is also found in cholangiocarcinoma which to consider in the differential diagnosis of hepatic and metastatic tumors [16].

Immunoprofile of hepatobiliary tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (±)	+ in 10–50% (∓)	+ in <10% (−)
Hepatocellular adenoma	Arginase-1, Hep Par-1, *CD34*[a]	ER, PgR		*Glypican-3, AFP*, HSP70
Hepatocellular carcinoma	*Arginase*-1[b], *Hep Par-1, BSEP, MDR-3, glypican-3,* CK8/18, *CD34*[a]	*AFP,* SATB-2, EMA, CD138, CD10[c], CK7[d] CEA[e], HSP70, ER, MAGE-1, osteonectin, CD66a, CD56, CD68[f]	CK19[g], CK20[g], BER-EP4, PgR, vimentin	CK5/6, CK14, EMA, inhibin, Melan A, EPCAM[h]
Hepatoblastoma	*Hep Par-1*, pan-CK, *glypican-3*	CK18, AFP, CEA, CD34, EMA, chromogranin, vimentin	S100	
Cholangiocarcinoma	*CK7*, CK8, CK18, CK19, CEA, EMA, CK17, S100P, *PDX*-1	CK20[i], CDH17, CD5	CDX-2, TTF-1[i], vimentin	AFP, CK5/6, CD56[j],
Biliary mucinous cystic neoplasm • Cystadenoma • Cystadenocarcinoma	CK7, CA125, CA19.9, CEA *Ovarian type stroma*: ER, PgR, CD10	CK20		
Angiomyolipoma	*HMB45,* HMB50, *Melan A,* actin, CD63 (NK1-C3), calponin, PgR	CD117	MIFT, ER	EMA, pan-CK
Adenocarcinoma of the gallbladder	*CK7,* CK18, CK19, EMA, CEA, S100P		CK20	Arginase-1, BSEP, MDR-3, CK5/6

[a]Labels sinusoidal endothelium lining neoplastic trabeculae, which are absent or rare in normal liver parenchyma
[b]The intensity of expression correlates with the differentiation of HCC
[c]Apical canalicular staining pattern
[d]Strong positive in fibrolamellar hepatocellular carcinoma and up to 50 in conventional hepatocellular carcinoma but usually negative in normal hepatocytes
[e]Only polyclonal CEA antibody exhibiting canalicular staining pattern but negative with monoclonal antibody
[f]Positive in fibrolamellar hepatocellular carcinoma, negative in conventional HHC and normal hepatocytes
[g]Negative in normal hepatocytes
[h]EPCAM (BerEp-4) usually positive in hepatoid carcinomas but negative in hepatocellular carcinoma
[i]The expression of CK20 and TTF-1 is only characteristic for carcinomas originated from extrahepatic bile ducts. Carcinomas of intrahepatic bile ducts are usually negative for these markers [16]
[j]The expression of CD56 is found only in a very small subset of carcinomas originated from intrahepatic bile ducts [17]

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Markers and Immunoprofile of Breast Tumors

10

Contents

Normal breast tissue consists of mesenchymal and epithelial components, which in their turn includes ductal and acinar (lobular) and myoepithelial components, each cell type having its characteristic immunoprofile. The immunoprofile of breast tumors depends on the origin of neoplastic cells.

10.1 Diagnostic Antibody Panel for Breast Carcinoma

Cytokeratin profile, estrogen and progesterone receptors, GATA-3, mammaglobin, GCFPD-15, E-cadherin, NY-BR-1, S100P, and HER-2.

10.2 Diagnostic Antibody Panel for Fibroepithelial Tumors

Cytokeratin profile, proliferation index (Ki-67).

10.3 Diagnostic Antibody Panel for Mesenchymal Tumors

See panels of other mesenchymal tumors.

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Estrogen receptor		
Expression pattern: nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Breast and endometrial carcinoma	Ovarian serous, mucinous, and endometrioid carcinoma, transitional cell carcinoma, hepatocellular carcinoma, gastric adenocarcinoma, skin adnexal tumors, uterine leiomyoma and leiomyosarcoma	Breast, endometrium, myometrium, and endometrial stromal cells, fallopian tube mucosa, sweat glands, salivary glands, hepatocytes, pituitary gland
Positive control: normal breast tissue		

Diagnostic Approach Estrogen receptors (ER) are a member of the steroid family of ligand-dependent transcription factors and include two types encoded by two different genes on different chromosomes, the alpha type (ER-α) and beta type (ER-β); each type includes different splice variants. Both types have different distributions in different organs and different tissue types [1]. The ER-α type is mainly expressed in both epithelial and stromal cells of the breast, uterus, placenta, liver, CNS, endothelium, and bone, whereas the ER-β type is mainly expressed in the prostate, testes, ovary, spleen, thymus, skin, and endocrine glands including thyroid and parathyroid glands, adrenal glands, and the pancreas. Anyway, many tissue types show the expression of both receptor types.

The expression of estrogen receptors (ER) is a good marker for the majority of breast carcinomas in addition to tumors of uterine and ovarian origin. Adequate and rapid tissue fixation with buffered neutral formalin is required for optimal stain results. For all steroid receptors, any stain pattern other than nuclear must be interpreted as negative. The expression of ER-α type is an important predictor for the response to the anti-hormone therapy (Fig. 10.1) [2]. Few scoring systems were suggested for semiquantitative estimation of estrogen and progesterone receptors. The modified scoring system suggested in 1987 by Remmele, the modified scoring system suggested in 1985 by McCarty, and the Allred scoring system proved to be the most practical and simplest systems. The three systems depend on

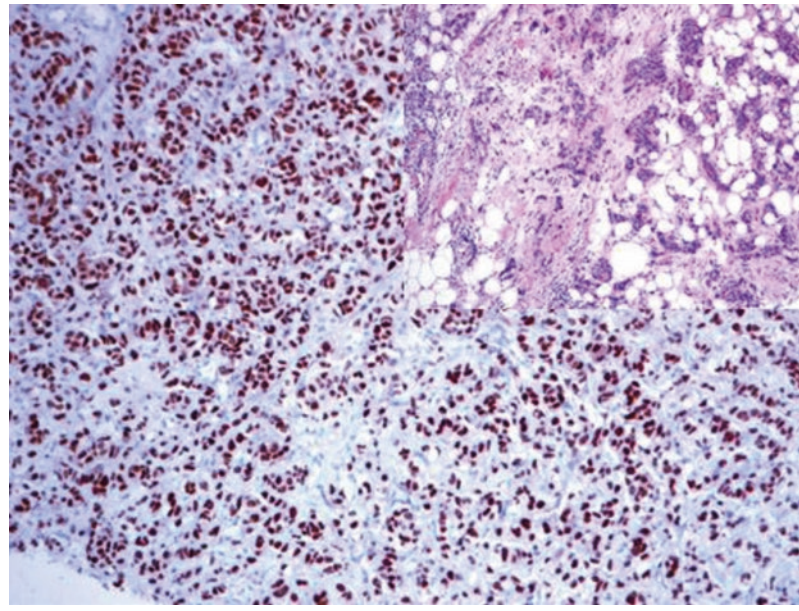

Fig. 10.1 Strong nuclear expression of estrogen receptors in breast carcinoma

the evaluation of the nuclear stain intensity and the percentage of positive tumor cells.

Remmele Scoring System This simple scoring system [2–4] has a 12-point scale (0–12). To calculate the score, one of the numbers 0, 1, 2, or 3 is given according to the intensity of the nuclear stain, and one of the numbers 0, 1, 2, 3, or 4 is given according to the percentage of positive tumor cells (see table). The score is calculated by multiplying the number reflecting the dominant stain intensity by the number reflecting the percentage of these positive tumor cells with a maximum score value of 12 (3 × 4). Tumors with a score of less than 3 show usually a poor response to the antiestrogen therapy.

Calculation of Remmele score

Percentage of positive cells		Intensity of the stain	
0	No positive cells	0	No detectable stain
1	Positive cells less than 10%	1	Weak nuclear stain
2	Positive cells 10–50%	2	Moderate nuclear stain
3	Positive cells 51–80%	3	Strong nuclear stain
4	Positive cells more than 80%		

McCarty Scoring System This scoring system [5] has a 300-point scale (0–300). The McCarty histoscore is the total value of each percentage of positive cells (0–100) multiplied by the number reflecting the intensity of the immunohistochemical stain (0, no detectable staining; 1, weak nuclear staining; 2, moderate nuclear staining; 3, strong nuclear staining) and calculated as the following:

- Percentage of tumor cells with strong positivity X 3 = **A**
- Percentage of tumor cells with moderate positivity X 2 = **B**
- Percentage of tumor cells with weak positivity X 1 = **C**

The value of the histoscore = **A** + **B** + **C**.

The clinical significance of this histoscore is explained as the following:

50 or less: negative (−)
51–100: weakly positive (+)
101–200: moderately positive (++)
201–300: strongly positive (+++)

Allred Scoring System The Allred scoring system has an 8-point scale (0–8). This scoring system is calculated by adding the number representing the proportion of positive cells 0, 1, 2, 3, 4, or 5 to the number reflecting the intensity of the nuclear stain 0, 1, 2, or 3 (see table below). Tumors with a score of less than 3 show usually a poor response to the antiestrogen therapy.

Calculation of Allred score

Percentage of positive cells		Intensity of the stain	
0	No positive cells	0	No detectable stain
1	Positive cells less than 1%	1	Weak nuclear stain
2	Positive cells 1–10%	2	Moderate nuclear stain
3	Positive cells 10–33%	3	Strong nuclear stain
4	Positive cells 33–66%		
5	Positive cells more than 66%		

Diagnostic Pitfall The expression of ER depends on the histological type and differentiation grade of the breast tumor. Additionally, the expression of ER is not restricted to the above-mentioned organs and tissue types but also can be found in other tumors such as hepatocellular carcinoma and transitional cell carcinoma. Additional markers such as GATA-3, mammaglobin, GCDFP15, and progesterone receptors as well as the cytokeratin profile are helpful to confirm the diagnosis of primary breast carcinoma.

Progesterone receptor		
Expression pattern: nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Breast carcinoma, endometrial carcinoma	Skin adnexal tumors, meningioma, solid pseudopapillary tumor of pancreas, stroma of mixed epithelial stromal tumor of the kidney, stromal tumors of the prostate	Breast and endometrial cells, endometrium stromal cells
Positive control: normal breast tissue		

Diagnostic Approach Progesterone is a steroid hormone involved in the differentiation of breast parenchyma and endometrium in addition to milk protein synthesis. Progesterone receptors (PgR) are good marker for breast carcinomas and have more specificity than estrogen receptors as they are expressed only in a limited number of tumors such as endometrial carcinoma. The progesterone receptor status is one of the important prognostic factors in breast, endometrial, and ovarian cancers [2]. A high expression level of both estrogen and progesterone hormone receptors is a positive prognostic factor for breast and endometrial cancers and predicts good response to antiestrogenic therapy.

Diagnostic Pitfalls Similar to the estrogen receptors, the expression of PgR depends on the grade of tumor differentiation. High-grade carcinomas are often negative for steroid receptors.

GATA-3		
Expression pattern: nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Breast carcinoma, transitional cell carcinoma of the urinary tract, tumors of skin adnexa, yolk sac tumor	Endometrioid carcinoma, trophoblastic tumors/ choriocarcinoma, basal cell carcinoma, mesothelioma, pancreatic ductal adenocarcinoma, colorectal adenocarcinoma, different salivary gland tumors, chromophobe renal cell carcinoma, bladder small cell carcinoma, paraganglioma, neuroblastoma, pheochromocytoma, adrenal cortical carcinoma, squamous cell carcinoma of different locations, peripheral T-cell lymphoma	Adult breast, terminal ducts of parotid gland, urinary bladder and renal pelvis mucosa, prostatic basal cells and seminal vesicle epithelium, cortex and medulla of adrenal gland, ductal epithelium of skin adnexa and salivary glands, trophoblasts, T-lymphocytes
Positive control: normal breast tissue		

Diagnostic Approach GATA-3, also known as endothelial transcription factor 3, is one of the six members of the GATA family of transcription factors taking part in the regulation of proliferation and differentiation of luminal epithelium of breast glands. GATA-3 is also involved in the differentiation of T-lymphocytes and skin adnexa. In diagnostic immunohistochemistry, GATA-3 is widely used as a marker for primary and metastatic breast carcinoma and transitional cell carcinoma (Fig. 10.2) [6, 7]. In breast carcinomas, the expression of GATA-3 strongly correlates with the expression of the estrogen receptors but lacks the therapeutic and prognostic value. The expression of GATA-3 is found in up to 90% of breast carcinoma while the lowest expression level is found in triple negative breast carcinomas as well as metaplastic and sarcomatoid breast carcinomas (<70%). Only one third of male breast carcinomas are positive for GATA-3 [8]. Generally, high expression levels of GATA-3 in breast cancer predict a good prognostic outcome. GATA-3 as a marker for urothelial tumors is discussed in a later section.

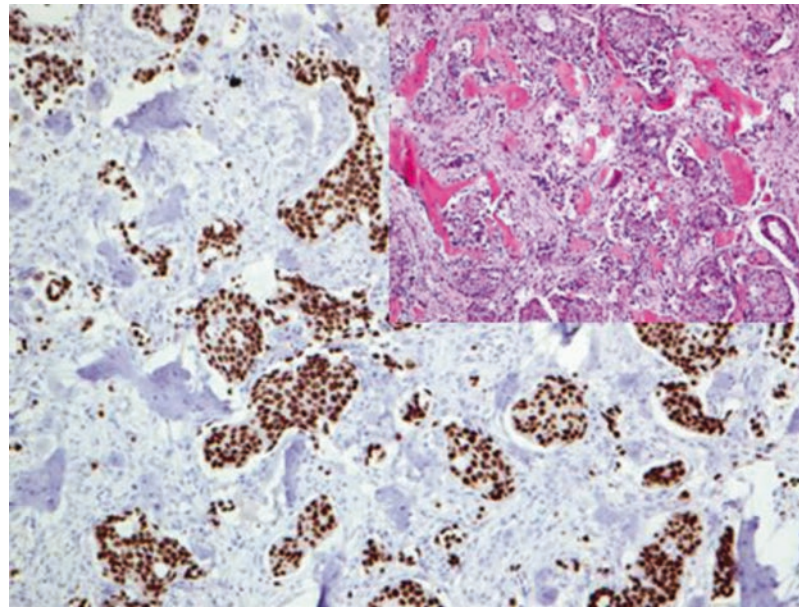

Fig. 10.2 Bone metastases of invasive ductal breast carcinoma. Tumor cells with strong nuclear GATA-3 expression

Diagnostic Pitfalls The expression of GATA-3 is not restricted to breast and urothelial tumors but also found in a wide range of tissue and tumor types, which to consider the interpretation of this marker [9]. Different expression intensities of GATA-3 are found in mesotheliomas, squamous cell carcinoma of different origin, pancreatic ductal adenocarcinoma, tumors of skin adnexa, and various types of benign and malignant salivary gland tumors including salivary duct carcinoma, acinic cell carcinoma, adenoid cystic carcinoma, and epithelial-myoepithelial carcinoma [10, 11]. Minor cases of endometrium carcinoma are also reported to express GATA-3. Furthermore, the expression of GATA-3 is characteristic for T-lymphocytes and peripheral T-cell lymphomas. Noteworthy is the expression of GATA-3 in the epithelium of seminal vessels and reactive mesothelium, which can be the source of misinterpretation. Accordingly, GATA-3 is a multilineage marker that lacks the specificity to breast and urothelial tumors, and the abovementioned notes must be considered in the interpretation of the GATA-3 stain.

Mammaglobin		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Breast carcinoma	Endometrioid adenocarcinoma, endocervical adenocarcinoma, sweat gland carcinoma, salivary gland carcinoma	Adult breast
Positive control: normal breast tissue		

Diagnostic Approach Mammaglobin is a low molecular protein and a member of the secretoglobin-uteroglobin family, homologous to the human Clara cell protein expressed in adult breast tissue [12]. Monoclonal antibodies to mammaglobin are good markers for tumors of breast origin, but the expression of mammaglobin is found only in 80–90% of primary breast carcinoma and lymph node metastases [13, 14].

Diagnostic Pitfalls Similar to the other breast markers, the expression of mammaglobin is

not restricted to breast tissue and breast tumors, but can be found in a subset of other tumor types including endometrioid carcinoma, sweat gland carcinoma, salivary gland tumors and in a small subset gastrointestinal cholangiocellular and pulmonary adenocarcinomas. Mesothelioma constantly lacks the expression of mammaglobin.

Gross cystic disease fluid protein 15 (GCDFP-15)		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Breast carcinoma	Salivary gland tumors, apocrine skin adnexal tumors, apocrine tumors, pulmonary adenocarcinoma, renal cell carcinoma, ovarian and endometrial carcinomas	Apocrine, lacrimal, ceruminous, Moll's, and cutaneous eccrine glands; serous cells of submandibular, sublingual, and minor salivary glands; serous cells of nasal and bronchial glands
Positive control: breast tissue/skin (apocrine cells)		

Diagnostic Approach Gross cystic disease fluid protein 15 (GCDFP-15) is a prolactin-inducible protein initially isolated from the fluid of cystic disease of the human breast. GCFP-15 is expressed by apocrine cells or cells with apocrine metaplasia and regulated by the androgen receptor [15]. Ductal and lobular cells lack the expression of GCFP-15. Antibody to GCDFP-15 reacts with apocrine cells of different origin and related tumors. According different reports, 30–90% of primary and metastatic breast carcinomas are positive for GCDFP-15. Triple negative breast carcinoma is usually negative for GCFP-15.

Diagnostic Pitfalls GCDFP-15 is also expressed in other apocrine, eccrine, and serous glandular epithelium and carcinomas derived from these glands including tumors of skin adnexa, which to consider in the differential diagnosis between primary skin tumors and metastases of breast carcinoma [16].

NY-BR-1: NY-BR-1 is a breast differentiation antigen expressed in normal breast epithelium and in up to 60% of breast carcinomas. The immunohistochemical reaction shows cytoplasmic and occasional nuclear stain pattern, and the expression intensity correlates with the differentiation of the tumor and the expression grade of estrogen receptors [17]. Sweat glands and about one third of sweat gland tumors are also positive for NY-BR-1.

HER-2 (c-erb-2)		
Expression pattern: membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Breast carcinoma with HER-2 overexpression for immunotherapy	Gastrointestinal adenocarcinomas, carcinomas of the salivary glands, ovarian and endometrial carcinomas, subset of pulmonary adenocarcinoma	Breast epithelium
Positive control: HER-2-positive tumors/brain tissue		

Diagnostic Approach Human epidermal growth factor receptor-2 (HER-2), also known as p185, ERBB-2, or c-erbB-2 (**c**hicken **er**ythro**b**lastic viral oncogene homolog 2), is one of the four members of the epidermal growth factor receptor family clustered as CD340. The HER-2 receptor consists of extracellular, transmembrane, and intracellular domains. In contrast to the other

members of this family, HER-2 does not have a ligand-binding domain, and the activation of this receptor appears by its dimerization. The HER-2 molecule is a part of the membrane of normal epithelial cells, and 20×10^3 to 50×10^3 receptors are generally found on the surface of normal breast epithelial cells. During carcinogenesis, the amplification of the HER-2 gene located on chromosome 17 (17q12) occurs, causing the overexpression of the HER-2 receptor, and up to 3×10^6 receptors may be expressed on the membrane of these tumor cells. The overexpression of HER-2 is characteristic for few types of human carcinomas, mainly breast and gastric adenocarcinomas in addition to a subset of other carcinoma types such as ovarian carcinoma, non-small cell carcinoma of the lung and salivary gland carcinoma, and urinary bladder transitional cell carcinoma [18]. The amplification of the HER-2 gene can be detected by the FISH assay. A good alternative is the semiquantitative detection using specific antibodies. Immunohistochemistry is an easy test to estimate of the corresponding overexpression of the HER-2 molecule on the membrane of tumor cells. The immunohistochemical expression score is an important parameter for immunotherapy of breast carcinomas and other HER-2 positive carcinomas. For the precise estimation of the HER-2 expression score, the following factors are to be considered:

- Only tissue with optimal fixation is used for HER-2 immunostaining.
- The interpretation of the immunostain must begin with the evaluation of standardized control slides with the scores 0, 1+, and 3+.
- Only membranous staining should be evaluated. Cytoplasmic or nuclear stain must be neglected. Staining caused by edge artifacts should also be ignored.
- Only invasive tumor components can be considered.

The following table shows the criteria for the estimation of the HER-2 score in breast cancer. Note that the criteria for HER-2 score evaluation in other tumors (specifically gastric cancer) vary and may depend on specimen type.

Scoring of HER-2 expression in breast cancer

Score	HER-2 overexpression	Staining result
0	Negative No gene amplification	No detectable staining or membrane staining in less than 10% of tumor cells
1+	Negative No gene amplification	A faint partial membrane staining in more than 10% of tumor cells
2+	Positive	A weak to moderate staining of the entire membrane in more than 10% of tumor cells
3+	Positive High gene amplification	A strong staining of the entire membrane in more than 10% of tumor cells (Fig. 10.3)

Tumors with the scores 0 or 1+ have no HER-2 overexpression and consequently no evidence for gene amplification, and are not sensitive for the specific immunotherapy. Tumors with the score 3+ are associated with HER-2 overexpression and show a good response to the specific antibody therapy, whereas tumors with the score 2+ needs a further confirmation to estimate the number of gene copies in the tumor cells. This can be achieved by genetic or chromosomal studies such as fluorescent in situ hybridization (FISH), chromogenic in situ hybridization (CISH), and real-time PCR assays. The presence of less than two gene copies in the examined tumor cells indicates no gene amplification, whereas 2–6 copies signify low-level amplification, and more than six gene copies signify strong gene amplification.

Diagnostic Pitfalls HER-2 is not a specific marker for breast tissue or breast carcinomas and the overexpression of HER-2 found only in up to 30% of breast carcinomas mainly in high-grade carcinoma of no special type. Similar amplification maybe also noted in other carcinoma types of different origin.

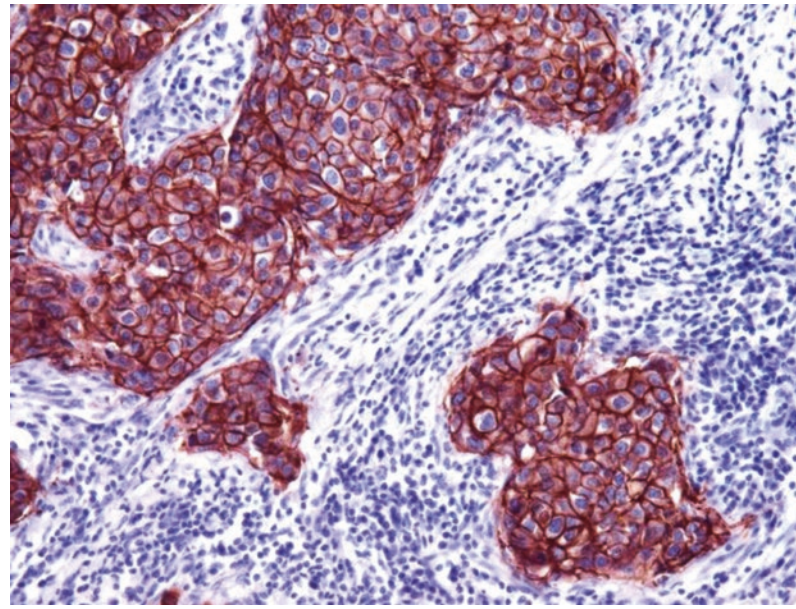

Fig. 10.3 Breast carcinoma with strong expression of HER-2 in all tumor cells (score 3+)

E-Cadherin: E-cadherin is a transmembrane glycoprotein, a member of the cadherin superfamily, and the major calcium-dependent cell adhesion molecule of epithelial cells. The expression of E-cadherin is associated with epithelial stratification and polarization in addition to gland formation [19]. E-cadherin is expressed in various types of epithelial cells and carcinomas originated from these cells. In routine histopathology, E-cadherin is a useful marker to discriminate between ductal and lobular breast carcinoma as lobular breast neoplasms lack the E-cadherin expression. The absence of E-cadherin in the cells of lobular neoplasms leads to the intracytoplasmic accumulation of p120 catenin, making it an interesting marker for lobular carcinomas of the breast. E-cadherin is also used as a marker to differentiate between reactive mesothelial proliferation, usually negative for E-cadherin, and mesotheliomas, mostly positive for E-cadherin. E-cadherin is also a prognostic marker for various carcinoma types such as breast and transitional carcinoma as the loss of E-cadherin expression is associated with aggressive behavior.

Immunoprofile of breast tumors				
Tumor types	+ in >90% (+)	+ in 50–90% (±)	+ in 10–50% (∓)	+ in <10% (−)
Ductal hyperplasia (usual ductal hyperplasia; UDH)	UDH composed of heterogeneous cell populations: 1. Glandular epithelial cells: positive for CK7, CK8, CK18, and CK19 2. Intermediate myoepithelial cells positive for CK5/6, CK14, CK8, CK18, CK19, and actin 3. Myoepithelial cells positive for CK5/6, CK14, p63, actin, and myosin Intact basement membrane positive for laminin			
Atypical ductal hyperplasia (ADH)	1. Clonal proliferation of luminal glandular epithelial cells: CK7, CK8, CK18, CK19 2. No luminal or only rare residual CK5/6/14, p63 positive intermediate myoepithelial cells 3. Intact layer of basal myoepithelial cells positive for CK5/6, CK14, p63, actin, and myosin 4. Intact basement membrane positive for laminin Luminal glandular cells positive for estrogen (ER) and progesterone receptors (PgR) and negative for HER-2 and p53			

Immunoprofile of breast tumors				
Ductal carcinoma in situ[a] (DCIS) low grade	CK7, CK8, CK18, CK19, ER Preserved basal myoepithelial cell layer positive for myoepithelial markers (p63, CK5/6/14, actin, myosin)	PgR, bcl-2	HER-2, p53, cyclin D1	
Ductal carcinoma in situ[a] (DCIS) high grade	CK7, CK8, CK18, CK19, *E-cadherin* Preserved basal myoepithelial cell layer positive for myoepithelial markers (p63, CK5/6/14, actin, myosin)	Cyclin D1, HER-2, p53	ER, PgR, bcl-2	
Lobular carcinoma in situ[a] (LCIS)	CK7, CK8, CK18, CK19, GATA-3 Preserved basal myoepithelial cell layer positive for myoepithelial markers (p63, CK5/6/14, actin, myosin)	ER expression in 80–95%	Cyclin D1	CK20, p53, *E-cadherin*[b], HER-2
Invasive carcinoma of no special type (invasive ductal carcinoma)	CK7, CK8, CK18, CK19, CD44, *GATA-3, E-cadherin,* β-catenin	Maspin, human milk fat globule, EGFR ER expression in 70–80% PgR in 70–80%	GCDFP15, bcl-2, CK10/13 HER-2 overexpression in 15–20%	CK1, CK14, CK17, CK20
Invasive lobular carcinoma	*CK7*, CK8, CK18, CK19, *GATA-3, p120 catenin*	GCPF15, CEA, cyclin D1, maspin, ER expression in 80–95% PgR in 80–90% AR in 80%	HER-2, EGFR	*E-cadherin*, CK5/6, CK14, CK20
Tubular carcinoma	*CK7*, CK18, CK19, *GATA-3* Absent of basal myoepithelial cell layer positive for myoepithelial markers (p63, CK5/6/14, actin, myosin)	ER and PgR expression in 90–100%	HER-2	HER-2, CK5/6, CK20
Cribriform carcinoma	*CK7*, CK8, CK18, CK19, *GATA-3*	Human milk fat globule ER expression in 80–100%, PgR expression in ~70%	CK10/13	HER-2, CK14, CK20

Immunoprofile of breast tumors				
Mucinous carcinoma	CK7, CK18, CK19, CEA, NSE	ER expression in ~90%, PgR in 70–80% WT-1	EGFR	HER-2, CK20, CDX-2
Papillary carcinoma	CK7, CK18, CK19, CEA	ER and PgR expression in ~90–100%		CK5/6, CK14
Medullary carcinoma and carcinoma with medullary features	CK 8, CK 18	p53, EGFR	Vimentin, S100, CK5/6, CK14 ER and PgR[c] expression in 0–10%	HER-2, CK7, CK19, CK20, GCDFP15
Carcinoma with apocrine differentiation	CK8, CK18, CK19, *androgen receptors*	*GCDFP15*, CEA, ER-β		HER-2, ER-α, PgR, S100
Breast tumors of salivary gland type: • Adenoid cystic carcinoma • Mucoepidermoid carcinoma • Polymorphous carcinoma	See salivary gland tumors			
Secretory carcinoma	CK8, CK18, CK19, EMA, lactalbumin	CK5/6, S100, GATA-3, CEA, vimentin	ER and PgR expression in <10%	HER-2
Oncocytic carcinoma	CK8, CK18, EMA	CK7, ER, PgR	HER-2, GCDFP-15	
Metaplastic carcinoma	Vimentin, Pan-CK	CK7, CD44	EMA, Actin, S100, GATA-3	ER, PgR
Basal-like phenotype of invasive ductal carcinoma	CK5/6, CK14, p63, EGFR	Vimentin, CK17, *SOX-10*		ER, PgR, HER-2
Paget's disease of the nipple	CK7, CK8, CK18, EMA (MUC-1), CD63 (NK1-C3)	CEA, GCDFP15, HER-2	ER, PgR	CK5/6, CK20, MUC-2
Myofibroblastoma of the breast	Desmin, CD34, CD99, bcl2, vimentin	CD10, androgen receptors, actin	PgR	Pan-CK, S100, ER
Phyllodes tumor	*Stromal cells*: vimentin *Epithelial cells*: CK 5/6, CK14, CK8/18, Pan-CK, EMA Proliferation index (Ki-67) in benign type usually <20% In malignant type usually >20%	bcl-2 CEA	CD34, actin, desmin, CD10, CD117	S100, Pan-CK, EMA

[a]No luminal or only residual of CK5/6/14 positive intermediate myoepithelial cells. Intact layer of basal myoepithelial cells positive for CK5/6, CK14, p63, Actin and myosin, h-caldesmon or calponin

[b]E-cadherin is positive in normal nonneoplastic breast lobular cells

[c]ER and PgR are usually negative in typical medullary carcinoma

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Markers and Immunoprofile of Tumors of Female Reproductive Organs

11

Contents

11.1 Diagnostic Antibody Panel for Tumors of the Vulva and Vagina

Cytokeratin profile, p63, CEA, p16, HPV, steroid hormone receptors, desmin, myogenin, and melanoma markers.

11.2 Diagnostic Antibody Panel for Tumors of the Uterine Cervix

Cytokeratin profile, p63, CEA, PAX-8, PAX-2, p16, p53, HPV, and steroid hormone receptors.

11.3 Diagnostic Antibody Panel for Epithelial Tumors of the Uterine Corpus, Fallopian Tube, and Uterine Ligament

Cytokeratin profile, CEA, PAX-8, p16, p53, HNF-1β, and steroid hormone receptors.

M.S.A. Tuffaha et al., *Immunohistochemistry in Tumor Diagnostics*,
DOI 10.1007/978-3-319-53577-7_11

11.4 Diagnostic Antibody Panel for Uterine Mesenchymal Tumors

Smooth muscle markers, CD10, and steroid hormone receptors.

p16		
Expression pattern: nuclear/cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
HPV-associated oropharynx and uterine cervix squamous cell carcinoma, atypical lipomatous tumors and liposarcoma	Endometrial serous carcinoma, clear cell carcinoma, melanocytic nevi and melanoma, adenoid cystic carcinoma, malignant mesenchymal tumors	
Positive control: cervical squamous cell carcinoma		

Diagnostic Approach P16 (also known as INK4a or cyclin-dependent kinase inhibitor 2A) is a tumor suppressor protein encoded by the $p16^{INK4a}$ gene. p16 inhibits the cyclin-dependent kinases [1, 2] involved in in cell cycle regulation and progression (G1 to S). p16 plays role in the pathogenesis of different malignancies. The expression of p16 is regulated by the retinoblastoma (Rb) gene, which in turn is affected by the E7 oncogene of the HPV gene. p16 is overexpressed in HPV-associated intraepithelial dysplasia and squamous cell carcinomas of different origins including vulvar, vaginal, and cervical squamous cell carcinoma in addition to oropharynx carcinoma. In routine immunohistochemistry, p16 reveals cytoplasmic and nuclear staining pattern and the intensity of the stain correlates with grade of HPV infection and grade of associated dysplasia. p16 is also highly expressed in uterine serous carcinoma and a helpful marker that labels the cells of serous tubal intraepithelial carcinoma (STIC) [3].

p16 is also a useful marker to discriminate between atypical lipomatous tumors (well-differentiated liposarcoma) or other liposarcoma types positive for p16 and benign adipocytic tumors lacking the expression of p16 [4, 5].

PAX-8: PAX-8 is a transcriptional factor involved in the fetal development of the brain, eye, thyroid tissue, kidney, and upper urinary system as well as the Müllerian organs. PAX-8 is listed in detail in a next chapter.

Hepatocyte Nuclear Factor-1β (HNF-1β): HNF-1β is a member of the hepatocyte nuclear factor family regulating the growth and differentiation of hepatocytes and cells of the biliary system. The expression of different hepatocyte nuclear factors is not restricted to the liver but variously found in other organs including the pancreas, kidney, prostate, and female genital system [6]. HNF-1β is used in diagnostic immunohistochemistry to differentiate between different types of ovarian and endometrial carcinomas. The strong nuclear HNF-1β expression is characteristic for both endometrial and ovarian clear cell carcinomas but usually negative in reactive lesions with clear cell appearance such as clear cell metaplasia and Arias-Stella phenomenon [7]. However, we must consider that focal weak to moderate HNF-1β expression can be also found in other endometrial and ovarian carcinoma types such as endometrioid and serous carcinomas [8]. Additionally, different HNF-1β expression intensity is also found in other carcinomas of different origin including colorectal, pancreatobiliary, prostatic, and renal cell carcinomas.

Phosphatase and Tensin Homolog (PTEN): PTEN is a widely expressed enzyme in mammalian cells that catalyzes the dephosporylation of the 3` phosphate of the inositol ring, an essential reaction that causes the inhibition of the protein kinase (AKT) signaling pathway involved in the regulation of apoptosis. Mutations that inactivate the PTEN gene cause the inhibition of the apoptotic cascade increasing cell proliferation. Inactivating mutations within the PTEN are commonly seen in different human neoplasias

such as urogenital, breast, and lung carcinomas in addition to melanoma and glial tumors [9]. The immunohistochemical staining of PTEN (cytoplasmic pattern) is a simple way to detect the loss of this enzyme. The loss of PTEN expression is found in 30–50% of endometrial carcinoma and in about 25% of endometrium with atypical complex hyperplasia, which indicates that the loss of PTEN is not a specific marker of malignant transformation [10, 11]. Normal proliferative endometrium shows usually strong PTEN expression. The loss of PTEN expression is also found in a subset of ovarian endometrioid carcinoma (~20%), high-grade serous carcinoma, and clear cell carcinoma.

A fraction of high Gleason prostatic carcinoma is also associated with PTEN loss (see markers of prostatic carcinoma) [9]. PTEN mutations are found in primary glioblastoma but rare in secondary glioblastoma.

Steroid Receptors: Both estrogen and progesterone receptors were discussed in details with the markers of breast tumors. Endometrial adenocarcinoma and serous endometrial carcinoma are sex hormone-dependent tumors, and the expression of estrogen and progesterone is characteristic for both carcinoma types [12]. Myometrium is also a target tissue for steroid hormones; accordingly the majority of uterine leiomyomas and leiomyosarcomas are positive for estrogen receptors, progesterone receptors, or both. This characteristic feature can be used to differentiate between uterine and soft tissue leiomyosarcoma [13]. Squamous cell carcinoma and adenocarcinoma of uterine cervix usually lack the expression of both receptors [14].

Immunoprofile of tumors of the uterine cervix, uterine corpus, and fallopian tube				
Tumor type	+ in >90% (+)	+ in 50–90% (±)	+ in 10–50% (∓)	+ in <10% (−)
A. Tumors of the vulva and vagina				
Paget's disease of the vulva	CK7, EMA (MUC1), CEA, androgen receptors	ER	GCFP-15	CK5/6/14, CK20
Squamous cell carcinoma	*CK5, CK6,* CK18, CK19, *P16*			CK7, CK20
Bartholin gland carcinoma • Adenocarcinoma • Squamous cell carcinoma • Adenoid cystic carcinoma • Transitional cell carcinoma	See immunoprofile of similar carcinomas of other locations			
Adenocarcinoma of mammary type	See immunoprofile of breast carcinoma			
Adenocarcinoma of Skene gland type	Pan-CK, PSA			PAX-8
Clear cell carcinoma	CK7, EMA, CEA			CK20
Sebaceous carcinoma	*Adipophilin*, EMA, androgen receptors	Perilipin, CK5/14, CK8/18, CK7, CK19, CD15, p16		CK20, CEA, S100
Angiomyofibroblastoma	Desmin	ER, PgR	CD34	Actin

Immunoprofile of tumors of the uterine cervix, uterine corpus, and fallopian tube				
Cellular angiofibroma		CD34, ER, PgR	Actin	
Superficial angiomyxoma	CD34			Actin, desmin, S100
Deep aggressive angiomyxoma	Desmin, HMGA2	Actin, ER, PgR	CD34, actin, S100	Myogenin, MyoD1
Epithelioid sarcoma	See miscellaneous soft tissue tumors			
Rhabdomyosarcoma	See soft tissue rhabdomyosarcoma			
B. Tumors of the uterine cervix				
Squamous cell carcinoma of the cervix and uterus	*CK5, CK6*, CK13, CK17, CK18, CK19, *P16*	CK14		CK7, CK20, ER, PgR
Endocervical adenocarcinoma	*CK7*, CK8, CK18, CK19, *CEA*, EMA, *p16*, *PAX-8*		CK20, vimentin	*ER*, PgR, CK5/6, WT-1, PAX-2[a], GFAP
Endometrioid adenocarcinoma	*CK7*, CK8, CK18, CK19, EMA	*ER*, PgR, vimentin, GFAP	p16, CD56	CK20, CK5/6, *CEA*, CDX-2
Mesonephric adenocarcinoma	CK5/6, CK7, CK8, CK18, EMA, CD15	CD10, p16, *calretinin*, vimentin, bcl-2	Androgen receptors, PAX-8, TTF-1	ER, PgR, CK20, CEA
Adenosquamous carcinoma/ glassy cell carcinoma	CK7[b], CK5/6/14[c]			ER, PgR
Adenoid basal carcinoma	CK5/14, p63, p16			
Neuroendocrine tumors • NET(c) G1 • NET(d) G2 • NEC(e) G3 (small cell carcinoma)[i, k, l]	Pan-CK, *CD56*, NSE, PGP9.5 Proliferation index (Ki-67) in NET G1: <2% NET G2: 3–20% NEC G3: >20%	*Synaptophysin, chromogranin*	TTF-1	CK7, CK20
C. Tumors of the uterine corpus				
Endometrial adenocarcinoma	*CK7*, CK8, CK18, CK19, *PAX-8*, EMA, CA125	PgR, *ER*, vimentin, GFAP	CD56, p53, P16	CK20, CK5/6, CEA, WT-1, IMP3, CDX-2[d]
Serous endometrial carcinoma	CK7, CK8, CK18, CK19, EMA, CA125, *p16, p53, PAX-8*, β catenin Proliferation index (Ki-67): >75%	*IMP3*, PgR, ER	ER, PgR, Sox-2, WT-1	CK5/6, CK20, HNF1-β
Clear cell carcinoma	CK 7, EMA, CA125, *PAX-8, hepatocyte nuclear factor 1-β (HNF1-β)*, p504s (AMACR)	Vimentin, CD15	ER, AFP, CEA, p16, p53, Sox-2	PgR, WT-1, CK20, CD10
Undifferentiated carcinoma	EMA, vimentin	Pan-Cytokeratin, CK8/18, p53	PAX-8, synaptophysin, chromogranin	ER, PgR
Low-grade endometrial stromal sarcoma	*CD10*, β-catenin, vimentin	ERα, PgR, bcl-2, WT-1, TLE-1	Cyclin D1, androgen receptors, actin, desmin, pan-CK	*h-Caldesmon, calponin*, CD34, EMA, inhibin, oxytocin receptor

Immunoprofile of tumors of the uterine cervix, uterine corpus, and fallopian tube				
High-grade endometrial stromal sarcoma	Cyclin D1	CD117		CD10, ER, PgR
Uterine leiomyoma/ leiomyosarcoma	Desmin, *actin, calponin*, oxytocin receptor, p16[e], p53[e], vimentin Proliferation index (Ki-67) in uterine leiomyoma: <5% Proliferation index (Ki-67) in atypical uterine smooth muscle tumors: 5–10% Proliferation index (Ki-67) in uterine leiomyosarcoma: >15%	*h-Caldesmon*, ER, PgR	Pan-CK	CD10, EMA
Perivascular epithelioid tumor of the uterus (PEComa)	*HMB45, Melan A*, tyrosinase, MITF[f], CD63 (NK1-C3)		Actin, desmin	CD10, CD34, pan-CK, S100
Placental site trophoblastic tumor	*Human placental lactogen*, CD146, inhibin, pan-CK Proliferation index (Ki-67): >10%[g]		ßhcG	
Gestational choriocarcinoma	See choriocarcinoma of the ovary			
D. Tumors of the fallopian tube				
Serous tubal intraepithelial carcinoma (STIC)[h]	*p53*, p16, stathmin 1[i] Ki-67 > 15%			
Serous carcinoma	CK7, CK8, CK18, CK19, EMA, WT-1, *p53*, p16	ER, PgR		CK5/6, CK20
Endometrioid adenocarcinoma	*CK7*, CK8, CK18, CK19, EMA, *ER*	PgR, GFAP, vimentin	p53, CD56	P16, CK20, CK5/6, CEA, CDX-2
Undifferentiated carcinoma	EMA, vimentin	Pan-cytokeratin, CK8/18	Synaptophysin, chromogranin	ER, PgR
E. Tumors of uterine ligaments				
Epithelial tumors of Müllerian type	See uterine tumors			

[a]PAX-2 is usually expressed in benign proliferating endocervical glands
[b]CK7 positive in glandular components
[c]CK5/6/14 positive in squamous components
[d]CDX-2 may be positive in mucinous-type endometrioid adenocarcinoma
[e]P16 and p53 usually positive only in leiomyosarcoma
[f]Microphthalmia transcription factor
[g]Proliferation index (Ki-67) in placental site nodule and exaggerated placental site <1% and >50% in choriocarcinoma
[h]See Fig. 11.1
[i]Diffuse expression in STIC lesions but few scattered cells in normal fallopian mucosa [3]
[j]Well-differentiated neuroendocrine tumor (carcinoid)
[k]Well-differentiated neuroendocrine tumor (atypical carcinoid)
[l]Poorly differentiated neuroendocrine carcinoma

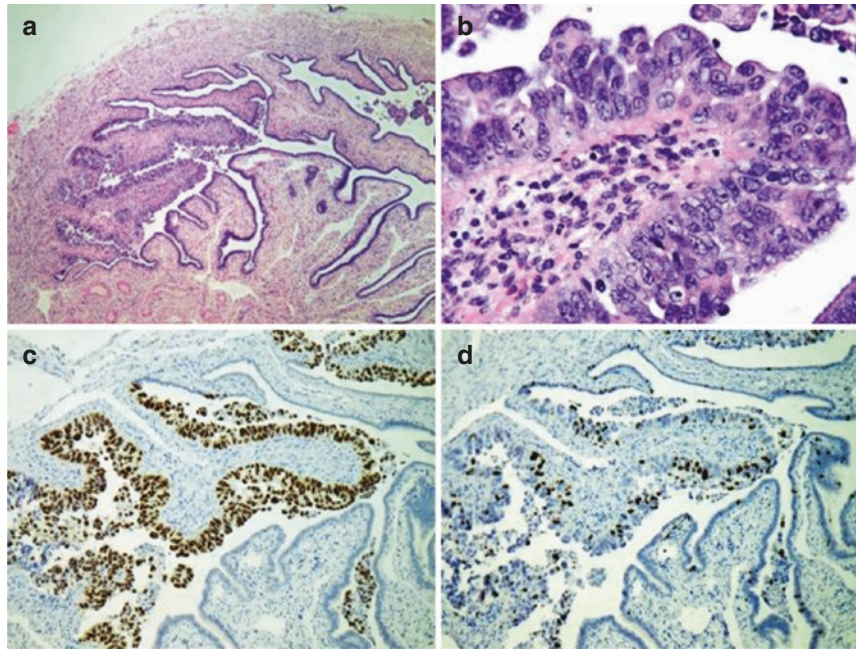

Fig. 11.1 Serous tubal intraepithelial carcinoma (STIC). (**a**, **b**) H&E 40X and 200X showing the fallopian tube with marked atypia of tubal epithelium, (**c**) same section with strong diffuse nuclear p53 expression, (**d**) Ki-67 expression in ~15% of epithelial cells

11.5 Tumors of the Ovary

11.5.1 Diagnostic Antibody Panel for Ovarian Epithelial Tumors

Cytokeratin profile, CEA, CA125, PAX-8, WT-1, p53, p16, GATA-3, S100P, steroid hormone receptors, and HNF-1β.

11.5.2 Diagnostic Antibody Panel for Ovarian Germ Cell Tumors

CD117, PLAP, Oct-4, SALL-4, Sox-2, AFP, CD30, βhcG, and cytokeratin profile (see also testicular germ cell tumors).

11.5.3 Diagnostic Antibody Panel for Ovarian Sex Cord-Stromal Tumors

Inhibin, anti-Müllerian hormone, FOXL-2, Melan A, CD56, CD99 (see also testicular sex cord-stroma tumors).

Wilms' tumor protein-1 (WT-1)		
Expression pattern: nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Nephroblastoma, mesothelioma, malignant melanoma, metanephric adenoma, ovarian serous carcinoma, carcinoma of the fallopian tube	Acute myeloid leukemia, Burkitt lymphoma and subset of ALL, desmoplastic small round cell tumor, endometrial stromal sarcoma, uterine leiomyosarcoma, sex cord-stromal tumors (granulosa cell tumor, fibroma, fibrothecoma, Sertoli cell tumor), Brenner tumor, ovarian small cell carcinoma of hypercalcemic type, neuroblastoma, rhabdoid tumor, rhabdomyosarcoma	Renal tissue (glomerular podocytes), mesothelial cells, granulosa cells, Sertoli cells, fallopian tube, endometrial stroma, spleen, breast tissue, bone marrow stem cells
Positive control: appendix		

Diagnostic Approach Wilms' tumor protein-1 (WT-1) is a transcriptional regulator encoded by the WT-1 gene on chromosome 11p13 with four isoforms. WT-1 plays an important role in the regulation of growth factors and development of tissues from the inner layer of intermediate mesoderm including the genitourinary system, mesothelial cells, and spleen. Mutation within the WT-1 gene affecting the DNA-binding domain can cause the development of nephroblastoma. In routine immunohistochemistry, WT-1 shows two different expression patterns: first, a true nuclear expression pattern characteristic for different tumors such as serous carcinomas of ovarian, tubal, and peritoneal origin and mesothelioma (Fig. 11.2); secondly a cytoplasmic staining pattern found in endothelium and vascular tumors in addition to some carcinoma types such as pulmonary adenocarcinoma [1]. The cytoplasmic expression pattern appears to result from a cross reactivity with other epitopes unrelated to the WT-1 transcription factor. Endometrioid, clear cell, transitional, and mucinous carcinomas are usually WT-1 negative or show focal weak positivity. WT-1 is a helpful marker to differentiate between WT-1 positive tumors and many other WT-1 negative tumors with similar morphology such as neuroblastoma and the PNET tumor group.

Diagnostic Pitfalls WT-1 labels a high percentage of epithelioid mesotheliomas, which to consider in the differential diagnosis between ovarian peritoneal carcinosis and primary peritoneal mesotheliomas. For differential diagnosis, other antibodies such as PAX-8, Ber-EP4, and calretinin are helpful.

CA125 (MUC-16)		
Expression pattern: membranous (luminal surface)		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Ovarian carcinoma (serous, endometrioid and clear cell carcinomas)	Lung, breast, gastrointestinal, uterine, and seminal vesicle adenocarcinomas, yolk sac tumor, epithelioid mesothelioma, anaplastic large cell lymphoma, desmoplastic small round cell tumor	Breast ductal epithelium, epithelium of the lung, gastrointestinal tract, biliary system, pancreas, female genital tract and apocrine glands, mesothelial cells
Positive control: serous ovarian carcinoma		

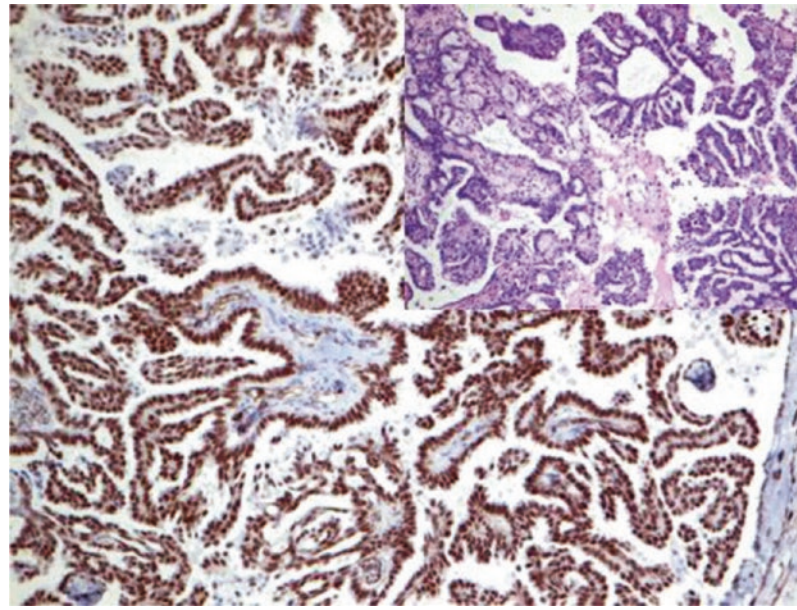

Fig. 11.2 Serous ovarian carcinoma with strong nuclear WT-1 expression

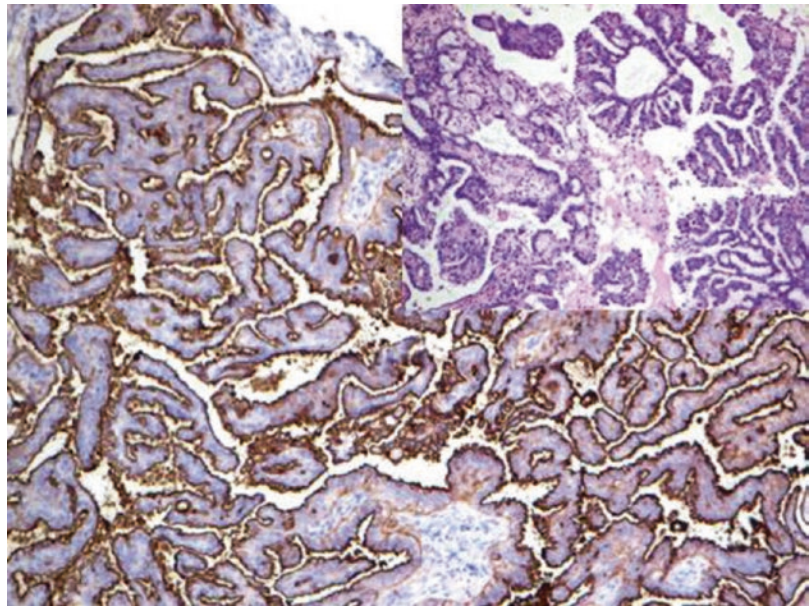

Fig. 11.3 Serous ovarian carcinoma with membranous CA125 expression

Diagnostic Approach Carbohydrate antigen 125 (CA125) is a high molecular weight glycoprotein classified as mucin 16 (MUC-16). CA125 is normally expressed by glandular epithelium of different organs and is highly expressed in ovarian serous and clear cell carcinomas (Fig. 11.3). Serum CA125 is also used to monitor the progression of ovarian carcinoma.

Diagnostic Pitfall CA125 is expressed by different epithelial and non-epithelial malignancies and lacks the specificity to ovarian carcinoma. Mesotheliomas can also be positive to CA125.

PAX-8: PAX-8 is a transcriptional factor and a member of the paired box (PAX) family listed in detail with the markers of renal cell tumors. PAX-8 is highly expressed in Müllerian glandular epithelia as well as in renal tubules and upper urinary system. PAX-8 strongly labels all uterine, endocervical, and ovarian tumors of Müllerian origin including serous, clear cell, and endometrioid carcinomas.

Hepatocyte Nuclear Factor-1β (HNF-1β): See the previous chapter (Chap. 10).

FOXL2		
Expression pattern: nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Sex cord-stromal tumors	Breast cancer, pituitary gland adenoma	Granulosa cells, subset of pituitary cells
Positive control: ovarian tissue (granulosa cells)		

Diagnostic Approach FOXL2 (**f**orkhead b**ox** transcription factor **L2**) is a transcriptional factor involved in the development of the ovaries and female genital tract. FOXL2 is highly expressed in testicular and ovarian sex cord-stromal tumors including adult and juvenile granulosa cell tumors, thecoma/fibroma, Sertoli/Leydig cell tumors and sclerosing stromal tumor. Subset of pituitary gland adenomas is also positive for FOXL2, namely, gonadotropins producing adenomas and majority of null cell adenomas [2, 15, 16]. Ovarian surface epithelial tumors and germ cell tumor are FOXL2 negative.

Immunoprofile of ovarian tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (±)	+ in 10–50% (∓)	+ in <10% (−)
A. Ovarian epithelial tumors				
Serous ovarian neoplasms • Adenoma • Borderline • Low-grade carcinoma • High-grade carcinoma	CK7, CK8, CK18, CK19, EMA, CA125, *WT-1, PAX-8, p53*[a], *p16*[a], HAM56 Median proliferation index (Ki-67) in serous carcinoma: Low grade ~ 2,5% High grade ~ 22%	CK5/6, mesothelin	Vimentin, ER, PgR, calretinin, S100, TTF-1, CD99	Villin, CK20, *CEA*, MUC-2, CDX-2, inhibin
Mucinous ovarian neoplasms (adenoma, borderline, and carcinoma)	CK7, CK8, CK18, CK19, EMA	CK20[b], CDX-2[b], MUC-2, MUC5AC, *CEA*, PAX-8, p53[c]	Villin	*WT-1, p16*, ER, PgR, CK17, vimentin, inhibin, TTF-1
Endometrioid carcinoma	CK7, CK8, CK18, CK19, EMA, *PAX-8*, ER, CA125	Vimentin, mesothelin, CD99	WT-1, p16, CK5	CK20, *WT-1*, CEA, inhibin, TTF-1
Clear cell adenocarcinoma	*Hepatocyte nuclear factor 1-β (HNF1-β), PAX-8*, CK7, EMA	Vimentin, CD15, CA125	AFP, CEA, napsin A, p53	*WT-1, p16, ER*, PgR, CK20, CD10
Brenner tumor (benign/malignant)	*Epithelial components*: EMA, *CK7*, p63, CEA, *CK5/6/14*[d], CA125, *Uroplakin III* *Fibrous stroma*: vimentin	*WT-1*, S100P, PAX-8 bcl-2		CK19, CK20, thrombomodulin (CD141), vimentin Pan-CK
B. Sex cord-stromal tumors				
Granulosa cell tumor	*FOXL2, adrenal 4 binding protein (SF-1), inhibin*, vimentin	Calretinin, *CD99*, actin, S100, CD56, *WT-1*, ERβ, PgR	Pan-CK, CK8, CK18, ERγ	CK7, EMA, CEA, anti-Müllerian hormone, desmin
Thecoma/Fibroma	*Inhibin, FOXL2, adrenal 4 binding protein (SF-1), WT-1*, calretinin, vimentin	sm-actin	ER, PgR	Pan-CK
Sclerosing stromal tumor	sm-Actin, PgR, *FOXL2*, vimentin	*Inhibin*, calretinin, desmin	ER	Pan-CK
Leydig cell tumor	*Inhibin*, Melan A, calretinin, vimentin	CD99, CD56	Pan-CK, S100, actin, desmin, synaptophysin, chromogranin, EMA	PLAP, AFP, CEA
Sertoli cell tumor	*Inhibin, adrenal 4 binding protein (SF-1), FOXL2, anti-Müllerian hormone*, WT-1, Melan A, vimentin	AFP, CD56, CD99, pan-CK, calretinin, NSE, S100	Synaptophysin, chromogranin	EMA, PLAP, CEA
Sex cord tumor with annular tubules	*Inhibin, adrenal 4 binding protein (SF-1)*, WT-1, calretinin	CD56	Pan-CK	EMA

Immunoprofile of ovarian tumors				
C. Germ cell tumors				
Dysgerminoma	*SAL4, Oct-4, NANOG, PLAP, CD117*	Pan-CK, D2-40	CK8/18	AFP, ßhcG, Sox-2, inhibin, S100, EMA
Embryonal carcinoma	*SALL-4, NANOG, Sox-2, PLAP, AFP, CD30, Oct-4*, pan-CK	CK19, NSE		ßhcG, *EMA*, CEA, CD117, vimentin
Yolk sac tumor	*AFP, SALL-4,* pan-CK, CD10, glypican-3	PLAP	CDX2, HepPar1	*EMA*, CD30, ßhcG, Oct-4, *Sox-2*, CK7, vimentin
Choriocarcinoma	*Syncytiotrophoblastic cells*: *ßhcG*, inhibin, CD10, pan-CK, CK8/18, CK19, GATA-3, EGFR *Cytotrophoblastic cells*: CD10, pan-CK, CK8/18, CK19, CEA	*PLAP,* human placental lactogen, EMA, CEA PLAP	Vimentin	CD30, AFP, Oct-4 ßhcG, inhibin, EMA, CD30, AFP, Oct-4
Polyembryoma	*In embryonal bodies*: *AFP*, pan-CK	PLAP		
Gonadoblastoma	*Germ cells*: PLAP, CD117, Oct-4, NANOG, D2-40 *Sex cord cells*: inhibin, WT-1, vimentin	Pan-CK		
D. Miscellaneous tumors				
Female adnexal tumor of probable Wolffian origin (ovarian Wolffian tumor)	Pan-CK, CK7, *androgen receptors*, vimentin	Calretinin, CD10, Melan A	Inhibin	EMA, CK5/6, CK20, CEA
Small cell carcinoma, hypercalcemic type	EMA, *WT-1*	Calretinin, CD56	Synaptophysin, chromogranin	CD10, inhibin
Small cell carcinoma, pulmonary type	NSE, *CD56*	TTF-1	Synaptophysin, chromogranin	

[a]High expression level characteristic for high-grade serous carcinoma, low expression level or negative in low-grade carcinoma
[b]CDX-2 and CK20 positive in mucinous adenocarcinoma and intestinal type adenoma
[c]Usually negative in adenoma and borderline tumors
[d]CK5/6/14 positive in basal epithelial cells

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Markers and Immunoprofile of Renal and Urinary Tract Tumors

12

Contents

12.1 Renal Tumors

Diagnostic Antibody Panel for Renal Tumors RCC, PAX-8, PAX-2, GATA-3, CD10, CD117, AMACR, human kidney injury molecule-1 (KIM-1), carbonic anhydrase IX (CAIX), TFE-3, DOG-1, cytokeratin profile, and vimentin [1, 2].

PAX-8		
Expression pattern: nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Renal cell carcinoma (clear cell, papillary, chromophobe, and collecting duct carcinomas), nephroblastoma, thyroid carcinoma (papillary, follicular, and anaplastic), ovarian carcinoma (serous, clear cell, and endometrioid carcinoma), adenocarcinoma of seminal vesicle and rete testis	Pancreatic neuroendocrine tumors and other NET of the gastrointestinal tract, parathyroid adenoma and carcinoma, endocervical adenocarcinoma, thymoma (types A and B, thymic carcinoma), seminoma, yolk sac tumor, Merkel cell carcinoma, B-cell lymphomas, medulloblastoma	Thyroid follicular cells, parathyroid cells, thymic cells, of renal tubules, endocervical and endometrial epithelial cells, non-ciliated epithelium of fallopian tubes, epididymal cells, seminal vesicles, a subset of B-lymphocytes
Positive control: thyroid tissue		

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DOI 10.1007/978-3-319-53577-7_12

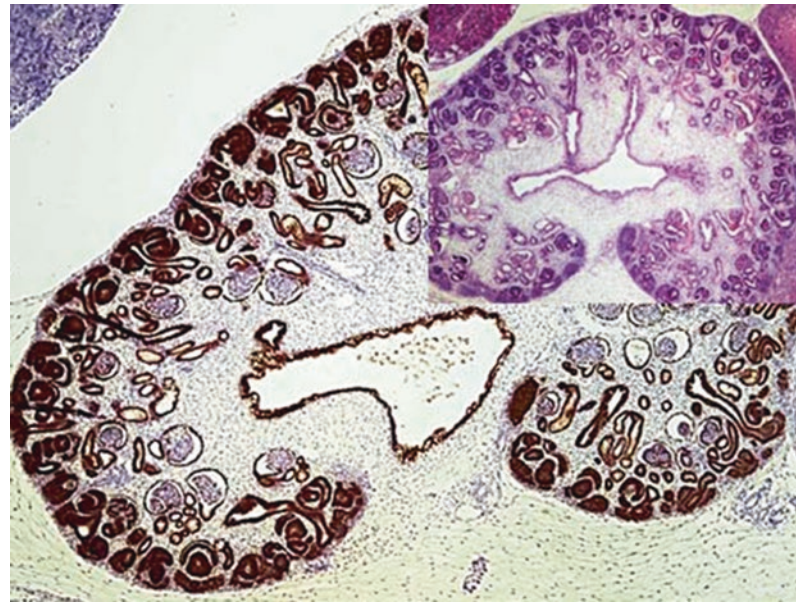

Fig. 12.1 The kidney of a 12-week-old embryo; PAX-8 highlighting the urothelium of the renal collecting system and renal pelvis

Diagnostic Approach PAX-8 is a transcriptional factor and a member of the **pa**ired bo**x** (PAX) family consisting of nine members (PAX-1–9). PAX-8 is involved in the fetal development of the central nervous system, eye, inner ear, thyroid gland, kidney, and upper urinary system as well as the Müllerian organs and organs derived from the mesonephric duct [3]. In normal tissue, PAX-8 is highly expressed in thyroid follicular cells, parathyroid cells, and non-ciliated cells of fallopian tubes, mucosa, and renal tubules; consequently, tumors developed from these tissue types are generally positive for PAX-8. Follicular and papillary thyroid carcinomas show high expression level of PAX-8 but not medullary thyroid carcinoma. Clear cell, papillary, and chromophobe renal cell carcinomas besides nephroblastoma are also positive for PAX-8 in addition to the majority of collecting duct carcinoma and oncocytomas and about 50% of sarcomatoid renal cell carcinoma. PAX-8 is also highly expressed in serous, endometrioid, and clear cell ovarian carcinomas, while mucinous carcinoma is usually negative. The expression of PAX-8 is also reported in different percentage of well-differentiated neuroendocrine tumors of pancreatic, gastroduodenal, appendicular, and rectal origin. [4]

Diagnostic Pitfalls As mentioned, PAX-8 is expressed of a wide range of tumors and must be used as a part of diagnostic panel. A diagnostic panel of PAX-8, WT-1, and two cytokeratins is necessary to confirm the diagnosis of ovarian carcinoma. The PAX-8 expression was noted in about 23% of transitional cell carcinoma of the renal pelvis (Fig. 12.1), which is important to consider in the differential diagnosis of primary renal tumors [5]. PAX-8 is useful to exclude pulmonary adenocarcinoma and breast carcinomas, which usually lack the expression of PAX-8 but express TTF-1 and GATA-3, respectively. The expression of PAX-8 in B-lymphocytes must be also considered in the interpretation of the PAX-8 stain, which is also a useful positive internal control.

PAX-2: PAX-2 is a further member of the paired box family of transcription factors analogous to PAX-8, is also involved in the renal development, and appears slightly later than PAX-8. PAX-2 has a wide expression range and is found in most renal cell carcinomas with the exception of chromophobe renal cell carcinoma and in tumors of Müllerian origin including ovarian, endometrioid, and endocervical carcinomas in addition to lobular breast carcinoma, hepatocellular carcinoma, epididymal tumor, and Merkel cell carci-

noma. PAX-2 is a useful marker to differentiate between benign cervical glandular proliferation positive for PAX-2 and endocervical adenocarcinoma usually lacking the PAX-2 expression. PAX-2 is also expressed in parathyroid cells and parathyroid tumors but constantly negative in thyroid tissue and thyroid carcinomas. Similar to PAX-8, PAX-2 is also positive in B-lymphocytes and related lymphoma types.

Renal cell carcinoma marker (RCC; gp200)		
Expression pattern: cytoplasmic/membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Renal cell carcinoma (clear cell, chromophobe, and papillary renal cell carcinoma)	Parathyroid adenoma, breast carcinoma, embryonal carcinoma	Renal proximal tubular brush border, epididymal tubular epithelium, breast parenchyma, thyroid follicles
Positive control: renal tissue or renal cell carcinoma		

Diagnostic Approach Renal cell carcinoma marker (RCC) is a glycoprotein expressed on the brush border of proximal renal tubules but absent in other renal areas. RCC is detected in about 90% of primary but less frequently in metastatic renal cell carcinoma, including clear cell, chromophobe, and papillary renal cell carcinoma, whereas the highest expression intensity is noted in clear cell carcinoma [6, 7]. Collecting duct carcinoma, sarcomatoid (spindle cell) carcinoma, oncocytoma, mesoblastic nephroma, nephroblastoma, and transitional cell carcinoma are negative for RCC.

Diagnostic Pitfalls RCC is occasionally detected in rare tumors other than renal cell carcinoma such as primary and metastatic breast carcinoma, embryonal carcinoma, and parathyroid adenoma, which are to be considered in the differential diagnosis.

CD10: CD10 is listed in detail with the lymphoma markers. CD10 it is also a helpful marker in the differential diagnosis of renal cell tumors. CD10 is positive in the majority of clear cell and papillary renal cell carcinomas in addition to collecting duct carcinoma demonstrating a typical apical expression but negative in chromophobe renal cell carcinoma, which is usually positive for CD117 (Fig. 12.2) [7, 8].

Diagnostic Pitfalls CD10 is also expressed in tumors with similar morphology such as tumors

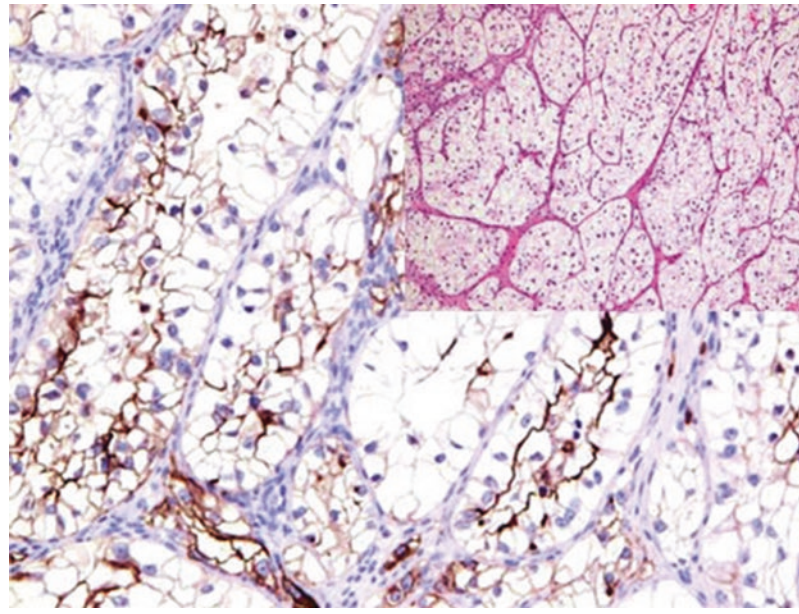

Fig. 12.2 Clear cell renal cell carcinoma stained by CD10 with expression accentuated on the apical side of the cell membrane

of the adrenal cortex and hepatocellular carcinoma and later lacks the expression of PAX-8 that can be used to discriminate between both tumors.

Paxillin: Paxillin is a cytoskeletal protein involved in the formation of focal adhesion complexes between F-actin and integrin and widely expressed in epithelial, neuronal, and mesenchymal cells. Paxillin is a helpful marker to differentiate between chromophobe renal cell carcinoma and renal oncocytoma both positive for paxillin and clear cell and papillary renal cell carcinoma negative for this marker [9]. Paxillin is not a specific renal cell carcinoma marker and can be expressed in different carcinoma types of the breast, lung, and liver.

Carbonic anhydrase IX (CA IX)		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Renal cell carcinoma (clear cell and papillary renal cell carcinoma)	Cervical and endometrial carcinoma, transitional cell carcinoma, breast carcinoma, alveolar soft part sarcoma	Gastric and gall bladder mucosa
Positive control: renal cell carcinoma		

Diagnostic Approach Carbonic anhydrase IX (CA IX) is member of the carbonic anhydrases family zinc metalloenzymes catalyzing the hydration of carbon dioxide. CA IX is a transmembrane isoenzyme taking part in the cell proliferation and cell adhesion as well as the regulation of intra- and extracellular pH. Normally, the expression of CA IX is suppressed by the wild type of von Hippel-Lindau protein and is negative in normal renal tissue. The expression of CA IX is activated during the malignant transformation, and CA IX is markedly expressed in clear cell and a part of papillary renal cell carcinomas. CA IX is helpful in the interpretation of small renal biopsies. It is also a useful marker to discriminate between benign renal cysts generally negative for CA IX and cystic renal cell neoplasm (Fig. 12.3) [10]. Chromophobe cell carcinoma and renal oncocytoma usually lack the expression of CA IX.

Diagnostic Pitfalls Carbonic anhydrase IX is not a specific marker for renal cell tumors, and different expression levels are found in various tumors of different origin including pulmonary carcinoma, esophageal carcinoma, renal transitional cell carcinoma, breast carcinoma, neuroen-

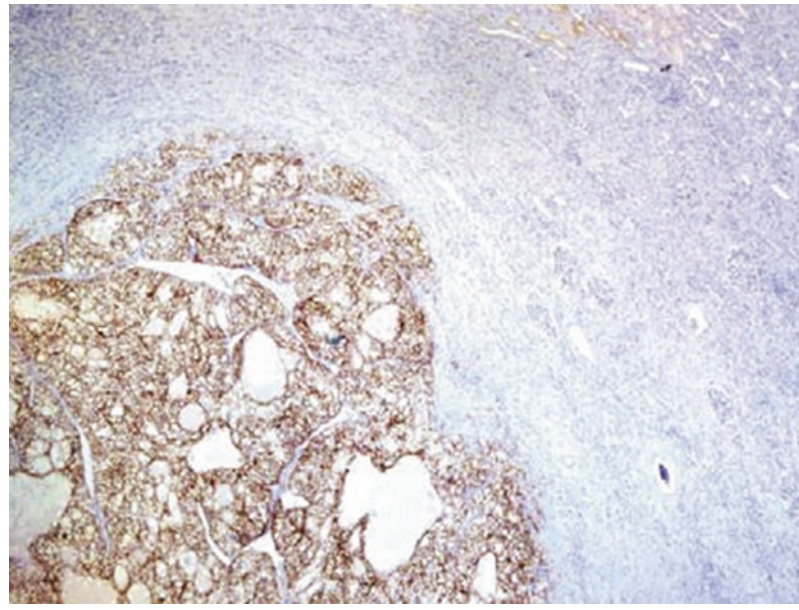

Fig. 12.3 CA IX expression in clear cell carcinoma. The expression is restricted to tumor areas; normal renal tissue is negative

docrine tumors, cervical squamous cell carcinoma and high-grade intraepithelial neoplasia, endometrial carcinoma, embryonal carcinoma, mesothelioma, Sertoli cell tumor, and adrenocortical carcinoma [11].

Human Kidney Injury Molecule-1: KIM-1 (also known as hepatitis A virus cellular receptor 1) is a type I transmembrane glycoprotein usually not detectable in normal renal tissue but expressed in the epithelial cells of proximal tubules after acute or chronic toxic or ischemic injury. KIM-1 is expressed in different renal cell carcinoma types [12]. In extrarenal tumors, KIM-1 is positive ovarian and uterine clear cell carcinoma, hepatocellular carcinoma, and a subset of colorectal carcinomas in addition to germ cell tumors, which may have a similar morphology to clear cell renal cell carcinoma [13].

Transcription Factor-E3: TFE-3 a transcription factor encoded by a gene located on Xp11.2. TFE-3 reacts with other transcription factors regulating macrophage and osteoclast differentiation and cell proliferation in addition to activation of B- lymphocytes. The t(X;17) translocation associated with one of the rare types of renal cell carcinoma causes the overexpression of this transcriptional factor, which is considered as a specific immunohistochemical marker for the Xp11.2 translocation-associated renal cell carcinoma [14]. The expression of TFE-3 is also characteristic for the alveolar soft part sarcoma due to an equivalent translocation.

Immunoprofile of renal tumors

Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Oncocytoma	CK8, CK14, CK18, *E-cadherin*, claudin-8, MSH-2[a]	*CK20*, PAX-2, PAX-8, EMA, *CD15, CD117,* DOG-1, cyclin D1, S100A1	CK7	Vimentin, RCC38, PAX-2, CD10, KIM-1, CA IX
Papillary adenoma	CK5, CK8, CK14, CK18, EMA	P504S (AMACR)		
Metanephric adenoma	CK8, CK18, vimentin, *CD57*, S100, *PAX-2*, *PAX-8*	*WT-1*		CK7, CK19, EMA, p504S (AMACR)
Multilocular cystic renal neoplasm of low malignant potential	PAX-8, *CA IX*			
Clear cell renal cell carcinoma	CK8, CK18, *PAX-2*, *PAX-8*, KIM-1, MOC31, α B-crystallin	Vimentin, *RCC* (gp200), CD10, CA IX, CK19, EMA	CD9	p504S (AMACR), CK7, CK13, CK20, CD117, inhibin, MSH-2
Chromophobe renal cell carcinoma	*CK7*, CK8, CK18, EMA, *CD117*, paxillin	CK19, CD9, MSH-2[b], DOG-1 E-cadherin, Ki-67 (MIB-1 clone) [b], parvalbumin	RCC (gp200), PAX-8, PAX-2, CD10[c]	Vimentin, *CD15*, p504S (AMACR), cyclin D1, *CA IX*, KIM-1, claudin-8, CK13, *CK20*
Papillary (chromophile) renal cell carcinoma	CK8, CK18, *PAX-8*	EMA, *p504S* (AMACR), *PAX-2*, *RCC* (pg200), CK19, CD9, CD10, *CK7*, CK19, CAIX, vimentin	Napsin	CK5/6, CK13, CK20, CD57, CD117, WT-1
Clear cell papillary renal cell carcinoma	CK7, CA IX, PAX-2, PAX-8		CD10	p504S (AMACR)

Immunoprofile of renal tumors				
Renal medullary carcinoma	CEA	CK20, CK7, PAX-8, OCT-4		
Collecting duct carcinoma (Bellini duct carcinoma)	CK8, CK18, CK19, UEA-1, lectin	CK7, EMA, vimentin, *PAX-2*, *PAX-8*, CK7, CD15, HER-2		RCC, CD10, GATA-3, CK5/6, CK13, CK17
Xp11.2 translocation-associated renal cell carcinoma	*TFE-3*	*Cathepsin-K*, CD10, p504S (AMACR), RCC	CK7, PAX-2, PAX-8	
t(6;11)-associated renal cell carcinoma	*TFEB, cathepsin-K, Melan A*	CD117, CD10, PAX-8, vimentin	HMB45	CA IX
Mucinous tubular and spindle cell carcinoma	CK7, PAX-2, p504S (AMACR)			
Acquired cystic disease-associated renal cell carcinoma	P504S			CK7
Tubulocystic renal cell carcinoma	CK7, CK8, CK19	CD10, p504S (AMACR)		
Spindle cell (sarcomatoid) carcinoma	CK8, CK18, vimentin	EMA	CD10, PAX-8	RCC
Neuroendocrine carcinoma	CK8, CK18, CD56, S100, chromogranin, synaptophysin, NSE			CK19
Juxtaglomerular cell tumor	Renin, CD31, CD34, actin, CD117	Calponin		Pan-CK, desmin, synaptophysin, chromogranin, S100
Mucinous tubular and spindle cell carcinoma (loopoma)	CK7, CK8, p504S, EMA, vimentin			CD10, RCC
Nephroblastoma (Wilms' tumor)	WT-1, CD56, vimentin	Myogenin, PAX-2, PAX-8, S100, pan-CK, NSE		CD57, CK19
Angiomyolipoma	*HMB45*[d], HMB50, *Melan A*, actin, CD63 (NK1-C3), calponin	CD117, PgR	MIFT, ER	EMA, pan-CK
Clear cell sarcoma of the kidney	Vimentin, cyclin D1			CK7, CK8, CK18, CK19, EMA, RCC, CD34
Rhabdoid tumor	Pan-CK, vimentin	CK8, EMA, CD99, NSE	Synaptophysin, actin, desmin	PAX-2, PAX-8, myoglobin, CD34, S100
Mixed epithelial and stromal tumor	*Epithelial components*: pan-CK, EMA *Stromal components*: actin	CEA desmin, ER, PgR		HMB45, CD34
Transitional cell (urothelial) carcinoma of renal pelvis	CK5, CK7, CK8, CK13, CK17, CK18, CK19, *GATA-3, thrombomodulin*	*Uroplakin* (Ia, II, and III), S100P	CK20, *PAX-8*, calretinin	*PAX-2*, WT-1

[a]Nuclear and apical stain
[b]Cytoplasmic stain
[c]Positive in aggressive tumor types
[d]See Fig. 12.4

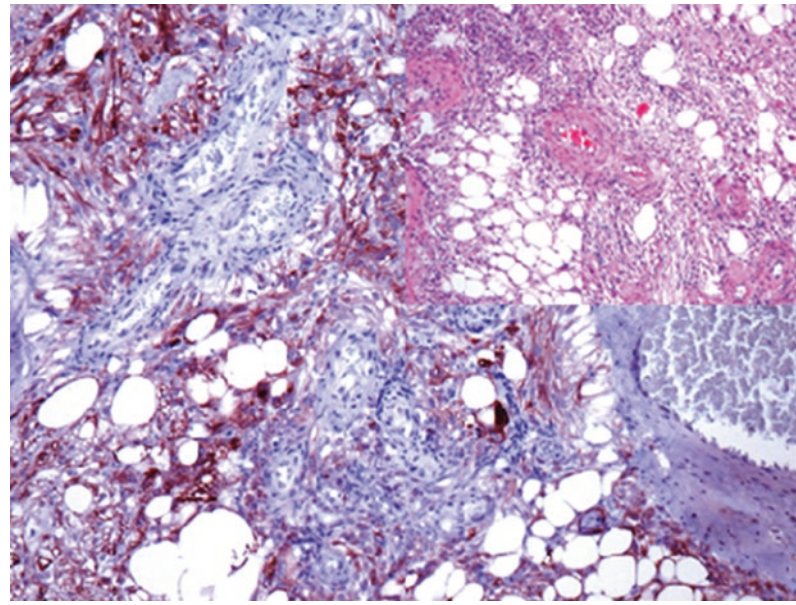

Fig. 12.4 HMB45 staining the perivascular epithelioid tumor cells in angiomyolipoma

Differential diagnosis between histological types of renal cell carcinoma

	CK7	CK20	CD10	CD117	E-cadherin	AMACR	RCC	PAX8	KIM1	CA IX	DOG-1
Clear cell renal carcinoma	–	–	+	–	–	–	+	+	+	+	–
Chromophobe renal cell carcinoma	+	–	–	+	+	–	–	+	–	–	+
Papillary renal cell carcinoma	+/–	+/–	+	–	+	+	+	+	+	–/+	–

Differential diagnosis clear cell renal carcinoma vs. tumors with clear cell appearance

	Pan-CK	PAX-8	CD10	p16	Inhibin	Arginase	HMB45 Sox-10	TEF-3
Renal cell carcinoma	+	+	+	–	–	–	–	-[a]
Adrenocortical tumors	–/+	–	–	–	+	–	–	–
Ovarian and endometrial clear cell carcinoma	+	+	–	+/–	–	–	–	–
Hepatocellular carcinoma	+	–	+	–	–	+	–	–
Clear cell sarcoma	–	–	–	–	–	–	+	–
Epithelioid sarcoma	+	–	–	–	–	–	–	–
Alveolar soft part sarcoma	–/+	–	–	–	–	–	–	+

[a]Positive in Xp11.2 translocation-associated renal cell carcinoma

12.2 Urinary Tract Tumors

Diagnostic Antibody Panel for Transitional Cell Carcinoma Cytokeratin profile (CK5/6/7/20), GATA-3, uroplakin, S100P, p63, and thrombomodulin (CD141).

Uroplakins		
Expression pattern: membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Transitional cell tumors		Normal urothelium
Positive control: urinary bladder mucosa		

Diagnostic Approach Uroplakins are transmembrane proteins expressed as rigid 0.2–0.5 μm plaques on the apical surface of mammalian urothelium and take part in the strengthening of the urothelial apical surface during distention of the urinary bladder and urinary tract [15, 16]. Uroplakins are divided into four subtypes, Ia, Ib, II, and III, all of which are expressed by the urothelium of the urinary tract, and the majority of tumors originate from the urothelium. Uroplakin subtypes Ia and II are specific for urothelium and are not detected in any tissue or carcinoma type other than transitional cell carcinoma (Fig. 12.5). Both uroplakins are also absent in primary squamous cell carcinoma and adenocarcinoma of the urinary bladder [17]. The uroplakin subtype Ib is detected in some other epithelial cells such as tracheal and bronchial epithelium and in the mucosa exhibiting squamous metaplasia. Uroplakin III is detected in prostatic glandular epithelium. Uroplakin, GATA-3, and CD141 (thrombomodulin) are negative in renal cell carcinoma and can discriminate between transitional cell carcinoma and renal cell carcinoma [18].

Diagnostic Pitfalls Antibodies to different uroplakins are specific markers for transitional cell carcinoma, but these markers are generally positive in only about 60% of transitional cell carcinoma, and a complete panel including the cytokeratins CK5/6/7/20, p63, GATA-3, and thrombomodulin is required for the appropriate diagnosis. Uroplakin II

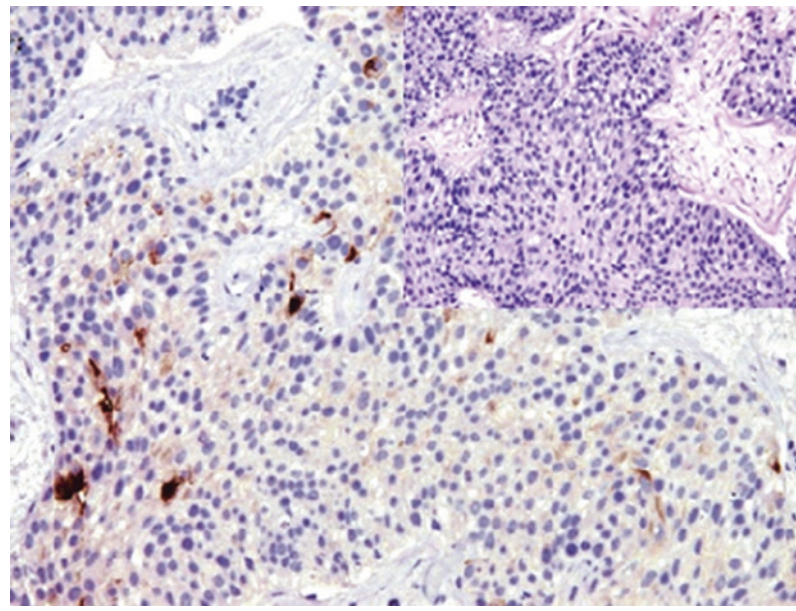

Fig. 12.5 Uroplakin highlights the cell membrane of transitional cell carcinoma

is the most used uroplakin in routine immunohistochemistry. The expression of uroplakins Ib and III is not diagnostic for transitional cell carcinoma, and other carcinoma types must be also considered in the differential diagnosis.

GATA-3: GATA-3 is a transcription factor listed in a previous section involved in the differentiation and proliferation of breast luminal epithelium, urothelium, and subsets of T-lymphocytes. GATA-3 is a useful screening marker to characterize metastases of unknown primary. Because of the wide expression spectrum of GATA-3, the diagnosis of transitional cell carcinoma must be confirmed by the cytokeratin profile and the expression of other urothelial markers such as thrombomodulin, uroplakin, and S100P (Fig. 12.6) [19, 20]. The co-expression of GATA-3, CDX-2, and CK7 in addition to membranous β-catenin is characteristic for primary adenocarcinoma of the bladder [21, 22].

Placental S100: S100P is one of the members of the S100 protein family listed in details in a previous section. S100P is found in normal urothelium and transitional cell carcinoma, while prostatic carcinoma lacks the expression of S100P. S100P is not specific for transitional cell carcinoma and must be used in a panel with other antibodies as it reacts with many other tissue and tumor types.

Thrombomodulin: Thrombomodulin is an endothelial anticoagulant protein clustered as CD141. It is a transmembrane glycoprotein expressed on the surface of endothelial cells and in other different cell types including mesothelial cells, stratified squamous epithelium, and transitional epithelium of the urinary tract. Thrombomodulin is a useful screening antibody for mesothelioma, transitional cell carcinoma, and vascular tumors (Fig. 12.7). Thrombomodulin is listed in details with the mesothelioma markers.

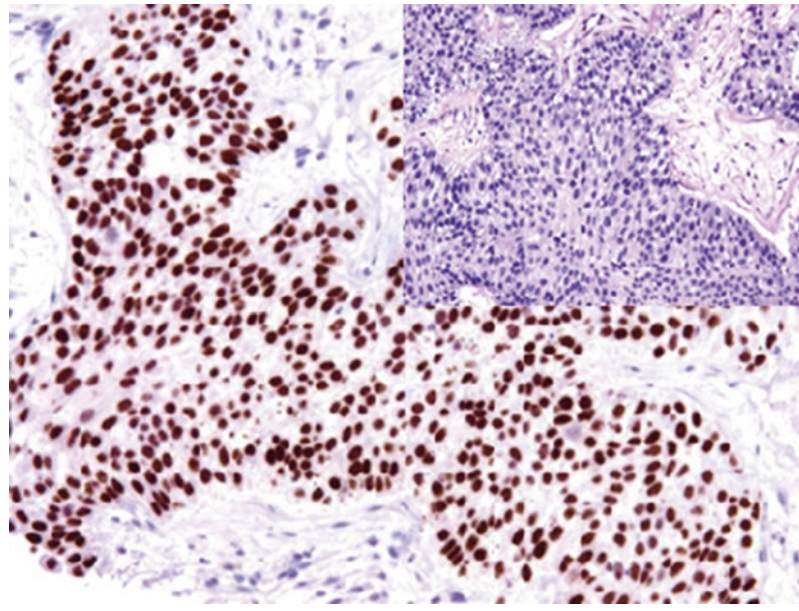

Fig. 12.6 Nuclear GATA-3 expression in transitional cell carcinoma

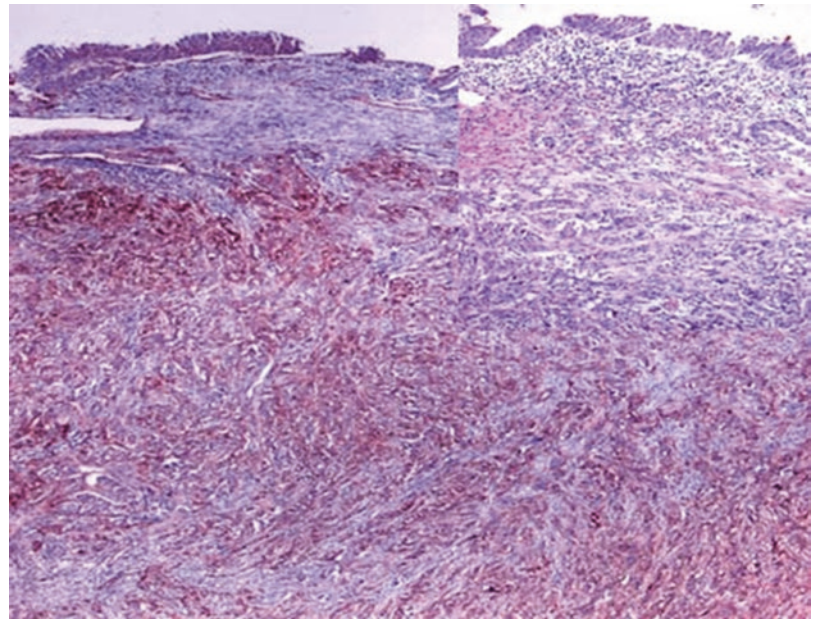

Fig. 12.7 Thrombomodulin expression in high-grade transitional cell carcinoma of the urinary bladder

Immunoprofile of urinary tract and urinary bladder tumors

Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Urothelial carcinoma in situ	CK20	*p53*, CEA		CD44[a]
Transitional cell (urothelial) carcinoma	CK5, CK7, CK8, CK13, CK17, CK18, CK19, *GATA-3, thrombomodulin*	*Uroplakin* (Ia, II, and III), CEA, S100P, fascin[b], CK20[c]	Calretinin	*PAX-8*[d], PAX-2, WT-1, vimentin
Adenocarcinoma of urinary bladder - Enteric type - Mucinous type - Signet ring cell type - Mixed type - NOS	CK8, CK18, CK19, β-catenin[e]	*Thrombomodulin*, CK7, CK20, CDX-2, CEA, CD15	GATA-3	CK5, PAP, PSA, PAX-8, NKX3.1
Urachal carcinoma	CK20, β-catenin, CD15	CDX-2, CEA, CK7	CK5/14	p63
Tumors of Müllerian type Clear cell adenocarcinoma	CK7, PAX-8, CA125, HNF1β, p53 Proliferation index (Ki-67): >15%	CK20, p504S (AMACR), PAX-2,	CD10	PSA
Squamous cell carcinoma of urinary bladder	CK5/6, *p40*, CK8, CK14, CK19			CK7, CK20
Small cell neuroendocrine carcinoma	Pan-CK, *CD56, synaptophysin, chromogranin,* NSE	EMA, CK7	TTF-1	CK20, uroplakin, CD44
Nephrogenic adenoma	PAX-2, PAX-8 Proliferation index (Ki-67): <3%		CK5/14, p63, p53	

Immunoprofile of urinary tract and urinary bladder tumors				
Botryoid fibroepithelial polyp of the urinary tract	Desmin, vimentin	ER, PR, actin, CD34		Pan-CK, S100, CD68, myogenin, Myo D1, CD 68, CD117

[a]CD44 positive in normal urothelium
[b]Fascin negative in normal urothelium
[c]CK20 negative in high-grade carcinoma and in inverted papilloma
[d]PAX-8 may be positive in transitional cell carcinoma of renal pelvis
[e]Membranous stain of β-catenin in bladder adenocarcinoma but nuclear stain in colorectal adenocarcinoma

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Markers and Immunoprofile of Male Genital Tract Tumors

13

Contents

13.1 Prostatic Tumors

Diagnostic Antibody Panel for Prostatic Adenocarcinoma (Acinar and Ductal) and Basal Cell Carcinoma

a. **Markers for prostatic epithelium:**
PSA, PAP, NKX3.1, prostein, androgen receptors, ERG, human glandular kallikrein-2 (hK2), AMACR (p504S)

b. **Basal cell markers:**
High molecular weight cytokeratins (CK5, CK6, CK14, CK34βE12), p40, p63

Prostate specific antigen (PSA)		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Carcinoma of the prostate	Salivary duct carcinoma, small cell carcinoma	Prostatic secretory and ductal epithelium, periurethral glands, male anal glands, Skene gland
Positive control: prostatic tissue		

Diagnostic Approach Prostate specific antigen (PSA) also known as kallikrin-3 is a single chain glycoprotein and a serine protease synthesized by the epithelium of prostatic gland and secreted into prostatic ducts. Normally, protease inhibitors

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rapidly inactivate PSA that enter the blood circulation. PSA is one of the most specific markers for prostatic parenchyma and prostatic carcinoma. Metastatic carcinoma positive for pancytokeratin but negative for cytokeratins 5/7/14/20 suggests a primary prostatic carcinoma, and the expression of PSA and/or NKX3.1 will confirm the prostatic origin.

Diagnostic Pitfalls About 10% of high-grade prostatic carcinomas are negative for PSA. In such cases, other prostate-specific markers such as NKX3.1, prostate-specific membrane antigen, prostatic acid phosphatase, and androgen receptors are useful to confirm the diagnosis. Low levels of PSA expression are reported in tumors other than prostatic carcinoma. Weak expression level of PSA is found in a subset of salivary duct carcinoma. Weak expression of PSA is also reported in small cell carcinoma and breast carcinoma in addition to endometrioid carcinoma.

Prostein (SLC45A3)		
Expression pattern: cytoplasmic, Golgi pattern		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Carcinoma of the prostate	None described	Prostatic secretory and ductal epithelium, periurethral glands, male anal glands
Positive control: prostatic tissue		

Diagnostic Approach Prostein (solute carrier family 45, type 4 (SLC45A4)) is a transmembrane transporter protein found in the Golgi apparatus of prostatic secretory epithelia. Prostein is more specific to determine a prostatic origin than PSA and slightly more sensitive. Prostein can thus be successfully used in a panel with NKX3.1 and PSA to classify metastases of unknown primary tumor or to discriminate between prostatic, urothelial, or colorectal carcinomas [1]. The loss of prostein expression is associated with unfavorable clinical course [2].

Diagnostic Pitfalls Negativity for prostein does not rule out prostatic origin.

Prostatic acid phosphatase (PAP)		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Carcinoma of the prostate	Neuroendocrine tumors, intravascular large B-cell lymphoma	Prostatic secretory and ductal epithelium, periurethral glands, male anal glands
Positive control: prostatic tissue		

Diagnostic Approach Prostatic acid phosphatase (PAP) is an enzyme secreted by prostatic epithelium and a major component of prostatic fluid. PAP is more sensitive but less specific than PSA for prostatic glands and prostatic carcinoma. PAP can be successfully used in a panel with PSA to classify metastases of unknown primary tumor.

Diagnostic Pitfalls Similar to PSA, PAP can be also expressed in neuroendocrine carcinomas of different origin. This feature is important for the differentiation between poorly differentiated prostatic carcinoma, prostatic carcinoma with neuroendocrine differentiation, and neuroendocrine tumors.

Androgen receptors		
Expression pattern: nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Prostatic carcinoma	Osteosarcoma, apocrine breast carcinoma, Paget's disease, salivary duct carcinoma, sebaceous carcinoma, basal cell carcinoma, mesonephric adenocarcinoma	Prostatic cells, Sertoli cells, Leydig cells, apocrine and sebaceous glands, skin, oral mucosa, hepatocytes
Positive control: prostatic tissue		

Diagnostic Approach Androgen receptor (AR) is a member of the steroid family of ligand-dependent transcription factors. Androgen receptor is expressed in different tissue types including prostatic glands and skin adnexa. Neoplastic prostatic glands are usually positive for AR, but studies show no direct correlation between the intensity of AR expression and the response to hormonal therapy [3]. The nuclear expression pattern of AR makes it useful for the immunohistochemical double stain with other antibodies with cytoplasmic or membranous expression pattern.

Diagnostic Pitfalls The expression of AR is not restricted to prostatic carcinoma and can be found in other carcinoma types with similar morphology such as salivary duct carcinoma, breast carcinoma, and apocrine carcinoma.

Alpha-methylacyl-CoA racemase (AMACR, p504S)		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Prostatic adenocarcinoma, high-grade PIN	Gastrointestinal adenocarcinoma, hepatocellular and papillary renal cell carcinoma, carcinoma of the breast and ovaries, endometrial clear cell carcinoma, urothelial carcinoma, extramammary Paget's disease, mesothelioma, lymphoma, pancreatic islet tumor	Periurethral glands, liver, salivary glands, sebaceous glands, renal tubular epithelium, pancreas epithelium, mesothelial cells
Positive control: prostatic carcinoma		

Diagnostic Approach Alpha-methylacyl-CoA racemase (also known as p504S) is a member of the isomerases enzyme family involved in the metabolism of branched-chain fatty acids and synthesis of bile acids. It is expressed in the mitochondria and peroxisomes of various normal and neoplastic cells. p504S is overexpressed in prostatic carcinoma compared to benign prostatic glands (Fig. 13.1) [4, 5]. In combination with p63, alpha-methylacyl-CoA racemase (AMACR) is now widely used for the diagnosis of prostatic carcinoma (so-called PIN cocktail). p63 is a myoepithelial marker exhibiting a nuclear stain [6]. The immunohistochemical double stain with the PIN cocktail can show one of the following three results:

- AMACR-positive prostatic glands lacking the p63-positive myoepithelial cells; a combination characteristic of neoplastic glands
- AMACR-positive glands surrounded by p63-positive myoepithelial cells; characteristic of prostatic glands with high-grade PIN
- AMACR-negative prostatic glands surrounded by p63-positive myoepithelial cells; characteristic of normal prostatic glands

Low molecular weight cytokeratins such as CK5/6/14 can be used as alternatives to p63 in a separate reaction (Fig. 13.2).

Diagnostic Pitfalls The expression of AMACR is found in many neoplasms types and cannot be considered as a specific marker of prostatic tumors [7].

NKX3.1		
Expression pattern: nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Prostatic adenocarcinoma	GCNIS, breast carcinomas, subset of T-ALL	Prostatic tissue, salivary glands, mucinous bronchial glands
Positive control: prostatic tissue		

Diagnostic Approach Is an androgen-regulated tumor suppressor gene located on chromosome 8p221.1 and strongly expressed in the nuclei of normal prostatic epithelium and persist in prostatic acinar carcinomas. NKX3.1 is a specific marker for primary prostatic carcinoma, and the

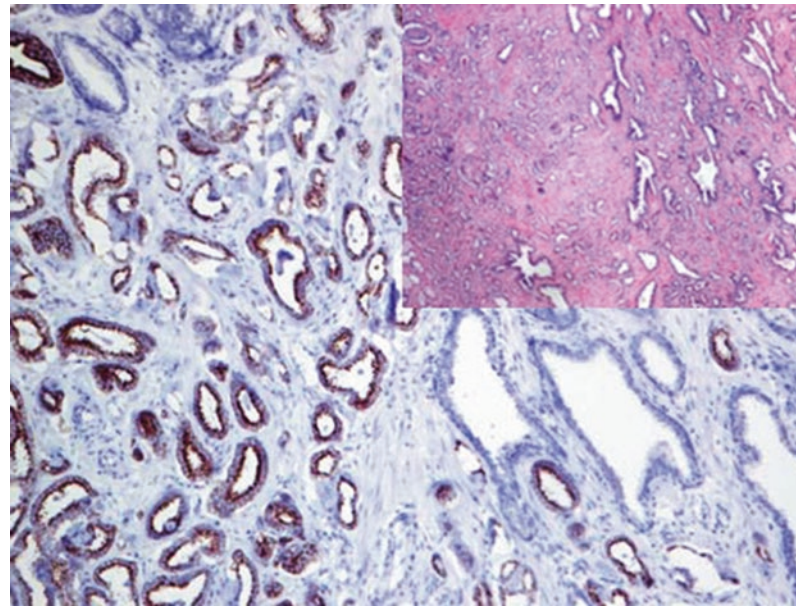


Fig. 13.1 AMACR expression in luminal cells of prostatic adenocarcinoma

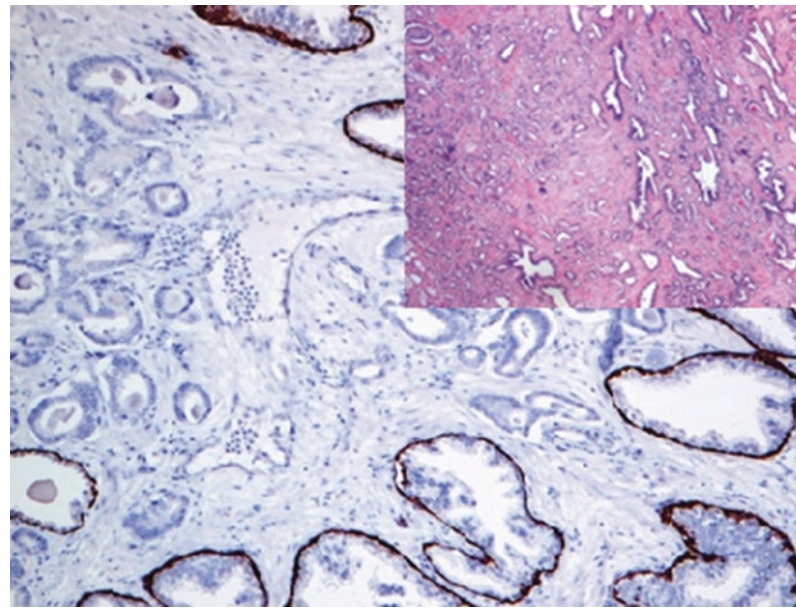


Fig. 13.2 Neoplastic glands of prostatic adenocarcinoma lacking the myoepithelial cells positive for high molecular weight cytokeratin (CK5/14)

intensity of the nuclear expression correlates with the differentiation grade of prostatic carcinoma, which can be very weak in poorly differentiated carcinoma (Fig. 13.3) [8].

Diagnostic Pitfalls NKX3.1 is also expressed in testicular germ cells and seminoma in situ (GCNIS) but lost in invasive seminoma and embryonal carcinoma. Different expression intensity of NKX3.1 is also found in estrogen- and androgen-positive breast carcinomas, i.e., invasive lobular carcinoma [9]. Mucinous units of salivary and bronchial glands also reveal a nuclear expression of NKX3.1 which to consider in the interpretation of small biopsies [10]. Furthermore, the TAL-1 genetic aberration associated with a subset of T-ALL causes the activation of NKX3.1 expression in neoplastic lymphocytes [11].

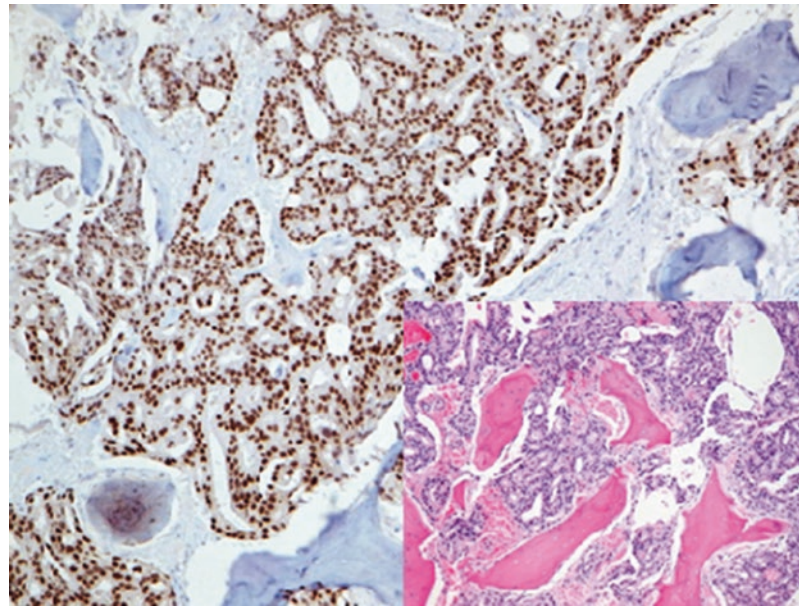


Fig. 13.3 Metastatic prostatic carcinoma with strong nuclear NKX3.1 expression

ERG		
Expression pattern: nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Prostatic adenocarcinoma, endothelial tumors/ angiosarcoma	Acute myeloid leukemia, solitary fibrous tumor, epithelioid sarcoma, meningioma	Endothelial cells
Positive control: blood vessels		

Diagnostic Approach V-ETS avian erythroblastosis virus E26 oncogene homolog (ERG) encoded by the gene located on chromosome 21q22.3. ERG is a member of the ETS family transcription factors, which also include Fli-1 and EST-1. ERG is normally expressed in endothelial cells and tumors derived from these cells [12].

The ERG gene is the fusion partner of the TMPRSS2 gene involved in the regulation of androgen response. This genetic mutation is the most frequent genetic abnormality associated with prostatic carcinoma and found in 40–80% of the cases. This mutation generates the TMPRSS2-ERG gene fusion causing the overexpression of the ERG protein detected by immunohistochemistry (Fig. 13.4) [13, 14].

The aberrant expression of ERG is also characteristic for the solitary fibrous tumor because of other genetic anomalies associated with this tumor. ERG expression is also reported in few other mesenchymal tumors including epithelioid sarcoma and fibrous meningioma [15, 16].

Despite this obvious lack of sensitivity, ERG positivity in a metastasis of unknown epithelial primary can be considered confirmative of prostate cancer.

Diagnostic Pitfalls The immunohistochemical results using this marker must be carefully interpreted as positive staining is observed in about 29% of high-grade PIN and occasionally benign glands; thus, the gold standard remains the labeling of the myoepithelial basal cells [17]. Both antibodies to ERG and p63 can be used as a cocktail for the diagnosis of prostatic carcinoma but has less sensitivity than the above disrobed PIN cocktail.

Phosphatase and Tensin Homolog (PTEN): PTEN is a tumor suppressor mentioned in a previous chapter. The loss of PTEN is found in a fraction of high Gleason prostatic carcinoma, which is usually resistant to the antiandrogen therapy. Furthermore, the loss PTEN expression is a useful marker to distinguish intraductal carcinoma from PIN usually positive for PTEN.

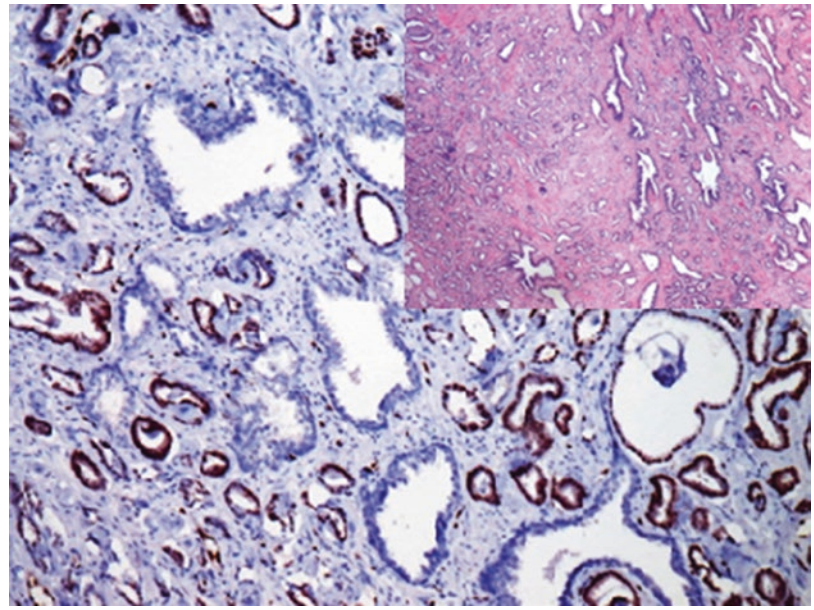


Fig. 13.4 Nuclear ERG expression in neoplastic cells of prostatic adenocarcinoma

Immunoprofile of prostatic and seminal vesicle tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Sclerosing adenosis of the prostate:	Preserved myoepithelial basal cells positive for high molecular weight cytokeratins (CK5/6/14, CK-34E12), p63, p40, LP34			
Adenocarcinoma of the prostate: – Acinar adenocarcinoma – Ductal adenocarcinoma	CK8, CK18, CK19, *PSA*, PAP, *NKX3.1*, *prostein*, hK2, *p504S (racemase)* *Diagnostic is the loss of basal myoepithelial cell layer: negativity for high molecular weight cytokeratins (CK5/6/14, CK-34E12), p63, p40*	*Androgen receptors*, *ERG*	CK7,	CK5, CK10, CK20, CEA, uroplakin
Basal cell carcinoma of the prostate:	CK8, CK18, *CK5/6/14*, *p63*, *p40*, HER-2, androgen receptors, bcl-2 CK7 in luminal cells			P504S (AMACR), CK 20, PSA
Neuroendocrine tumors: – Adenocarcinoma with neuroendocrine differentiation – Well-differentiated neuroendocrine tumor – Small and large cell neuroendocrine carcinoma	See neuroendocrine tumors			
Stromal tumor of uncertain malignant potential/stromal sarcoma:	CD34, PgR		ER	
Adenocarcinoma of seminal vesicle:	CK8, CK18, CK19, PAX-8, CA-125, CEA	CK7, CA 125 (MUC 16)		CK20, PAP, PSA

13.2 Testicular and Paratesticular Tumors

13.2.1 Germ Cell Tumors

Diagnostic Antibody Panel for Germ Cell Tumors Oct-3/4, SALL-4, NANOG, LIN28, Sox-2, CD117, PLAP, AFP, CD30, β-hcG, and cytokeratin profile.

13.2.2 Sex Cord-Stromal Tumors

Diagnostic Antibody Panel for Sex Cord-Stromal Tumors Inhibin, adrenal 4 binding protein (Ad4BP, SF-1), FOXL2, calretinin, CD56, anti-Müllerian hormone, Melan A, CD99.

SALL-4		
Expression pattern: nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Seminoma, intratubular germ cell neoplasms, embryonal carcinoma, yolk sac tumor, ovarian dysgerminoma, CNS germinoma	Subset of gastrointestinal, pulmonary adenocarcinoma, ovarian serous carcinomas, rhabdoid tumor, Wilms' tumor, B-cell ALL, AML	CD34 positive progenitor cells
Positive control: seminoma		

Diagnostic Approach Sal-like protein (SALL-4) is member of the **sp**a**l**t-**l**ike multi-zinc finger family functioning as a transcription factor encoded on chromosome 20q13. SALL-4 is involved in the development and maintenance of embryonic stem cell pluripotency by modulation of Oct-4 [18–20]. The expression of SALL-4 is an important sensitive and specific marker for testicular, ovarian, and extragonadal germ cell tumors including seminoma and dysgerminoma, embryonal carcinoma, immature teratoma, and mononuclear trophoblastic cells of choriocarcinoma. In contrast to Oct-4, SALL-4 strongly labels yolk sac tumor (Fig. 13.5). It is also strongly expressed in the neoplastic cells of intratubular germ cell neoplasms but not in the normal testicular intratubular germ cells. SALL-4 is negative in sex cord tumors [21].

Diagnostic Pitfalls The expression of SALL-4 is not restricted to germ cell tumors as it is found in a subset of other non-germ cell tumors such as serous ovarian carcinoma, pulmonary adenocarcinoma, cholangiocarcinoma, urothelial carcinoma, and small cell carcinoma, which are to consider in the interoperation of this marker [20].

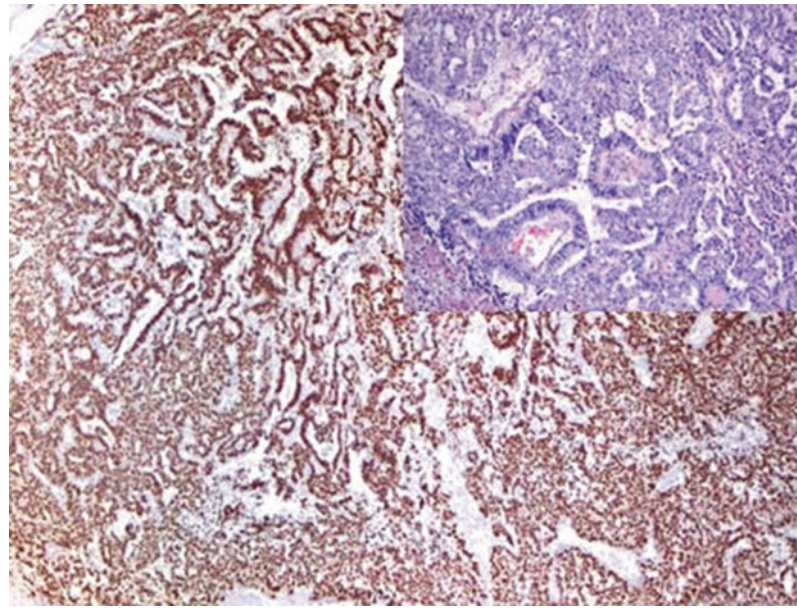


Fig. 13.5 SALL-4 labeling the nuclei of yolk sac tumor cells

Oct-4		
Expression pattern: nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Seminoma, intratubular germ cell neoplasms, embryonal carcinoma	Ovarian dysgerminoma, CNS germinoma, diffuse large B-cell lymphoma	Germ cells (pluripotent germ cells)
Positive control: seminoma		

Diagnostic Approach Octamer-binding transcription factor 4 (Oct-4) is a member of the POU family of transcription factors, expressed in early embryonic cells, and plays a role in the differentiation of pluripotent germ cells. A high expression level of Oct-4 is characteristic for seminoma and embryonal carcinoma, whereas spermatocytic seminoma lacks the expression of Oct-4 (Fig. 13.6) [22]. Oct-4 labels the nuclei of the majority of the dysplastic cells of intratubular germ cell neoplasms but not the nonneoplastic testicular cells, making Oct-4 a helpful and specific maker for intratubular germ cell neoplasms (Fig. 13.7) [23].

Diagnostic Pitfalls The expression of Oct-4 is found in a subset of pulmonary non-small cell carcinoma and breast carcinoma [24]. Oct-4 expression is also found in some cases of testicular and extratesticular diffuse large B-cell lymphoma, which to consider in the differential diagnosis [25].

Placental alkaline phosphatase (PLAP)		
Expression pattern: membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Seminoma, embryonal carcinoma	Proximal GI tumors, lung and ovarian carcinoma, tumors with myogenic differentiation	Placental syncytiotrophoblasts, endocervical and fallopian tube mucosa
Positive control: seminoma		

Diagnostic Approach Alkaline phosphatases are a group of metalloenzymes catalyzing the hydrolysis phosphoric acid monoesters. Placental alkaline phosphatase (PLAP) is a membrane-associated glycoprotein primarily expressed in placental syncytiotrophoblasts from the eighth week throughout

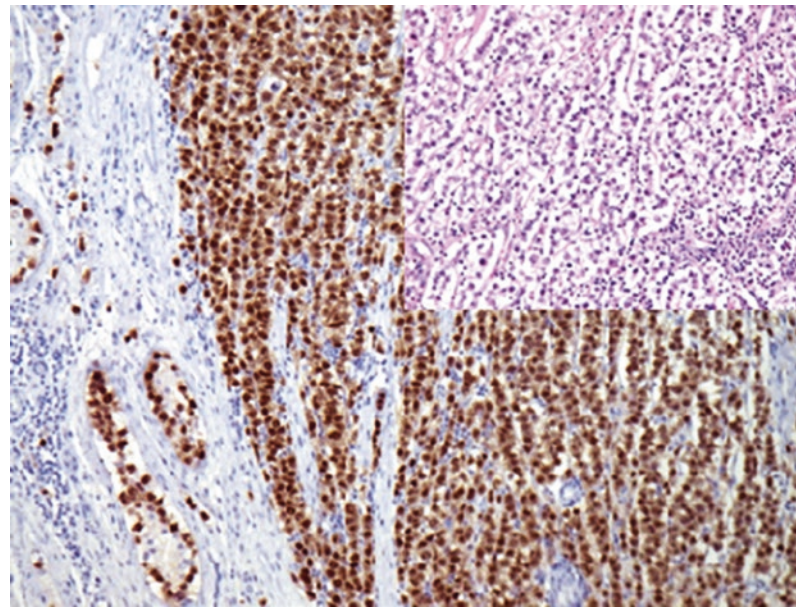


Fig. 13.6 Oct-4 labeling the nuclei of seminoma cells

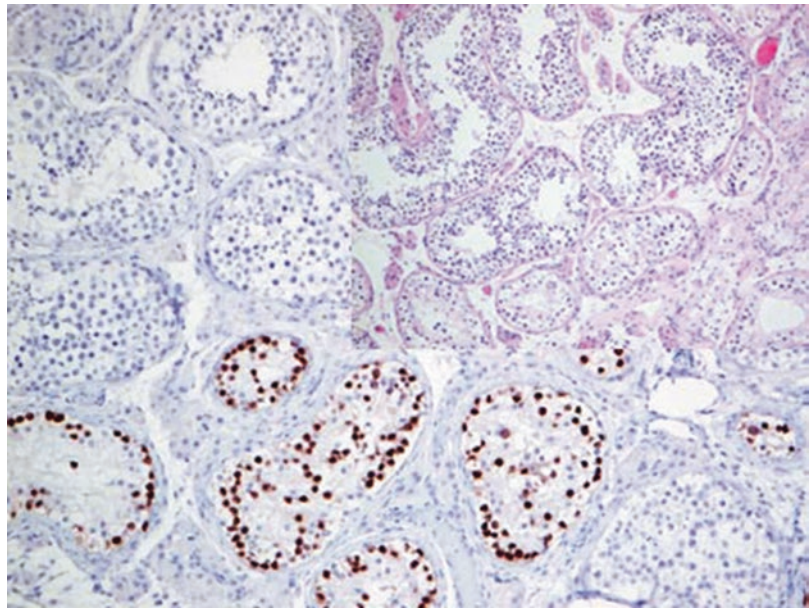


Fig. 13.7 Oct-4 highlighting the cells of germ cell neoplasia in situ (GCNIS)

the pregnancy. PLAP is a marker for several germ cell tumors such as seminoma, dysgerminoma, embryonal carcinoma, yolk sac tumor, and gonadoblastoma. Since PLAP is not specific for any specific germ cell tumor, a panel of antibodies is required to differentiate between the PLAP-positive germ cell tumors (see below) [26–28].

Diagnostic Pitfalls Aberrant PLAP expression is rarely found in other non-germ cell tumor types such as breast and lung carcinoma. Additionally, it is important to consider that a cytoplasmic PLAP stain is reported in tumors with myogenic differentiation such as embryonal rhabdomyosarcoma and smooth muscle tumors [29].

Sox-2		
Expression pattern: nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Embryonal carcinoma	Squamous cell carcinoma, prostatic carcinoma, neuroendocrine tumors, gliomas	Brain tissue
Positive control: seminoma		

Diagnostic Approach Sox-2 is a member of the Sox family of transcription factors (**s**ex determining region Y-b**ox 2**). Sox-2 forms a trimeric complex with Oct-4 on DNA and controls the expression of a number of genes involved in embryonic development of the respiratory tract, nervous system, and germ cells. In germ cell tumors, Sox-2 shows strong nuclear expression in embryonal carcinoma but negative in seminoma, yolk sac tumor, and choriocarcinoma [30]. Sox-2 is also expressed in glial brain tumors and supratentorial PNET [31]. Ectopic Sox-2 expression is found in a subset of pulmonary squamous cell carcinomas and adenocarcinomas. Variable PLAP expression is also reported in some neuroendocrine carcinomas [32].

Podoplanin (D2-40): D2-40 is a type I transmembrane mucoprotein listed in details with the markers of vascular tumors. D2-40 is an excellent seminoma maker negative in other germ cell tumors. As D2-40 stains both seminoma cells and lymphatic vessels, it can be used as a marker to highlight the lymphovascular invasion in surgical specimens.

Human chorionic gonadotropin (hCG)		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Syncytiotrophoblast in germ cell tumors (choriocarcinoma), non-seminomatous testicular tumors	Pulmonary large cell carcinoma and adenocarcinoma	Trophoblasts
Positive control: placenta		

Diagnostic Approach Human chorionic gonadotropin is a hormone produced by syncytiotrophoblasts composed of α- and β-chains. The β-chain reveals a unique structure and is more specific for syncytiotrophoblasts and related tumors. The α-chain shares amino acid sequences with other hormones such as LH, FSH, and TSH of the pituitary gland.

Diagnostic Pitfalls Low expression levels of β-hCG could be found in other non-syncytiotrophoblastic tumors such as pulmonary and colonic carcinomas and rarely lymphomas [33]. Generally, the expression of β-hCG in nontrophoblastic tumors indicate an aggressive behavior.

CD30: CD30 is listed in detail in a later section as an important marker for Hodgkin's and anaplastic lymphomas. Additionally, the expression of CD30 is characteristic for embryonal carcinoma (Fig. 13.8). In rare cases, CD30 may faintly stain yolk sac tumor, which to consider in differential diagnosis of combined germ cell tumors.

Inhibin A		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Sex cord-stromal tumors (granulosa cell tumor, Leydig, Sertoli, and steroid cell tumor, thecoma and fibrothecoma), adrenocortical tumors	Choriocarcinoma and trophoblastic lesions	Sertoli cells, granulosa cells, theca interna, adrenal cortex, brain tissue
Positive control: granulosa cell tumor/adrenal gland		

Diagnostic Approach Inhibin is a member of the transforming growth and differentiation factor family. It is a glycoprotein hormone composed of α- and β-subunits expressed in the gonads and adrenal gland functioning as inhibitor for the pituitary follicle-stimulating hormone (FSH) secretion and stimulates the synthesis of androgen in ovarian theca cells. Antibodies to inhibin A, anti-Müllerian

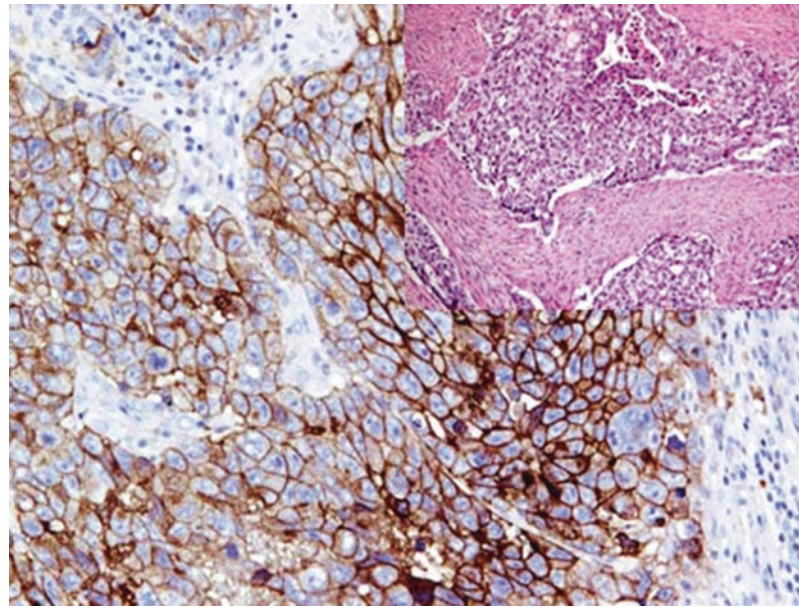


Fig. 13.8 Strong CD30 membranous stain of embryonal carcinoma

hormone, and Melan A are important diagnostic markers for sex cord tumors [34]. Inhibin and anti-Müllerian hormone are consistently negative in ovarian surface epithelial-stroma tumors, seminoma, and embryonal carcinoma.

Diagnostic Pitfalls Both inhibin and Melan A (MART-1) are also expressed in other tumors, mainly tumors of the adrenal cortex. Furthermore, Melan A is widely used as melanoma marker.

Anti-Müllerian Hormone: Anti-Müllerian hormone (AMH) is a member of the transforming growth factor-beta gene family. The expression of AMH is regulated by SF-1, GATA factors, DAX1, and follicle-stimulating hormone. AMH mediates the male sexual differentiation by inhibiting the development of Müllerian duct, preventing the transformation of the Müllerian duct into the uterus, fallopian tubes, and other Müllerian structures, and plays a role in the testicular differentiation. If no AMH is produced, the Müllerian ducts undergo differentiation, while the Wolffian ducts become atrophic. In the postnatal period, AMH is also expressed in both males and females by Sertoli cells and to a lesser degree by granulosa cells. Anti-Müllerian hormone is an immunohistochemical marker for Sertoli cell and granulosa cell tumors [35]. Other sex cord-stromal tumors are usually AMH negative.

Adrenal 4 Binding Protein (SF-1): SF-1 is listed in detail in the chapter of adrenocortical tumors. SF-1 is a sensitive marker for Sertoli cell tumors and granulosa cell tumor. Leydig cell tumor lacks the expression of SF-1.

Glypican-3: Glypican-3 was listed in detail in a previous chapter. In germ cell tumors, Glypican-3 is a specific marker for yolk sac tumor and choriocarcinoma, whereas embryonal carcinoma and seminoma usually lack the expression of Glypican-3.

CD56: CD56 (neural cell adhesion molecule) is listed in detail in a later section. CD56 is a sensitive marker for ovarian and testicular sex cord-stromal tumors but lacks the specificity as it is expressed in a wide range of other tumors. The combination of CD56 with inhibin and Melan A will make the diagnosis of sex cord tumors more precise.

Melan A and CD99 are further helpful markers for cord-stromal tumors and listed in detail in later chapters.

13.2.3 Paratesticular Tumors

Diagnostic Antibody Panel for Paratesticular Tumors PAX-8, calretinin, and cytokeratin profile.

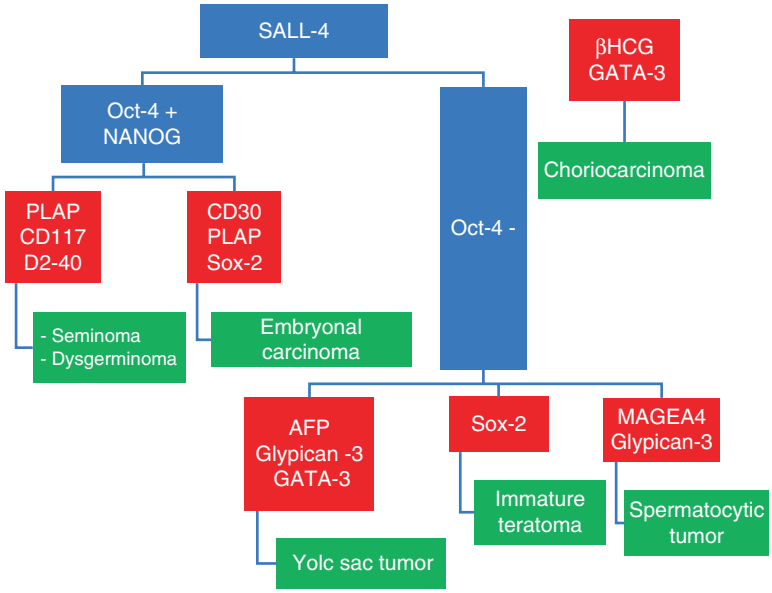
PAX-8: PAX-8 strongly stains epididymal and seminal vesicle cells and carcinomas derived from these cells and can be used to differentiate between prostatic carcinoma and carcinoma of seminal vesicles (see markers of renal cell tumors).

Immunoprofile of testicular and paratesticular tumors

Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
A. Germ cell tumors				
Germ cell neoplasia in situ (GCNIS)	*SALL-4*, *Oct-4*, *PLAP*, *TCL-1*, SOX-17, sCD143, LIN28, angiotensin-converting enzyme (ACE), NSE	Ferritin, CD117		AFP, ß-hCG, inhibin
Seminoma/dysgerminoma	*SALL-4*, *Oct-3/4*, *NANOG*, *PLAP*, Sox-17, *TCL-1*, LIN28, tCD143	CD117, *D2-40*, CK8, AP-2γ, Glut-3, vimentin	CK18, NSE, CK7	*CD30*, *Sox-2*, *glypican-3*, GATA-3EMA, CK19, CK20, CEA, AFP, ß-hCG, inhibin

Immunoprofile of testicular and paratesticular tumors				
Spermatocytic tumor (spermatocytic seminoma)	*SALL-4*, *MAGEA4*, CK8/18	Vimentin, NSE	CD117	AFP, *Oct-4*, PLAP, ß-hCG, CEA, EMA, CD30, CD143, D2-40
Embryonal carcinoma	*Oct-3/4*, *SALL-4*, *NANOG*, *Sox-2*, *PLAP*, *CD30*, LIN28, CK8, CK18	CK7, CK19, NSE	ß-hCG, AFP, vimentin	EMA, CK20, CEA, GATA-3, *Sox-17*, *CD117*, *glypican-3*, D2-40
Yolk sac tumor	*AFP*, *SALL-4*, *glypican-3*, pan-CK, GATA-3, LIN28	PLAP, CD34	CDX2, CD117, HepPar1, NSE, GFAP	*NANOG, Sox-2*, *Oct-3/4*, *CK7*, EMA, ß-hCG, CD30, CEA, vimentin
Choriocarcinoma – Syncytiotrophoblastic cells – Cytotrophoblastic cells	*ß-hCG*, inhibin, CD10, pan-CK, CK8/18, CK19, glypican-3, EGFR, GATA-3 CD10, pan-CK, CK8/18, CK19, CEA	PLAP, human placental lactogen, EMA, CEA, glypican-3, PLAP	Vimentin	CD30, AFP, Oct-4, NANOG, Sox-2, Sox-17 ß-hCG, inhibin, EMA, CD30, AFP, Oct-4
Immature teratoma	*SALL-4*, *Sox-2*			*Oct-4*, *NANOG*, CD117, CD30
Polyembryoma: Embryonal bodies	AFP, pan-CK	PLAP		
B. Sex cord-stromal tumors				
Leydig cell tumor	*Inhibin*, *CD56*, *Melan A*, *adrenal 4 binding protein (SF-1)*, calretinin, vimentin	CD99	Pan-CK, S100, synaptophysin, chromogranin	EMA, PLAP, AFP, anti-Müllerian hormone
Sertoli cell tumor	*Adrenal 4 binding protein (SF-1)*, *FOXL2*, *anti-Müllerian hormone*, CD56, vimentin	Inhibin, AFP, CD99, pan-CK, calretinin, SOX-9	NSE, S100, synaptophysin	Chromogranin, EMA, PLAP
Granulosa cell tumor	*Inhibin*, *FOXL2*, *adrenal 4 binding protein (SF-1)*, CD56, vimentin	CD99, anti-Müllerian hormone	CK8, CK18, actin, S100	EMA, desmin
Gonadoblastoma	The immunoprofile of both germ cell and sex cord-stromal components			
C. Paratesticular tumors				
Adenomatoid tumor	*Calretinin*, pan-CK, CK5/6, CK7, WT-1, thrombomodulin (CD141), vimentin			CD31, CD34, CEA
Adenocarcinoma of rete testis	Pan-CK, EMA, *PAX-8*		CEA	AFP, PLAP
Melanotic neuroectodermal tumor	*Large pigmented cells:* pan-CK, NSE, HMB45, synaptophysin *Small cells:* NSE, HMB45, synaptophysin, CD56	S100	GFAP Pan-CK, GFAP	

Algorithm 13.1: Immunoprofile of Germ Cell Tumors



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Markers and Immunoprofile of Endocrine and Neuroendocrine Tumors

14

Contents

14.1 General Endocrine and Neuroendocrine Markers

Chromogranin, synaptophysin, NSE, S100, PGP9.5, CD56, PAX-6, synaptic vesicle protein 2, and somatostatin receptor

The abovementioned immunohistochemical markers are used to screen for neuroendocrine differentiation in normal or tumor tissue; however, none of these antibodies are a universal marker for the neuroendocrine differentiation; consequently, screening for such differentiation must include two or more antibodies. In our practice, we found that a mixture of chromogranin A and synaptophysin gives better results and superior stain intensity.

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DOI 10.1007/978-3-319-53577-7_14

Chromogranin A		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Neuroendocrine tumors: pituitary adenomas, medullary thyroid carcinoma, parathyroid adenoma/carcinoma, pheochromocytoma, islet cell tumors, Merkel cell carcinoma, small cell carcinoma, carcinoid and neuroendocrine carcinoma	Oligodendroglioma, neuroblastoma, PNET, paraganglioma	Neuroendocrine cells: anterior pituitary gland, C cells of the thyroid gland, parathyroid gland, islet cells of the pancreas, adrenal medulla, gastrointestinal and bronchial endocrine cells, neuronal cells
Positive control: appendix		

Diagnostic Approach Chromogranin and synaptophysin are the most commonly used neuroendocrine markers. Chromogranins are glycosylated calcium-binding acidic proteins and members of the chromogranin/secretogranin family that includes chromogranin A, chromogranin B (also known as secretogranin I), and chromogranin C (also known as secretogranin II), located in the neurosecretory granules of neuroendocrine cells and synaptic vesicular walls. Chromogranin A is the most used marker in routine immunohistochemistry. Chromogranins are expressed in almost all neuroendocrine cells and neuroendocrine tumors. The intensity of the immunostain depends on the quantity of neurosecretory granules present in the cytoplasm of examined cells; an example is small cell carcinoma, which actively synthesizes chromogranin but, because of paucity of cytoplasm and scarcity of neurosecretory granules, shows usually very weak chromogranin stain.

Synaptophysin		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Neuroendocrine tumors: pituitary adenomas, medullary thyroid carcinoma, parathyroid adenoma/carcinoma, pheochromocytoma, islet cell tumors, small cell carcinoma, carcinoid and neuroendocrine carcinoma	Medulloblastoma, retinoblastoma, neurocytoma, ependymoma, neuroblastoma, adrenocortical tumors, Merkel cell carcinoma	Neuronal and neuroendocrine cells, carotid body cells, adrenal cortex and medulla
Positive control: appendix		

Diagnostic Approach Synaptophysin is a transmembrane calcium-binding glycoprotein present as a major component of presynaptic vesicles. Synaptophysin is a wide-spectrum marker for neuroendocrine cells and tumors with neuroendocrine differentiation. A mixture of antibodies to chromogranin and synaptophysin will increase the sensitivity.

Other synaptic vesicle proteins such as synaptic vesicle protein-2, synaptogranin, and vesicle-associated membrane protein are rarely used in routine immunohistochemistry.

Neuron-specific enolase (NSE) γ-subunit		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Neuroectodermal and neuroendocrine tumors	Melanoma, Merkel cell carcinoma, meningioma, renal cell carcinoma	Neurons, neuroendocrine cells, megakaryocytes, T-lymphocytes, smooth and striated muscle
Positive control: appendix		

Diagnostic Approach Neuron specific enolase (NSE) is a glycolytic enzyme catalyzing the reaction pathway between 2-phospho-glycerate and phosphophenol pyruvate playing role in intracellular energy metabolism. Enolases are homo- or heterodimers composed of the three subunits: alpha (α) subunit, beta (β) subunit, and gamma (γ) subunit, whereas antibodies to the γ-subunit are the most commonly used. The γ-subunits are primarily expressed in neurons and normal and neoplastic neuroendocrine cells. Different expression levels are also found in megakaryocytes and T-lymphocytes in addition to striated and smooth muscle cells.

Diagnostic Pitfall NSE has a low specificity to neuroendocrine tumors ("nonspecific enolase") and is usually used as a screening marker; therefore, the diagnosis must be supported by other more specific markers.

S100		
Expression pattern: cytoplasmic/nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Melanomas, schwannoma, histiocytic (Langerhans cell) neoplasms, neuroendocrine tumors	Liposarcoma, malignant peripheral nerve sheath tumors, neurofibroma, neurilemmoma, chondrosarcoma and chondroblastoma, clear cell sarcomas, myoepithelial tumors, granulosa cell tumor	Cells of neural crest (glial cells, Schwann cells, melanocytes and nevus cells), chondrocytes, adipocytes, myoepithelial cells, macrophages, adrenal medulla and paraganglia, Langerhans cells, dendritic cells
Positive control: appendix		

Diagnostic Approach S100 protein family consists of about 25 homologous low molecular weight intracellular calcium-binding proteins encoded by different genes located at different chromosomes, mainly chromosome 1. S100 is normally present in cells derived from the neural crest including glial cells, Schwann cells, melanocytes, chondrocytes, osteocytes, adipocytes, myoepithelial cells, dendritic cells, Langerhans cells, macrophages and some types of epithelial cells. S100 is a widely used broad-spectrum marker and different polyclonal or monoclonal antibodies directed to various members of the S100 family are available for routine immunohistochemistry.

Diagnostic Pitfalls S100 is a screening marker that lacks the specificity, and the final diagnosis must be confirmed by additional more specific markers.

Further markers for endocrine- and neuroendocrine tumors such as CD56 and PGP9.5 are listed in detail in other sections.

14.2 Pituitary Gland Tumors

14.2.1 Diagnostic Antibody Panel for Tumors of the Anterior Pituitary Gland (Adenohypophysis)

Neuroendocrine markers (see previous chapter), cytokeratin profile, and pituitary hormones.

The adenohypophysis is composed of six secretory cell types (α, β, δ, γ, ε cells), and all but one of them are able to produce only one of the anterior lobe hormones. The new classification of pituitary gland adenomas based on the hormonal activity of the adenoma cells, which can be detected using specific antibodies to the pituitary gland hormones and hormone precursor molecules.

14.2.2 Pituitary Hormones

- **Growth hormone (GH):** is a 191 amino acid polypeptide able to stimulate the release of insulin-like growth factor-1, which promotes the growth of long bones.

- **Prolactin (PRL):** PRL is a 198 amino acid polypeptide. Antibodies to PRL stain prolactin producing normal and neoplastic cells of pituitary gland. Prolactin producing cells may be also found in prostatic glands.
- **Thyroid-stimulating hormone (TSH):** a glycoprotein consisting of the β- and α-chain regulating the T4 production in the thyroid gland.
- **Adrenocorticotropic hormone (ACTH):** a 39 amino acid polypeptide that acts on the cells of adrenal cortex. Beside cells of adenohypophysis, ACTH can be synthesized by macrophages and lymphocytes in response to stress. Pulmonary small cell carcinoma can also be positive for ACTH.
- **Follicle stimulating hormone (FSH):** a glycoprotein consisting of the β and α chain regulating folliculogenesis, spermatogenesis, and proliferation of Sertoli cells.
- **Luteinizing hormone (LH):** a glycoprotein consisting of the β and α chain regulating folliculogenesis and the production of testosterone in Leydig cells.
- **α-hormone subunit (α-SU):** all glycoprotein hormones are composed of a 92 amino acid α-chain and a variable β-chain. The expression of the α-SU is found in the majority of the TSH-, FSH-, and LH-producing adenomas, whereas some of the pituitary gland adenomas exclusively express the α-SU.

14.2.3 Diagnostic Antibody Panel for Tumors of the Posterior Pituitary Gland (Neurohypophysis)

GFAP, S100, TTF-1 (see also tumors of the central nervous system)

Thyroid Transcription Factor-1 (TTF-1): TTF-1 was listed in details as a marker for pulmonary and thyroid carcinomas (see Chap. 3). In addition to lung and thyroid cells, TTF-1 is also expressed in the cells of neurohypophysis (Fig. 14.1); consequently, TTF-1 is also a diagnostic marker for tumors derived from these cells including pituicytoma and granular cell tumor of the sellar region [1, 2]. These tumors constantly lack the expression of cytokeratins, which is important to consider in the differential diagnosis.

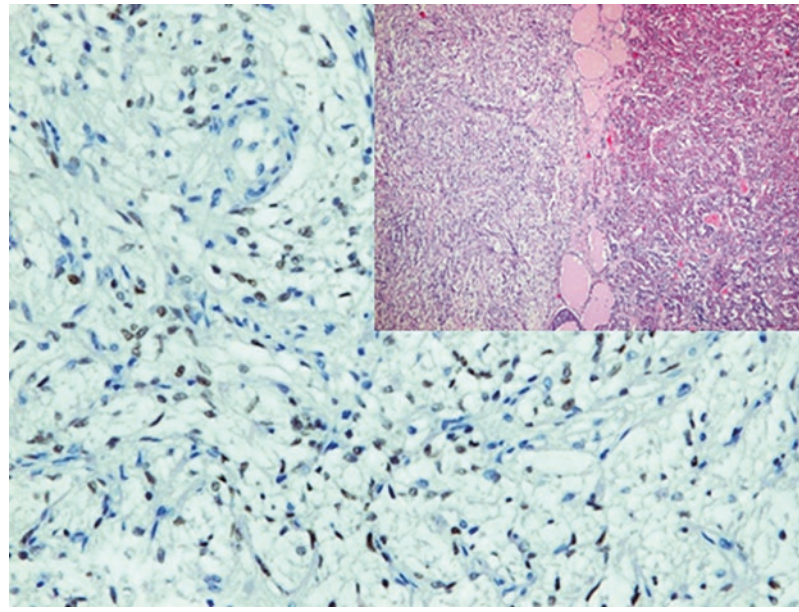

Fig. 14.1 TTF-1 staining the cells of the neurohypophysis

Immunoprofile of pituitary gland tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
A. Tumors of adenohypophysis				
Pituitary adenoma: (General markers)	Synaptophysin, NSE Proliferation index (Ki-67) <5% in pituitary adenoma, >12% in pituitary carcinoma	Chromogranin, Pan-CK, EMA	CD99	Vimentin, CK5/6, CK7, CEA
– Somatotrope adenoma:	*GH*	Prolactin, TSH, FSH, LH, α-subunit		
– Lactotrope adenoma:	*Prolactin*	α-subunit, galectin-3		
– Corticotrope adenoma:	*ACTH*	α-subunit		
– Gonadotrope adenoma:	*LH*, *FSH*	α-subunit		
– Thyrotrope adenoma:	*TSH*	Prolactin, α-subunit		
– Plurihormonal adenoma:		STH, TSH, LH, FSH, prolactin		
– Null cell adenoma:	Nonfunctional (no hormone secretion)			
– Oncocytoma/spindle cell oncocytoma:	Nonfunctional (no hormone secretion)			
B. Tumors of neurohypophysis				
Granular cell tumor of the sellar region (neurohypophysis):	*S100, TTF-1*	GFAP		Neurofilaments, *Pan-CK*, Olig-2, synaptophysin, chromogranin, pituitary hormones
Pituicytoma:	S100, *TTF-1*, vimentin	GFAP	EMA	Synaptophysin, chromogranin, neurofilaments, Pan-CK, pituitary hormones
Spindle cell oncocytoma:	S100, *TTF-1*, bcl-2		EMA	Synaptophysin, chromogranin, Pan-CK, pituitary hormones
C. Tumors from the Rathke pouch epithelium				
Craniopharyngioma:	CK5/6, CK7, CK17, CK19, claudin-1, β-catenin	p53	CK18	CK10, CK20, EMA, vimentin, GFAP
Rathke cleft cyst:	Pan-CK, CK7, β-catenin			

14.3 Tumors of the Thyroid Gland

14.3.1 Tumors of Follicular Cell Origin

Thyroglobulin, thyroperoxidase, TTF-1, PAX-8, galectin-3, HBME-1, CD56, Trop-2, cytokeratin 19, and cytokeratin profile [3]

14.3.2 Tumors of C-Cell Origin

Calcitonin, TTF-1, CEA, and other neuroendocrine markers

Thyroglobulin		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Follicular and papillary thyroid carcinomas		Thyroid follicular cells
Positive control: thyroid tissue		

Diagnostic Approach Thyroglobulin is a glycoprotein synthesized by the thyroid follicular cells and used as a substrate for the synthesis of thyroxin (T_4) and triiodothyronine (T_3). Thyroglobulin is a specific marker for thyroid follicular cells and follicular cell neoplasms. It is recommended to use thyroglobulin in a panel with TTF-1 and PAX-8 to discriminate between pulmonary and thyroid carcinoma. Anaplastic thyroid carcinoma is usually negative for thyroglobulin. Thyroid parafollicular C cells and related neoplasms constantly lack the expression of thyroglobulin.

Thyroperoxidase is a further specific marker for thyroid follicular cells. The expression of this enzyme correlates with the differentiation grade of thyroid tumors and can be lost in poorly differentiated thyroid carcinomas.

Thyroid Transcription Factor-1 (TTF-1): TTF-1 is mentioned in detail among the markers for pulmonary carcinomas. In addition to pulmonary carcinomas, the expression of TTF-1 is characteristic for thyroid tissue and thyroid carcinomas. Follicular, papillary, and medullary thyroid carcinomas are typically strongly positive for TTF-1, whereas undifferentiated (anaplastic) thyroid carcinoma is usually negative.

Thyroid Transcription Factor-2 (TTF-2): TTF-2 is a nuclear protein involved in the synthesis of thyroglobulin and thyroperoxidase, expressed in thyroid follicular cells and related thyroid tumors in addition to a small subset of parafollicular C cells, anterior pituitary gland, esophageal and tracheal mucosa, and seminiferous tubes [4]. Pulmonary parenchyma, gastrointestinal and hepatopancreatic epithelium, and corresponding tumors are constantly negative for TTF-2.

PAX-8: PAX-8 is a transcriptional factor involved in the fetal development of the brain, eye, thyroid tissue, and upper urinary system, as well as organs of Müllerian origin. PAX-8 labels more than 90% of follicular and papillary thyroid carcinomas in addition to the majority of poorly differentiated thyroid carcinoma and more than 50% of anaplastic thyroid carcinomas (Fig. 14.2). Pulmonary adenocarcinomas and breast carcinoma are constantly negative for PAX-8. It is important to consider that parathyroid tissue and parathyroid tumors are also positive for PAX-8 (Fig. 14.3). PAX-8 is listed in details with the markers of genitourinary tumors.

Trophoblastic cell surface antigen 2 (Trop-2)		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Papillary thyroid carcinoma, gastrointestinal and pancreatic carcinomas	Carcinomas of the breast, carcinomas of the lung, uterus, uterine cervix and ovaries, bladder, and prostatic carcinoma	Epithelium of salivary glands, pancreas, bile ducts, breast, uterus, prostate, squamous epithelium
Positive control: prostatic tissue		

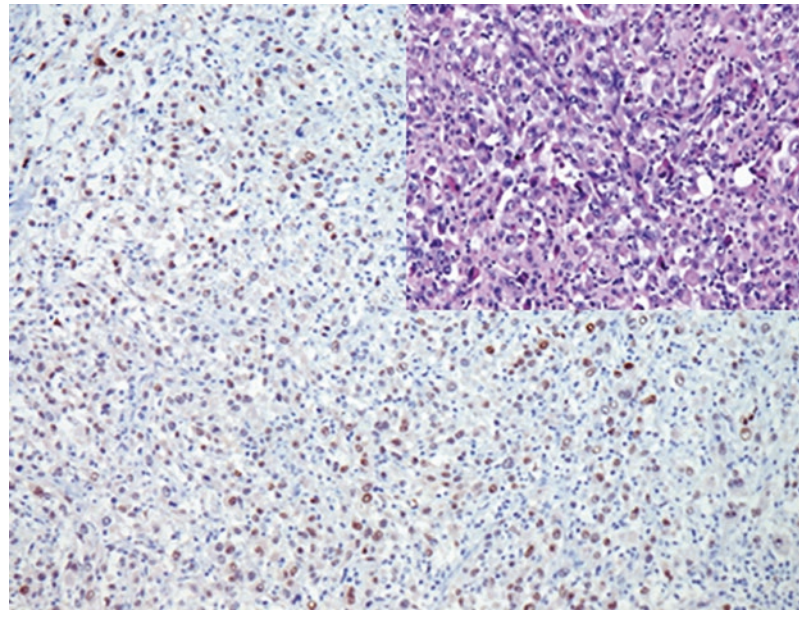

Fig. 14.2 PAX-8 staining the nuclei of anaplastic thyroid carcinoma cells

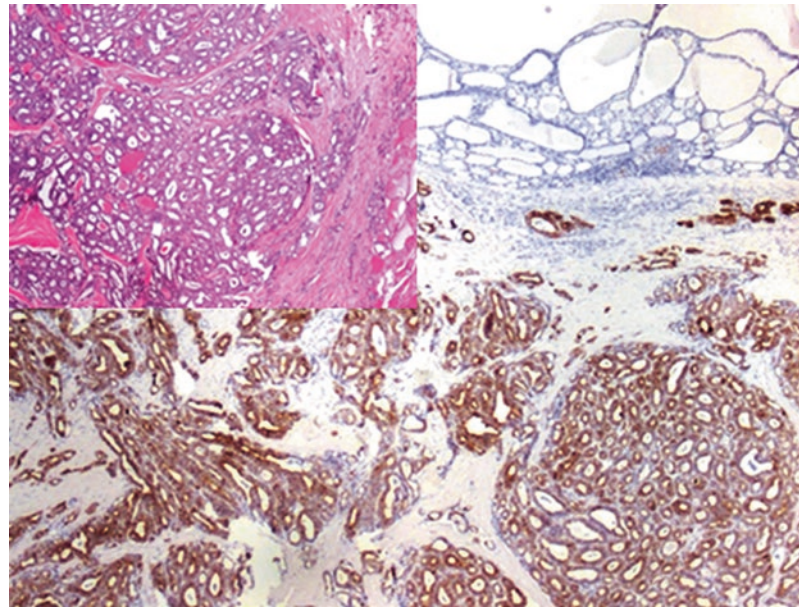

Fig. 14.3 CK19 highlighting the cells of papillary thyroid carcinoma. Normal thyroid tissue lacks CK19 expression

Diagnostic Approach Trop-2 is a transmembrane glycoprotein functioning as calcium signal transducer. The expression of Trop-2 is upregulated during malignant transformation [5]. The expression of Trop-2 is noticed in different carcinoma types including gastrointestinal, pulmonary, genitourinary, and breast carcinomas. More than 90% of papillary thyroid carcinomas express Trop-2 while follicular adenomas and follicular carcinomas usually lack the expression of this protein.

Galectin-3: Galectin-3 is one of the 14 members of the galactosidase-binding protein family normally expressed in endothelial cells and peripheral nerves. The galectin-3 expression is stimulated during malignant transformation, which makes it a helpful marker for the diagnosis of different carcinoma types. Galectin-3 is positive in the majority of papillary and follicular thyroid carcinomas, in parathyroid carcinoma as well as head and neck squamous cell carcinoma, and colorectal and hepatocellular carcinoma.

CD44v6: CD44v6 is a surface glycoprotein, expressed in different carcinoma types including papillary thyroid carcinoma. In combination with other markers, CD44v6 can be a helpful marker to differentiate between papillary carcinoma and other thyroid lesions mimicking this carcinoma type.

Calcitonin		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Medullary thyroid carcinoma	Neuroendocrine carcinoma	Thyroid parafollicular (C) cells
Positive control: thyroid tissue/medullary thyroid carcinoma		

Diagnostic Approach Calcitonin is a polypeptide hormone synthesized by the parafollicular (C) thyroid cells involved in the regulation of calcium and phosphorous metabolism principally contracting the effect of parathyroid hormone. Calcitonin is a specific marker for the parafollicular cells and tumors originating from these cells, namely, medullary thyroid carcinoma. Tumors originating from the thyroid follicular cells are constantly negative for calcitonin but also positive for TTF-1. Best stain results are obtained using monoclonal antibodies.

Diagnostic Pitfalls Some cases of neuroendocrine tumors such as pheochromocytoma are reported to be positive for calcitonin, but these tumors are usually negative for TTF-1.

Immunoprofile of thyroid tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Follicular thyroid adenoma:	*Thyroglobulin*, thyroid peroxidase, *TTF-1*, *PAX-8*, Pan-CK	CK7	CK19	CK5/6, CK20, calcitonin, CD44V6, Trop-2, galectin-3
Follicular thyroid carcinoma:	*Thyroglobulin*, thyroid peroxidase, *TTF-1*, *PAX-8*, CK7, CK8, CK18, CD44V6, S100	Galectin-3, vimentin, HBME1, E-cadherin, bcl-2,	CK19	Calcitonin, CK5/6, CK20, Trop-2, CEA, HER-2
Papillary thyroid carcinoma:	*Thyroglobulin*, thyroid peroxidase, *TTF-1*, *PAX-8*, *Trop-2*, CK1, CK7, CK8, CK18, CK19[a], p63[a], galectin-3[a], CD44V6, HBME-1	CK5/6/14, EMA, CD15, vimentin	CD34	CK20, CEA, calcitonin, synaptophysin, chromogranin, CD56[a]
Poorly differentiated thyroid carcinoma:	*Thyroglobulin*, thyroid peroxidase, *TTF-1*, *PAX-8*, Pan-CK, galectin-3, CD44V6	Vimentin, bcl-2		CK5/6, CK19, CK20, calcitonin

Immunoprofile of thyroid tumors				
Anaplastic thyroid carcinoma:	Pan-CK, CK8, CK18	CK19, *PAX-8*, CEA, vimentin	TTF-1, EMA, galectin-3, bcl-2	Thyroglobulin, calcitonin
Medullary thyroid carcinoma:	*Calcitonin*, chromogranin, synaptophysin, *TTF-1*, CD56, Leu7, S100, NSE, *CEA*, vimentin (in spindle cell components), CK7, CK8, CK18, HER-2, Synapsin I	bcl-2	CK19, galectin-3	*PAX-8*, CK5/6, thyroglobulin, CK20
Hyalinizing trabecular tumor:	*Thyroglobulin*, *TTF-1*, Ki-67 (MIB-1 clone)[b]	CK 7, galectin-3		

Immunohistochemical markers for differentiation between papillary thyroid carcinoma (PTC), benign pseudopapillary hyperplasia (BPH), and follicular neoplasms (FN)
CK19: positive in PTC but negative or weakly positive in FN with the exception of chronic lymphocytic thyroiditis (Fig. 14.3)
Galactin-3: positive in PTC follicular carcinoma but negative in benign thyroid tissue
CD56: negative in PTC but positive in benign thyroid tissue, BPH, and FN (Fig. 14.4) [6]
p63: focal expression in PTC, constantly negative in non-PTC lesions
Trop-2: positive in >90 PTC, negative in follicular adenoma/carcinoma

[a]See table below
[b]Atypical membranous and cytoplasmic staining pattern may be noted when the MIB-clone is used a characteristic staining pattern for this tumor type

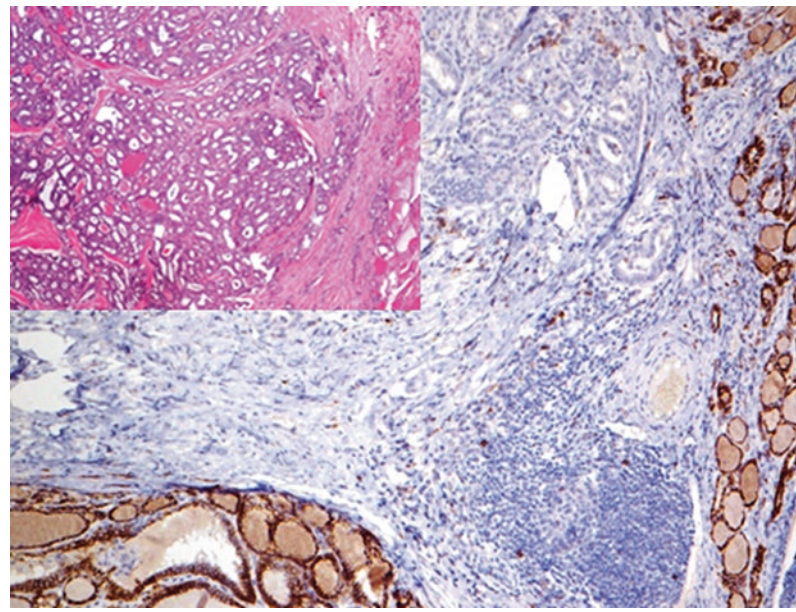

Fig. 14.4 CD56 staining normal thyroid tissue whereas areas infiltrated by papillary thyroid carcinoma lack CD56 expression

14.4 Tumors of the Parathyroid Gland

Markers and Immunoprofile of Parathyroid Neoplasms Parathyroid hormone, thyroglobulin, TTF-1, PAX-8 [7]

Parathyroid hormone (PTH)		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Parathyroid tissue and neoplasms	Ovarian small cell carcinoma of hypercalcemic type, pheochromocytoma	Parathyroid chief cells, fetal tissue (CNS, lung, gastrointestinal tract)
Positive control: parathyroid		

Diagnostic Approach Parathyroid hormone (parathormone, PTH) is a polypeptide hormone secreted by the chief cells of the parathyroid glands. PTH and calcitonin are directly responsible for the regulation of calcium and phosphate levels in the serum. Antibodies to PTH and related peptides are specific markers for the diagnosis of parathyroid neoplasms. PHT is helpful to recognize ectopic parathyroid tissue and tumors, which may be situated in the mediastinum or intrathymic (Fig. 14.5).

Diagnostic Pitfalls Parathyroid chief cells usually rapidly discharged PHT after the synthesis, which may cause false negative immunohistochemical reaction. More challenging are nonsecretory clear cell parathyroid carcinomas, which may resemble metastatic renal cell carcinoma or any other clear cell carcinoma. The diagnostic panel for thyroid/parathyroid tumors must include thyroid and parathyroid hormone in addition to other differentiation markers.

Parathyroid Hormone-Related Peptide: This polypeptide (PtHrP) is a member of the parathyroid hormone family also involved in the calcium metabolism and regulates the enchondral bone development. Antibodies to PtHrP stain parathyroid cells and parathyroid tumors in addition to a number of other malignant tumors such as breast carcinoma, cholangiocarcinoma, and transitional cell carcinoma especially poorly differentiated types. PtHrP can be also used as a marker to discriminate between cholangiocarcinoma and metastatic colorectal adenocarcinoma [8, 9].

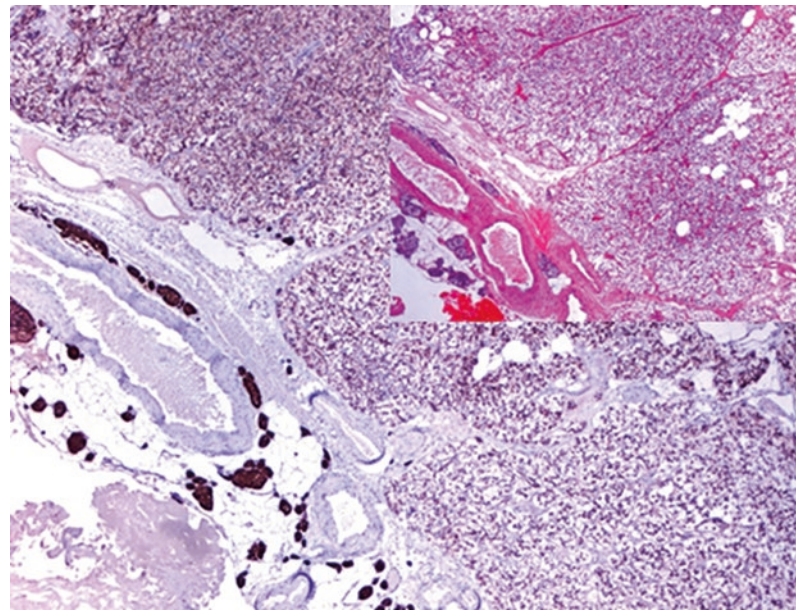

Fig. 14.5 Parathyroid hormone labeling parathyroid tissue and cells of parathyroid adenoma

PAX-8 and GATA-3 Both transcription factors were listed in detail in previous chapters as markers for breast, renal, and urinary tract tumors. PAX-8 and GATA-3 label also parathyroid tissue and parathyroid tumors including adenoma and carcinoma with the characteristic nuclear pattern and can be used in a panel as parathyroid markers (Figs. 14.6 and 14.7) [10]. It is important to remember that PAX-8 labels also thyroid follicular cells and tumors.

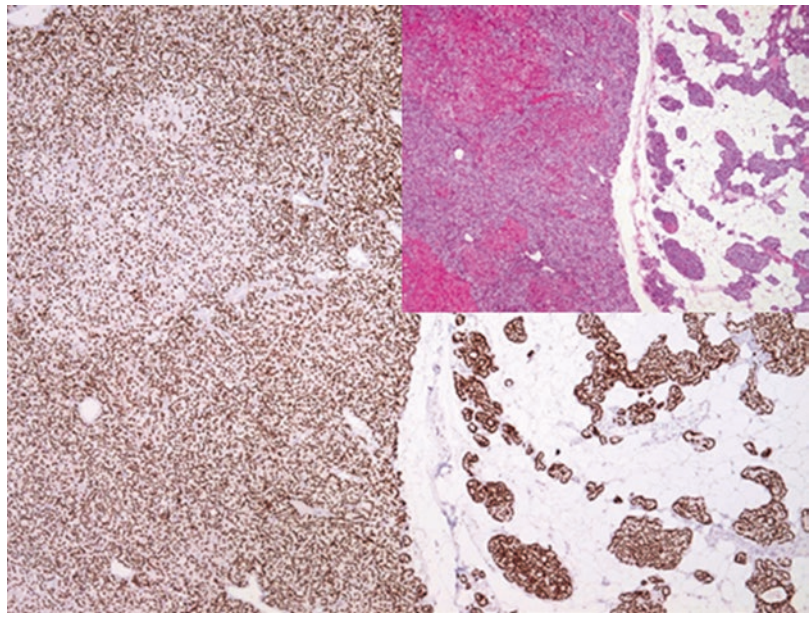

Fig. 14.6 GATA-3 staining cells of suppressed parathyroid gland and neighboring parathyroid adenoma

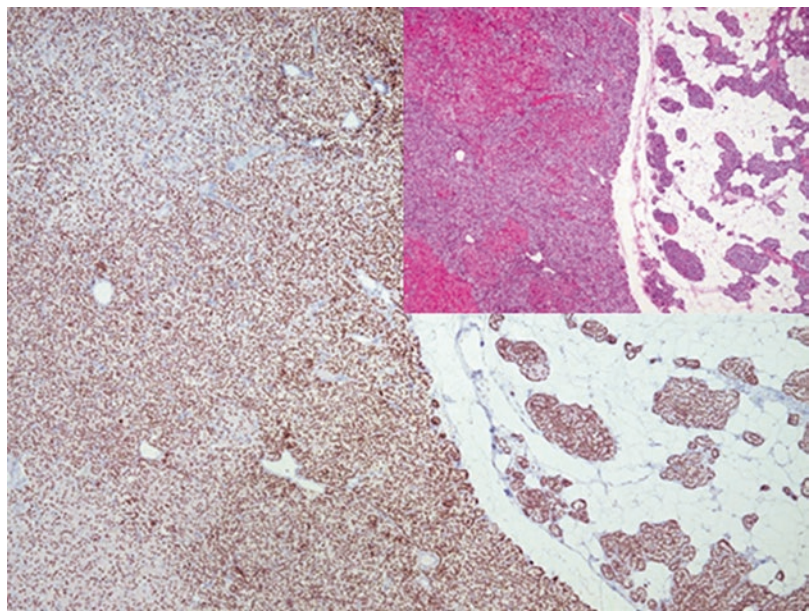

Fig. 14.7 PAX-8 staining cells of suppressed parathyroid gland and neighboring parathyroid adenoma

Immunoprofile of parathyroid tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Parathyroid adenoma:	*PTH*, synaptophysin, chromogranin, neurofilaments, Pan-CK, CK8, CK18, CK14[a]. PAX-8, GATA-3 Proliferation index (Ki-67): < 5%	CK19, RCC (gp200), vimentin	Cyclin D1, calcitonin, CK7, CK20	*TTF-1*, Thyroglobulin, CD56, CK5/6
Parathyroid carcinoma:	Synaptophysin, chromogranin, neurofilaments, Pan-CK Proliferation index (Ki-67): > 6%	*PTH*, CK19, PAX-8, GATA-3, cyclin D1, vimentin	Calcitonin, galectin-3, CK7	Thyroglobulin, CK5/6, CK14 TTF-1, CD56[b], CK20

[a]Negative in parathyroid carcinoma
[b]May be positive in oxyphil parathyroid adenoma

14.5 Pancreatic Endocrine Tumors

Diagnostic Antibody Panel for Pancreatic Endocrine Tumors PDX-1, insulin, gastrin, glucagon, somatostatin receptor, vasoactive intestinal polypeptide (VIP), and human pancreatic polypeptide (hPP)

Immunophenotype of pancreatic endocrine tumors is listed in the section of pancreatic tumors.

14.6 Tumors of the Adrenal Gland

14.6.1 Diagnostic Antibody Panel for Adrenocortical Tumors

Adrenal 4 binding protein (Ad4BP, SF-1), DAX-1, inhibin, Melan A, calretinin, synaptophysin, podoplanin, and WT-1 [11]

Adrenal 4 binding protein (Ad4BP, SF-1)		
Expression pattern: nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Adrenocortical tumors	Sex cord-stromal tumors (granulosa cell tumor, Sertoli cell tumor, fibroma and fibrothecoma), pituitary adenoma	Adrenal cortex, ovarian stromal cells, Sertoli cells, stromal cells of the spleen, gonadotrophic cell in the anterior pituitary gland
Positive control: adrenal gland		

Diagnostic Approach Adrenal 4 binding protein (Ad4BP), also known as steroid factor 1 (SF-1), is a member of the orphan nuclear receptor family and is a transcriptional factor regulating steroidogenesis. SF-1 is expressed in the adrenal cortex, pituitary gland, Sertoli cells, and different tumors derived from these tissue types. SF-1 is constantly negative in renal cell carcinoma, hepatocellular carcinoma, melanoma, and pheochromocytoma. Generally, the positivity to

synaptophysin, Melan A, inhibin, D2-40, and calretinin, and the co-expression of vimentin and cytokeratin 5 will support the adrenocortical origin of the tumor [12–14].

Diagnostic Pitfalls Clinical and paraclinical data must be considered for the diagnosis of metastatic adrenocortical carcinoma as the morphology and immunoprofile of sex cord-stromal tumors maybe very similar to those of adrenocortical tumors.

DAX-1: DAX-1 is a nuclear receptor protein and a member of the orphan nuclear receptor family encoded by the NR0B1 gene acting as suppressor for the steroid hormone production in the adrenal cortex by inhibiting the effect of the steroidogenic factor 1 (SF-1) [15, 16]. Furthermore, DAX-1 plays an active role in the development of hypothalamic-pituitary-adrenal-gonadal axis and the differentiation of osteoblasts. The expression of the DAX-1 transcription factor is restricted to steroid-producing cells including those of the adrenal cortex, pituitary gland and hypothalamus, testis, and ovary. Similar to SF-1, DAX-1 is a marker of the adrenocortical tumors and some other types of ovarian, testicular, and breast tumors.

DAX-1 is also found to be a specific marker for Ewing's sarcoma due to the genetic alterations caused by the EWS/Fli-1 translocation prompting the expression of DAX-1 [17, 18].

Inhibin: Inhibin is a glycoprotein hormone listed in a former chapter as a marker for sex cord tumors. Inhibin is normally expressed in the gonads and adrenal glands, whereas the strongest expression in the adrenal gland is found in the zona fasciculata and reticulares of the cortex. The adrenal medulla lacks the expression of inhibin.

Beside testicular and ovarian sex cord tumors, inhibin is an important marker for benign and malignant adrenocortical tumors [19] (Fig. 14.8).

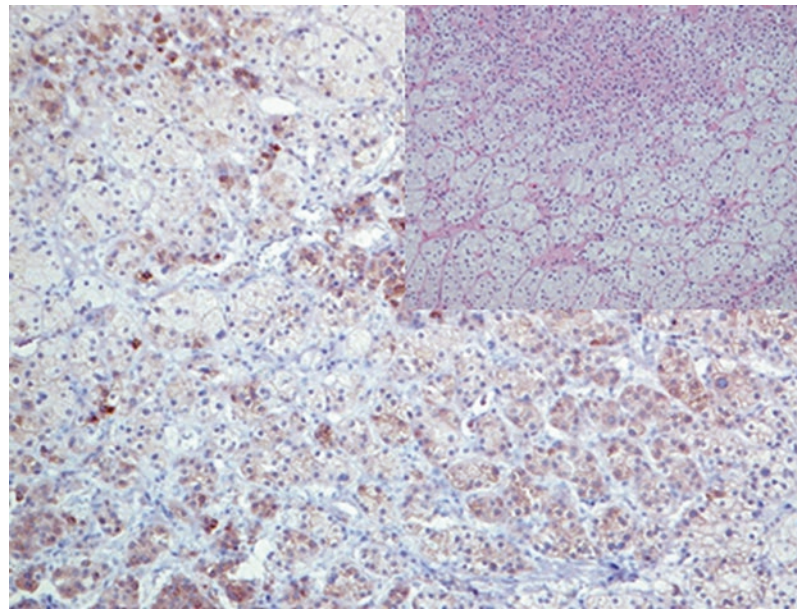

Fig. 14.8 Adrenocortical adenoma exhibiting cytoplasmic expression of inhibin

14.6.2 Markers and Immunoprofile of Tumors of Adrenal Medulla and Extra-adrenal Paraganglia

14.6.2.1 Diagnostic Antibody Panel for Pheochromocytoma and Extra-adrenal Paraganglia

Chromogranin, synaptophysin, CD56, NSE, S100, GATA-3. These antibodies were listed in details in other chapters

14.6.2.2 Diagnostic Antibody Panel for Neuroblastoma and Extra-adrenal Paraganglia

NSE, NB84, chromogranin, synaptophysin, CD56, PGP9.5, GATA-3, CD117, and neurofilaments (Figs. 14.9 and 14.10) [20]

NB84		
Expression pattern: membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Neuroblastoma	Ewing's sarcoma/PNET, medulloblastoma, desmoplastic small round cell tumor	
Positive control: neuroblastoma		

Diagnostic Approach: NB84 is a membranous antigen isolated from the human neuroblastoma cells. It stains about 100% of differentiated and about 90% of undifferentiated neuroblastomas. NB894 is more sensitive but less specific than synaptophysin [21]. For an appropriate diagnosis of adrenal or extra-adrenal tumors, a panel of three to four of the abovementioned antibodies is recommended.

Diagnostic Pitfalls NB84 may be positive in other tumors with similar morphology including PNET and desmoplastic small round cell tumor. To exclude these tumors, an antibody panel including CD99 and cytokeratins is required. It is important to consider that about 5% of undifferentiated neuroblastoma lacks the expression of NB84.

GATA-3: This transcription factor was listed in details in previous chapters as a marker for breast, salivary gland, parathyroid, and urothelial tumors. GATA-3 strongly labels the fetal sympathicoblasts and the chromaffin cells of adrenal medulla and sympathetic paraganglia derived from sympathicoblasts (Fig. 14.11). Consequently, GATA-3 is marker for tumors of the adrenal medulla and extra-adrenal paraganglia including pheochromocytoma and neuroblastoma (Figs. 14.12 and 14.13). Very low GATA-3 expression is also found in adrenal cortex and adrenocortical tumors.

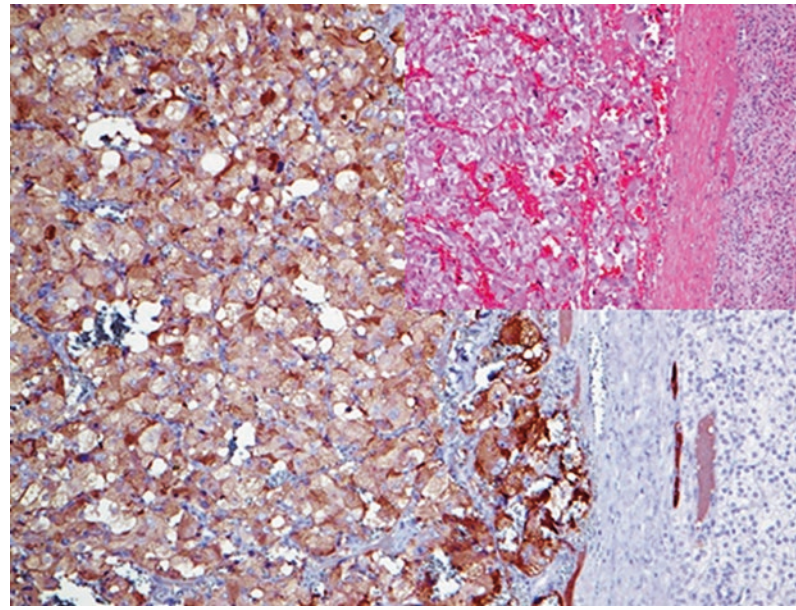

Fig. 14.9 Pheochromocytoma with strong CD56 expression

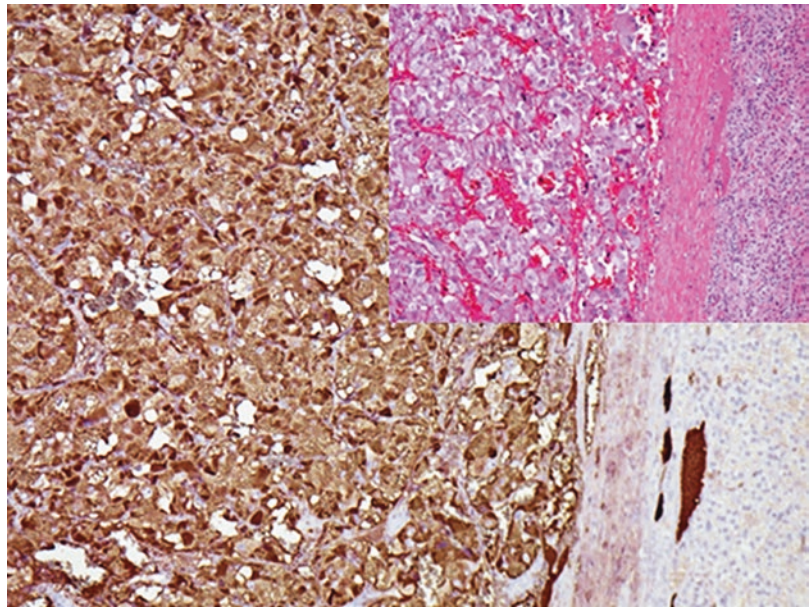

Fig. 14.10 Pheochromocytoma exhibiting strong synaptophysin expression

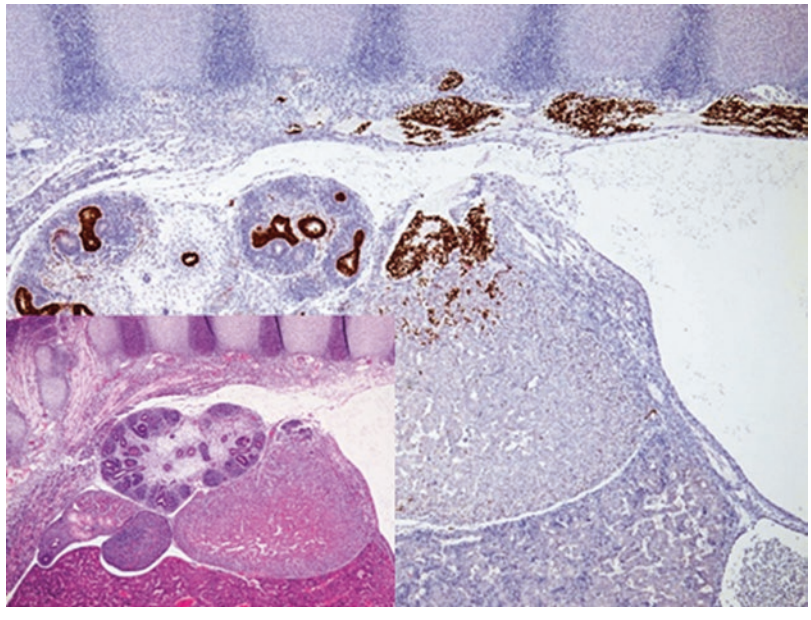

Fig. 14.11 Section through a 12-week embryo showing paravertebral sympathicoblasts of neural crest labeled by GATA-3. These cells are migrating into dorsomedial part of the primordial adrenal gland to form the adrenal medulla. GATA-3 is also highlighting the urothelium of the collecting system of the kidney

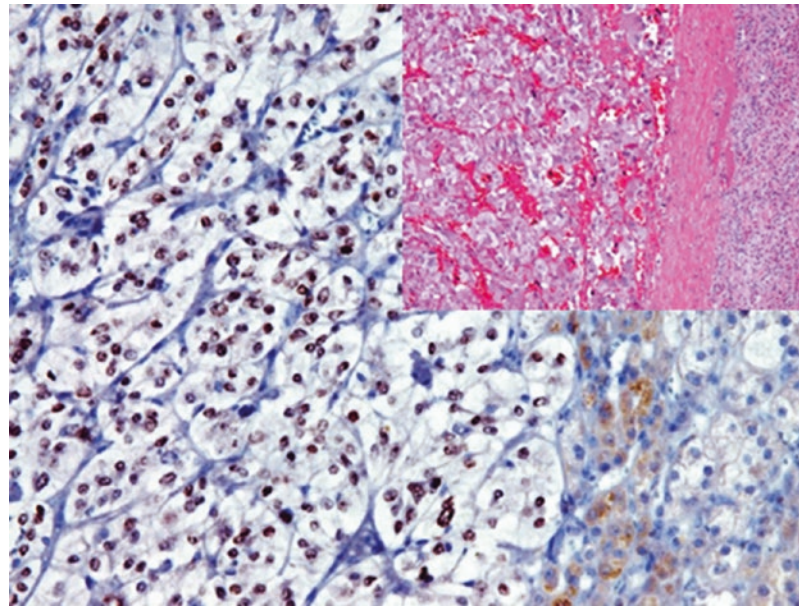

Fig. 14.12 GATA-3 staining the nuclei of pheochromocytoma cells

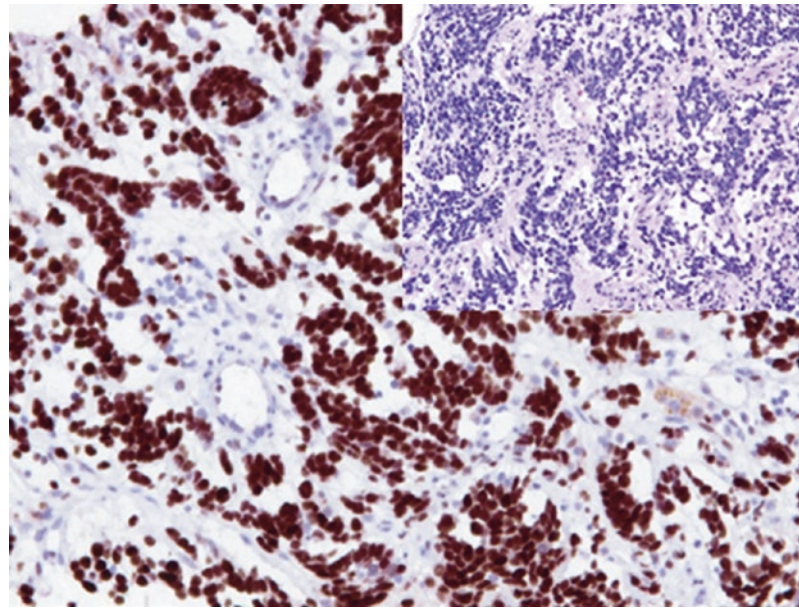

Fig. 14.13 GATA-3 highlighting the nuclei of neuroblastoma cells in an adrenal gland biopsy

Immunoprofile of adrenal gland tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Adrenocortical adenoma/carcinoma:	Adrenal 4 binding protein (Ad4BP, SF-1), Melan A, inhibin Proliferation index (Ki-67): in adrenocortical adenoma <2.5% In adrenocortical carcinoma >4%	Synaptophysin, NSE, calretinin, CD56, vimentin	Pan-CK, CK5, bcl-2	CK7, CK19, CK20, EMA, CEA, CD10, Chromogranin, RCC, PAX-8

Immunoprofile of adrenal gland tumors				
Pheochromocytoma and extra-adrenal paraganglia:	Chromogranin, synaptophysin, CD56, NSE Proliferation index (Ki-67): In benign pheochromocytoma <2% In malignant pheochromocytoma[b] >3%	*S100*[a], GFAP, GATA-3, bcl-2,	Vimentin, Pan-CK, Calcitonin	CK5/6, CK7, CK19, CK20, EMA, D11, PAX-8, CA IX, Melan A
Neuroblastoma:	*CD56*, NSE, neurofilaments, PGP9.5, NB84, *GATA-3, PHOX2B*, vimentin	*S100*, ALK, synaptophysin, chromogranin, CD117	Pan-CK, WT-1	CK5/6, CK7, CK20, CD99

[a]Strong nuclear stain in sustentacular cells
[b]This criterium cannot be used exclusively to define malignancy

14.7 Diagnostic Antibody Panel for Neuroendocrine Carcinomas (Small and Large Cell Types)

Cytokeratin profile, chromogranin, synaptophysin, NSE, S100, CD56, somatostatin receptor, and proliferation index (Ki67) [12, 22, 23]

CDX-2, Satb-2, PDX-1, PAX-6, and TTF-1 are helpful markers to ascertain the site of the primary tumor.

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Markers and Immunoprofile of Mesothelioma Tumors of the Peritoneum

15

Contents

15.1 Diagnostic Antibody Panel for Mesothelial Tumors

Calretinin, thrombomodulin (CD141), mesothelin, podoplanin, WT-1, GLUT1, BAP-1, h-caldesmon, CD146, and cytokeratin profile [1–3].

15.2 Diagnostic Antibody Panel for Epithelial Tumors of Müllerian Type

Cytokeratin profile, CEA, CA125, PAX-8, WT-1, p53, and p16 (see ovarian tumors).

15.3 Diagnostic Antibody Panel for Smooth Muscle Tumors

Actin, h-caldesmon, calponin, and cytokeratin profile.

15.4 Diagnostic Antibody Panel for Tumors of Uncertain Origin and Miscellaneous Peritoneal Primary Tumors

CD34, CD99, DOG-1, actin, h-caldesmon, desmin, ALK, and cytokeratin profile.

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Cytokeratin Profile All mesothelial tumors are positive for pan-cytokeratin and the cytokeratins 5/6/7/8/10/14/18 but typically lack the expression of cytokeratin 20. Consequently, the cytokeratin profile alone cannot discriminate between mesotheliomas and metastatic carcinomas. It is important to consider that submesothelial fibroblasts are usually positive for pan-cytokeratin and other keratins that maybe a source of misinterpretation.

Calretinin		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Mesothelioma, adrenocortical tumors, ovarian sex cord-stromal tumors	Squamous cell carcinoma, ameloblastoma, thymic tumors, transitional cell carcinoma, colonic carcinoma, granular cell tumor, fibrosarcoma, PEComa, myxoid chondrosarcoma, synovial sarcoma, desmoplastic small round cell tumor, atrial myxoma, lipogenic tumors, mast cell lesions	Central and peripheral neural cells, ganglion cells, neuroendocrine cells, mesothelial cells, mast cells, steroid-producing cells (Leydig and Sertoli cells, adrenal cortex cells, ovarian theca interna, and surface cells), endometrium, eccrine glands, thymus, adipose tissue
Positive control: appendix		

Diagnostic Approach Calretinin is an intracellular neuron-specific calcium-binding vitamin D-dependent protein expressed in various epithelial, mesenchymal, and central and peripheral neurogenic tissue types. Calretinin is strongly expressed in normal and neoplastic mesothelial cells and considered as an important mesothelioma marker (Fig. 15.1). Calretinin is also a marker for mast cells and steroid-producing cells and tumors derived from these cells, namely, sex cord-stromal tumors including granulosa cell tumor, Sertoli and Leydig cell tumors, gonadoblastoma, and gynandroblastoma in addition to adrenocortical tumors. About one third of squamous cell carcinomas shows also different calretinin expression intensity. Calretinin is also widely expressed in different soft tissue tumors such as synovial sarcoma, chondrosarcoma, desmoplastic small round cell tumor, lipoma, and liposarcoma [4, 5]. Moreover, calretinin is an optimal marker to highlight ganglion cells in colonic biopsies for the diagnosis of Hirschsprung disease.

Diagnostic Pitfalls Calretinin has a wide expression spectrum, and the calretinin positivity alone is not enough for the diagnosis of mesothelioma.

Thrombomodulin (CD141)		
Expression pattern: membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Mesothelioma, transitional cell carcinoma	Squamous cell carcinoma, trophoblastic tumors, vascular tumors, synovial sarcoma	Endothelial cells, urothelium, mesothelial cells, keratinizing epithelial cells, monocytes, neutrophils, platelets/megakaryocytes, meningeal cells, smooth muscle cells, syncytiotrophoblasts, synovial lining cells, osteoblasts
Positive control: appendix		

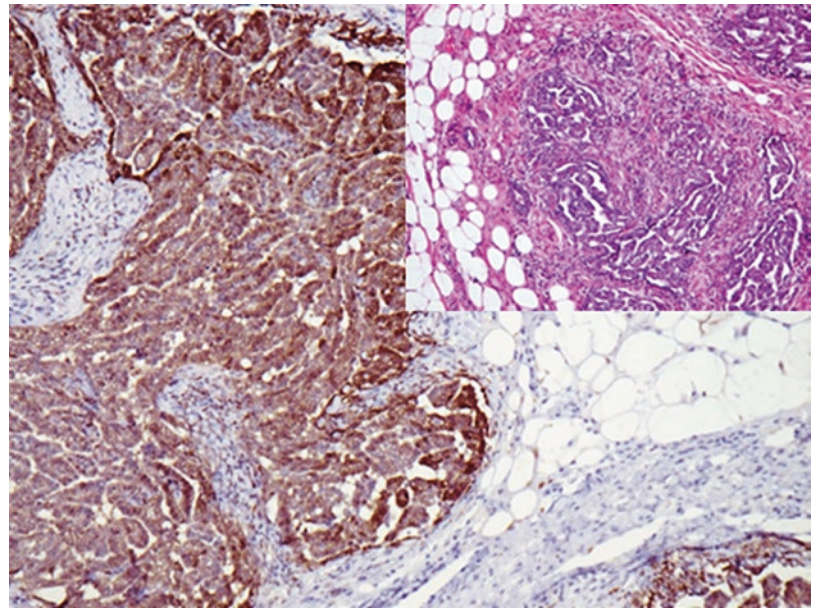

Fig. 15.1 Calretinin highlighting mesothelioma cells infiltrating the chest wall

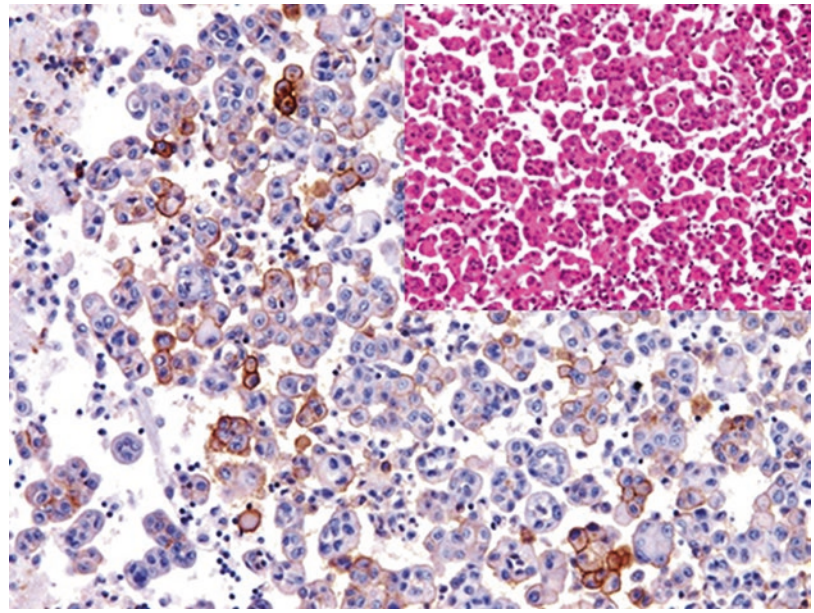

Fig. 15.2 Thrombomodulin labeling mesothelioma cells in malignant pleural effusion

Diagnostic Approach Thrombomodulin (also known as endothelial anticoagulant protein, clustered as CD141) is a transmembrane glycoprotein expressed on the surface of endothelial cells and taking part in the regulation of intravascular coagulation. The expression of thrombomodulin is characteristic for other cell and tissue types including mesothelial cells, squamous epithelial cells, and transitional epithelium of the urinary tract. Thrombomodulin is a useful screening antibody for mesothelioma, transitional cell carcinoma, and squamous cell carcinoma in addition to vascular tumors (Fig. 15.2). Thrombomodulin is usually negative in sarcomatoid mesothelioma.

To discriminate between thrombomodulin-positive tumors, it is important to use other more specific markers. Thrombomodulin is constantly negative in renal cell carcinoma, prostatic carcinoma, gastrointestinal adenocarcinoma, and endometrioid carcinoma.

Mesothelin		
Expression pattern: membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Mesothelioma, non-mucinous ovarian surface carcinomas	Adenocarcinoma of different origin, acinar cell carcinoma and squamous cell carcinoma	Mesothelial cells, renal tubules, tracheal and tonsil epithelial cells, fallopian tube mucosa
Positive control: appendix		

Diagnostic Approach Mesothelin is a glycoprotein located on the cell surface of mesothelial cells in addition of some other types of epithelial cells.

Diagnostic Pitfalls Mesothelin labels mesothelioma in addition to other carcinoma types including ovarian, pancreatic, and pulmonary carcinoma and adenocarcinomas. Generally, mesothelin is a screening antibody and cannot be considered as a specific mesothelioma marker. Sarcomatoid mesothelioma is negative for mesothelin.

WT-1: WT-1 is one of the important mesothelioma markers discussed in a previous chapter. In mesothelioma cells, WT-1 has a nuclear expression pattern and can be used as the double stain in combination with other markers exhibiting membranous stain.

Podoplanin: Podoplanin (also known as D2-40) is a mucoprotein expressed on the membrane of lymphatic endothelium discussed in the chapter of vascular tumors. Podoplanin is not specific for lymphatic endothelium but also expressed in other cell and tumor types such as meningeal cells, germ cells and germ cell tumors, mesothelial cells and mesothelioma in addition to many other mesenchymal tumors (Fig. 15.3) [6, 7].

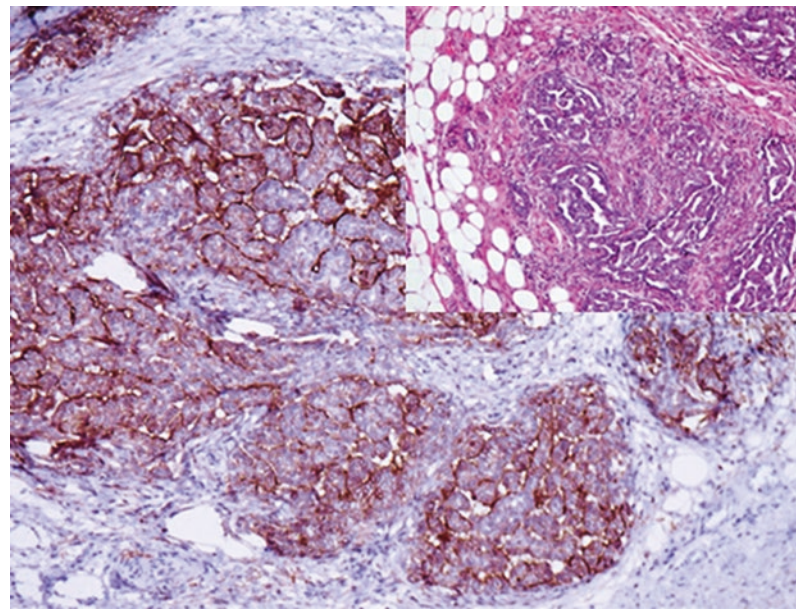

Fig. 15.3 Podoplanin (D2-40) labeling the cells of mesothelioma

GLUT1		
Expression pattern: membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Malignant mesothelioma vs. reactive mesothelial hyperplasia Benign endometrial hyperplasia vs. atypical hyperplasia	Perineurioma, hemangioma, chordoma, epithelioid sarcoma, wide range of carcinomas of different origin	Red blood cells, testicular germinal cells, renal tubules, placental trophoblasts, brain capillaries, perineural cells
Positive control: mesothelioma		

Diagnostic Approach Glucose transporter 1 (GLUT1) is a member of the GLUT transporter family and a membrane-associated erythrocyte glucose transport protein maintaining the basal glucose transport in most cell types. GLUT1 is not a tissue-specific marker but expressed in a wide range of epithelial and non-epithelial tumors. In diagnostic histopathology, GLUT1 is a potential marker for malignant transformation as it is overexpressed in many types of malignant epithelial and non-epithelial tumors. It is helpful marker to discriminate between malignant mesothelioma and reactive proliferation of mesothelial cells. GLUT1 is a helpful marker to distinguish between hemangioma usually positive for GLUT1 and vascular malformation, pyogenic granuloma, and granulation tissue lacking the expression of GLUT1.

Diagnostic Pitfalls GLUT1 is a hypoxia-inducible factor (HIF) target gene which is also induced by the hypoxia-inducible factor 1α (HIF-1α) [8]. Consequently, hypoxic areas will also overexpress GLUT1.

Insulin-Like Growth Factor II mRNA-Binding Protein 3: IMP3 is a cytoplasmic oncofetal protein mediating RNA trafficking and cell growth expressed in fetal tissue and different premalignant and malignant lesions. Benign adult tissue usually lacks the expression of IMP3 with the exception of the ovarian and testicular tissue, placenta, endocrine cells, and brain. In routine immunohistochemistry, IMP3 is used to discriminate between malignant and reactive proliferative lesions. Similar to GLUT1, IMP3 is a helpful marker to discriminate between mesothelioma and reactive mesothelial proliferation, as the majority of benign mesothelial cells are negative for IMP3 (Fig. 15.4) [9].

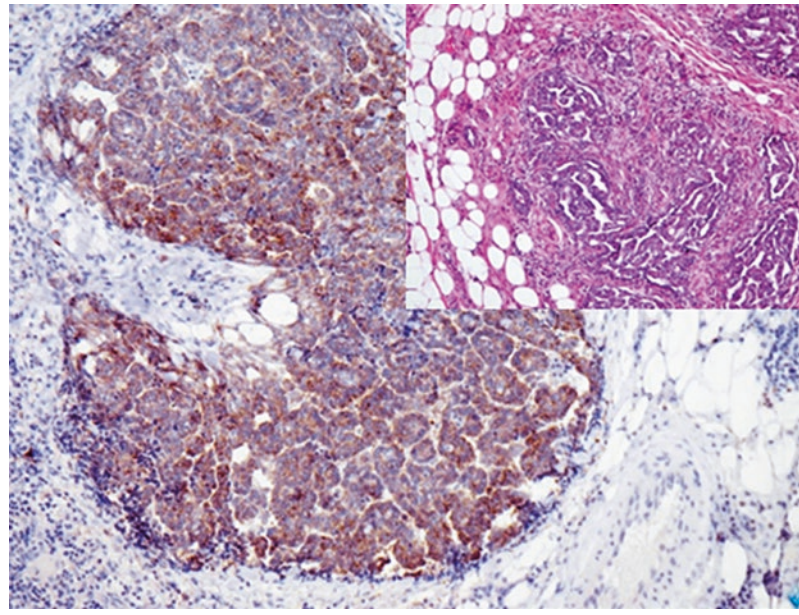

Fig. 15.4 IMP3 expression in malignant mesothelioma

IMP3 is also a selective marker for Hodgkin cells; however, it can be also found some extrafollicular blasts or cells of B-cell lymphoma. Furthermore, IMP3 is a helpful marker to discriminate between serous endometrial carcinoma positive for IMP3 and endometrioid carcinoma negative for IMP3 [10].

BRCA1-Associated Protein 1 (BAP-1): BAP-1 is a nuclear ubiquitin hydrolase involved in chromatin remodeling and functions as transcriptional regulator and tumor suppressor. BAP-1 is encoded by a gene located on chromosome 3p12.124; a genomic region found to be deleted in different fractions of several human malignancies, including mesotheliomas, uveal and cutaneous melanomas, clear cell renal cell carcinomas, pulmonary adenocarcinomas, and meningiomas [11, 12]. For different tumor types, the lack of BAP-1 expression has been associated with an aggressive behavior. In routine immunohistochemistry, BAP-1 is a helpful marker to discriminate between malignant mesothelioma and malignant melanoma (lacks the nuclear expression of BAP-1) and reactive mesothelial proliferation or benign melanocytic lesions (BAP-1 positive). The sensitivity of BAP-1 to differentiate between benign and malignant mesothelial lesion is reported to be up to 90%. The diagnosis can be supported by p16 FISH analysis [13, 14].

Diagnostic Criteria for Mesothelioma Initially, it is important to consider that mesothelioma has no uniform morphological appearance and may demonstrate epithelioid, sarcomatoid, desmoplastic, or mixed (biphasic) differentiation patterns with different immunophenotypes; consequently, it is always essential to exclude other tumors using more specific markers such as TTF-1, CDX-2, CEA, steroid receptors, and CD15, which are consistently negative in mesothelioma. Generally, it is advisable to confirm the diagnosis of mesothelioma by three to four mesothelioma markers [1]. Other markers such as GLUT1, BAP-1, and CD146 are helpful to confirm the neoplastic nature of the mesothelial proliferation.

Markers Constantly Negative in Reactive or Malignant Mesothelial Proliferation but Diagnostic or Specific for Different Carcinoma Types: Epithelial specific antigen (BerEp4), MOC-31, p63, claudin-4, CEA, TTF-1, napsin, CDX-2, SATB-2, GATA-3, PDX-1, PAX-8, and CD15.

Immunoprofile of peritoneal tumors				
Tumor type	+ in >90% (+)	+ in 50-90% (+/−)	+ in 10-50% (−/+)	+ in <10% (−)
A. Immunoprofile of mesothelioma				
Epithelioid mesothelioma and adenomatoid tumor	Pan-CK, CK5/6, CK7, CK8, CK14, CK18, CK19, *WT-1*, *calretinin*, podoplanin (D2-40), mesothelin, *h-caldesmon*, CD44s	*Thrombomodulin* (CD141), *IMP3*, *GLUT1*, HBME-1, vimentin	N-cadherin, E-cadherin, GATA-3, CD30, actin, EMA	p63, CD15, EPCAM (BerEp4), claudin-4, CK20, CEA, TTF-1, CDX-2, napsin, PAX-8. myoglobin, myogenin
Antibodies discriminating between malignant mesothelioma (MM) and benign/reactive mesothelial proliferation (BMP)	• *BAP-1* - in MM, + in BMP • GLUT1 + in MM, − in BMP • Desmin - in MM, +/− in BMP • CD146 + in MM, − in BMP • P53 +/− in MM, − in BMP • Osteonectin: +/− in MM, − in BMP • CD56 (NCAM): +/− in MM, − in BMP • IMP3 +/− in MM, − in BMP • bcl-2 −/+ in MM, − in BMP • p53 +/− in MM, −/+ in BMP • EMA +/− in MM (membranous stain), −/+ in BMP • Tenascin-X +/− in MM, −/+ in BMP			
B. Epithelial tumors of Müllerian type				
Serous/mucinous/ endometrioid/ clear cell and transitional cell tumors	See epithelial tumors of the ovary			
C. Smooth muscle tumors				
Leiomyomatosis peritonealis disseminata	Actin, h-caldesmon			CK5/14
D. Miscellaneous tumors				
Pseudomyxoma peritonei	CK20, CDX-2, SATB-2, CEA	MUC-2		CK7
Extra gastrointestinal stromal tumor	See gastrointestinal GIST			
Desmoplastic small round cell tumor	See miscellaneous soft tissue tumors			

	Differential diagnosis epithelioid mesothelioma versus metastatic carcinoma																
	BER-EP4	CK5/14	CK7	CK20	Calretinin	CD141	CEA	WT-1	PAX-8	CDX-2	ER / PR	PDX-1	p16	GATA-3	TTF-1	Oct-4	CD10
Mesothelioma	−	+	+	−	+	+/−	−	+	−	−	−	−	−	−/+	−	−	−
Ovarian serous carcinoma	+	−	+	−	−	−	−	+	+	−	+	−	+	−	−	−	−
Ovarian mucinous carcinoma	+	−	+	+/−	−	−	+	−	−	+/−	−	−	−	−	−	−	−
Ovarian clear cell carcinoma	+	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−
Endometrioid adenocarcinoma	+	−	+	−	−	−	−	−	+	−	+	−	−	−	−	−	−
Cervical adenocarcinoma	+	−	+	−	−	−	+	−	+	−	−	−	+	−	−	−	−
Embryonal carcinoma		−	+	−	−	−		−	−	−	−	−	−	−	−	+	−
Gastric adenocarcinoma	+	−	+	−	−	−	+	−	−	+	−	−/+	−	−	−	−	−
Colorectal adenocarcinoma	+	−	+	+	−	−	+	−	−	+	−	−/+	−	−	−	−	−
Pancreatic adenocarcinoma	+	−	+	−/+	−	−	+	−	−	−	−	+	−	−/+	−	−	−
Hepatocellular carcinoma	−/+	−		−	−	−		−	−	−	−/+	−	−	−		−	+
Cholangiocarcinoma	+	−	+	−/+	−	−	+	−	−	−	−	+	−	−/+	−/+	−	−
Clear cell renal carcinoma	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+
Pulmonary adenocarcinoma	+	−	+	−	−	−	+	−	−	−	−	−	−	−	+	−	−
Breast carcinoma (NST)	+	−	+	−	−	−		−	−	−	+	−	−	+	−	−	−

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Markers and Immunoprofile of Lymphoid Tissue Neoplasms

16

Contents

Lymphoid tissue is a microenvironment composed of B-, T-, and NK-lymphocytes in different maturation and differentiation stages, plasma cells, macrophages, dendritic cells, reticular cells, and granulocytes. For the diagnosis of lymphoma, all these components must be considered. For initial diagnosis, screening markers are helpful. Further specific markers must be used for the precise diagnosis. Markers listed in different parts of this chapter are essentially used for orientation. The final diagnosis must be done according to the histomorphology, immunophenotype (immunohistochemistry and flow cytometry), and genetic analysis. The 2016 revision of the World Health Organization classification of lymphoid neoplasms was considered in this chapter.

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16.1 Screening Markers for Lymphoma

CD45 (LCA), TdT, B-cell markers, T-cell markers, and Ki-67 [1–3].

CD45 (LCA)		
Expression pattern: membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Lymphoma/ leukemia	Granulocytic sarcoma, histiocytic sarcoma, dendrocytoma, interdigitating dendritic cell sarcoma, giant cell tumor of tendon sheet	Hematopoietic cells including B- and T-lymphocytes, monocytes, macrophages and mast cells, dendritic cells, medullary thymocytes, fibrocytes
Positive control: appendix		

Diagnostic Approach CD45, also known as leukocyte common antigen (LCA), is a family of high molecular mass integral membrane glycoprotein molecules expressed on all hematopoietic cells except mature red cells and their immediate progenitors, megakaryocytes, and platelets.

Diagnostic Pitfalls CD45 is a specific marker for hematopoietic and lymphatic tumors; nonetheless, less than 3% of B-cell lymphoma, about 10% of T-cell lymphoma, and about 30% of precursor B- and T-lymphoblastic lymphomas (ALL) lack the expression of CD45. In suspicious cases, the use of other lymphoid markers is required. Membranous CD45 expression is reported in very rare cases of undifferentiated, neuroendocrine, and small cell carcinomas. Necrotic carcinomas can also imitate a membranous LCA positivity, which also holds true for other markers, as in general, necrosis may display a false positivity.

TdT (Terminal deoxynucleotidyl transferase)		
Expression pattern: nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
B- and T-ALL	AML, CML, Merkel cell carcinoma	B- and T-cell precursors, cortical thymocytes
Positive control: ALL		

Diagnostic Approach Terminal deoxynucleotidyl transferase (TdT) is a DNA nuclear polymerase, catalyzing the template-independent polymerization of deoxynucleotidyl triphosphates to double-stranded gene segment DNA. TdT is mainly expressed in precursors of B- and T-lymphocytes. Therefore, antibodies to TdT are specific markers for precursor cell lymphomas of T- and B-cell origin, namely, acute lymphoblastic leukemia.

Diagnostic Pitfalls It is important to consider that TdT may be positive in some types of acute myeloid leukemia especially minimally differentiated AML (M0) and blast crisis of chronic myeloid leukemia (CML). Furthermore, the TdT expression is characteristic for the immature T-lymphocytes associated with the thymoma types A, B, and AB but not thymic carcinoma.

TdT is also positive in a large percentage of Merkel cell carcinoma, which may be also positive for PAX-5 [4, 5].

CD5 and CD10 are further markers for the diagnosis and classification of lymphomas. Both do not have lineage specificity and may be expressed in both B- and T-cell lymphomas in addition to other nonlymphoid neoplasms.

CD10 (CALLA)		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Burkitt lymphoma, acute lymphoblastic lymphoma/leukemia, angioimmunoblastic T-cell lymphoma, endometrial stromal tumors, renal cell carcinoma	Follicular lymphoma, plasma cell neoplasms, hepatocellular carcinoma, transitional cell carcinoma, colorectal adenocarcinoma, prostatic carcinoma, melanoma, placental site trophoblastic tumor, choriocarcinoma, myofibroblastoma, mesothelioma, rhabdomyosarcoma, leiomyosarcoma, Ewing's sarcoma, solitary fibrous tumor, atypical fibroxanthoma	Pre-B and pre-T cells, cells of germinal centers, granulocytes, adrenal cortex, endometrial stroma cells, hepatocytes and bile duct canaliculi, cells of proximal renal tubules and glomerular epithelial cells, endothelial cells, myoepithelial cells, fibroblasts, brain tissue, choroid plexus, fetal intestinal epithelium, mesonephric remnants
Positive control: appendix/tonsil		

Diagnostic Approach CD10 (neprilysin) is a zinc-dependent cell membrane metalloprotease involved in the post-secretory processing of neuropeptides and *vasoactive peptides*. Despite the name of CD10 as the common acute lymphoblastic leukemia antigen (CALLA), CD10 is not a cell line- or tumor-specific marker as it is expressed in a long list of tissue and tumor types of lymphoid, epithelial, and mesenchymal origin mentioned in the above table [6, 7]. In diagnostic immunohistochemistry, CD10 must be used in a panel with other tissue- and cell-specific markers [8]. The expression pattern of CD10 (membranous or cytoplasmic) is highly variable, depending on tumors type but also grade as the cytoplasmic stain is usually seen in poorly differentiated carcinomas.

CD5		
Expression pattern: membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Mantle cell lymphoma	B-CLL, T-ALL, T-cell lymphoma, prolymphocytic leukemia, adenocarcinomas of different origin, atypical thymoma, and thymic carcinoma	T cells, subset of B cells of mantle zone of the spleen and lymph nodes
Positive control: appendix/tonsil		

Diagnostic Approach CD5 (lymphocyte antigen T1, Leu-1) is a glycoprotein receptor expressed in the majority of T-lymphocytes and subset of B-lymphocytes including mantel zone lymphocytes. CD5 labels different T-cell neoplasms such as T-ALL, adult and peripheral T-cell lymphoma, mycosis fungoides, and T-cell large granular lymphocytic leukemia. The expression of CD5 is not restricted to T-lymphocytes but also found in a small subset of B-lymphocytes and lymphomas of B-cell origin mainly mantle cell lymphoma and B-CLL (Figs. 16.1 and 16.2).

Diagnostic Pitfalls The expression of CD5 is not limited to lymphoid tissue but found in adenocarcinomas of different origin, renal cell carcinoma, and adrenocortical carcinoma in addition to squamous cell carcinoma. Furthermore, CD5 is a diagnostic marker for atypical thymoma and thymic carcinoma; a focal weak expression of CD5 can be also found in mesothelioma, transitional carcinoma, squamous cell carcinoma, and adenocarcinomas of different origin [9].

Ki-67: Ki-67 is a nonhistone nuclear protein involved in the early steps of polymerase I-dependent ribosomal RNA synthesis and DNA replication expressed in active cell cycles. The expression of Ki-67 begins in the G_1 phase and persists during the active phases of cell cycle throughout the S, G_2, and M phases, whereas the peak of the Ki-67 expression appears in the early M phase. Ki-67 is rapidly catabolized at the end of the M phase with a half-life of 1–1.5 h and is undetectable in the G_0 phase or in the initial stage of the G_1 phase. Cells during the DNA repair also lack the Ki-67 expression.

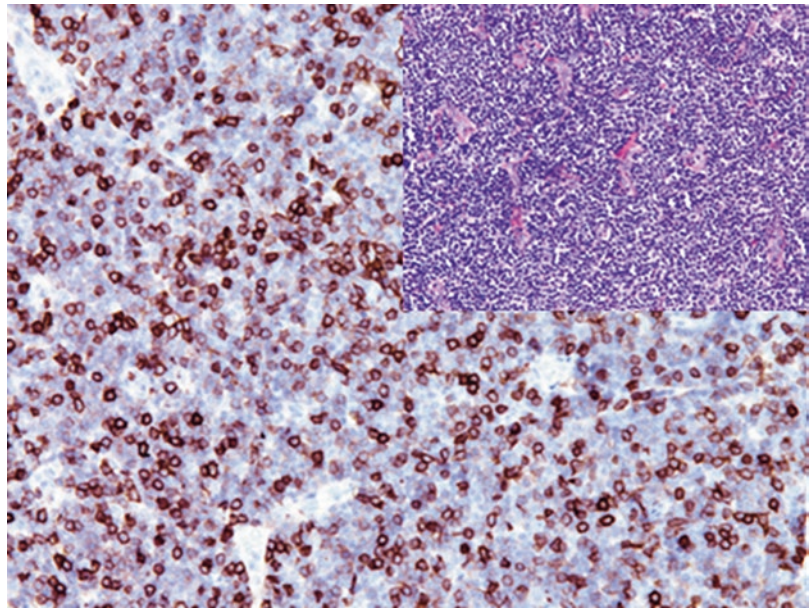


Fig. 16.1 Weak to moderate CD5 expression in cells of B-CLL. T-lymphocytes with strong CD5 expression

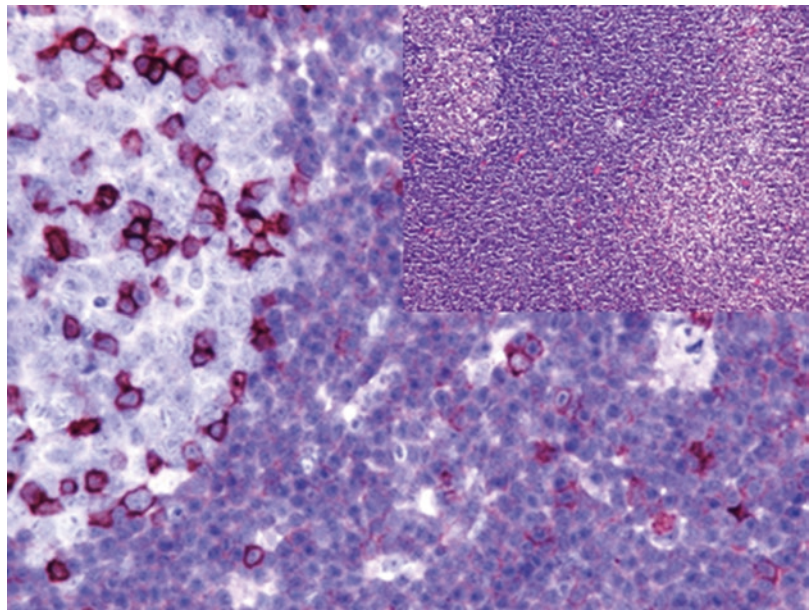


Fig. 16.2 Cells of mantel cell lymphoma showing moderate membranous CD5 expression. T-lymphocytes with strong CD5 expression

The expression of Ki-67 strongly correlates with the intensity of cell proliferation and tumor grade. In routine histopathology, Ki-67 is an important marker for the assessment of cell proliferation. The Ki-67 index is an important criterion for tumor diagnosis (benign, borderline, malignant, low- or high-grade tumor). Furthermore, it is a helpful marker to differentiate between atrophy or thermal alterations and dysplasia (Fig. 16.3). Few tumors show a Ki-67 index of nearly 100%, which can be used as a diagnostic clue; most representative examples are small cell lung carcinoma, Burkitt lymphoma, and plasmablastic lymphoma (Fig. 16.4). In routine hematopathology, the Ki-67 index is an important parameter to classify low and high malignant

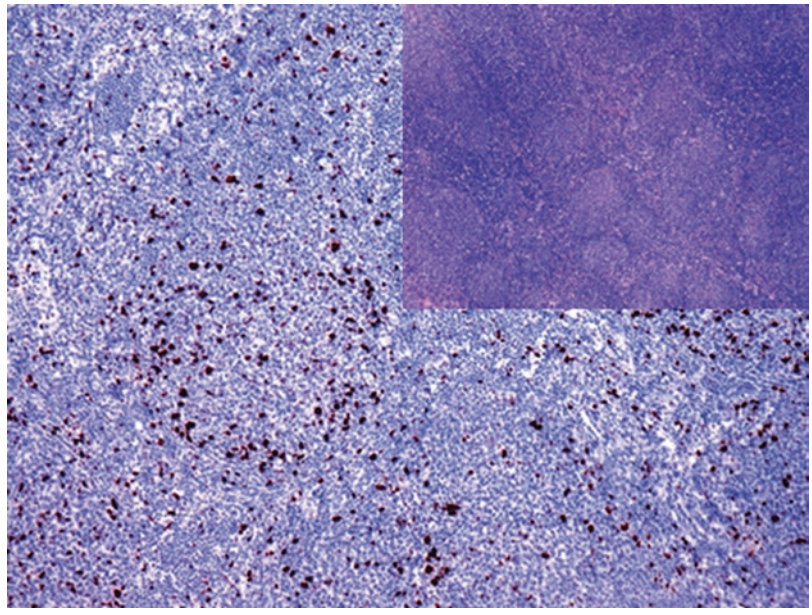


Fig. 16.3 Characteristic low proliferation index of neoplastic follicles in grade 1–2 follicular lymphoma

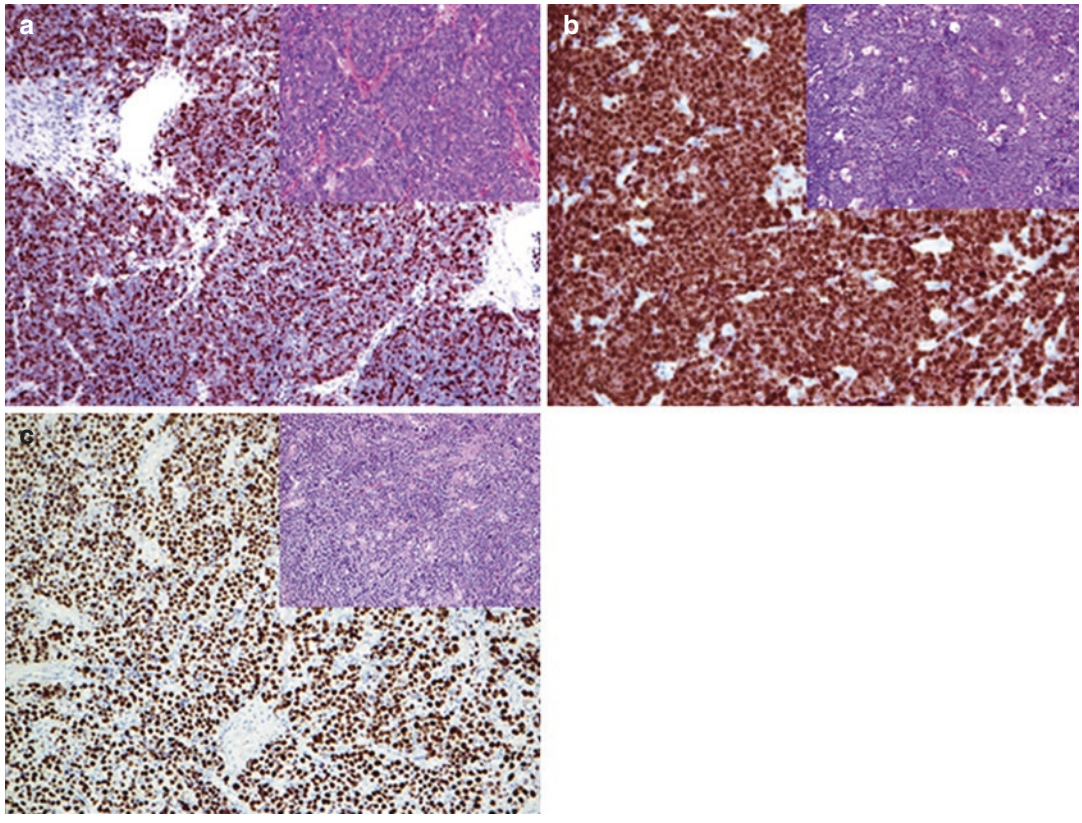


Fig. 16.4 Three tumor types with high Ki-67 index (~100%): (**a**) small cell carcinoma, (**b**) Burkett's lymphoma, and (**c**) plasmablastic lymphoma

lymphomas. Additionally, the Ki-67 index is a well-known prognostic marker correlating with the biological behavior of tumors such as breast carcinoma and neuroendocrine tumors. Nonetheless, it is a challenge to standardize Ki-67 staining and to establish a robust and reliable Ki-67 evaluation, which tends to show a considerable interlaboratory variability. This markedly hampers its clinical utility.

16.2 Markers and Immunoprofile of B-Cell Neoplasms

Immunohistochemical Markers for B-Cell Lymphoma CD5, CD10, CD19, CD20, CD23, CD79a, PAX-5, bcl-2, bcl-6, cyclin D1, Sox-11, ARTA1, and TdT [2, 3, 8, 10].

CD19 (B4)		
Expression pattern: membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
B-cell lymphoma/ leukemia	AML (M0), blast phase of CML	B cells, follicular dendritic cells
Positive control: appendix/tonsil		

Diagnostic Approach CD19 is a single chain glycoprotein and a member of the immunoglobulin family. CD19 is an early naïve B-lymphocyte antigen, which remains through the B-lymphocyte differentiation stages and disappears in the plasma cell stage. It is also expressed on the surface of follicular dendritic cells. CD19 is an excellent B-lymphocyte marker, and antibodies to CD19 are available for both flow cytometry and paraffin histology [11].

CD20 (B1 antigen)		
Expression pattern: membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
B-cell lymphoma/ leukemia		B cells, follicular dendritic cells
Positive control: appendix/tonsil		

Diagnostic Approach CD20 is a transmembrane non-glycosylated phosphoprotein acting as receptor during B-cell activation and differentiation. CD20 is expressed in B cells after CD19 in the naïve B-lymphocytes and remains until late stages of B-lymphocyte differentiation but disappears in the plasma cell stage.

Diagnostic Pitfalls CD20 is a pan-B-lymphocyte marker, but some types of B-cell lymphomas are CD20 negative or show a very weak expression level; consequently in doubtful cases, it is important to use two B-cell markers to assure or exclude the B-cell origin of the neoplasm. Optimal combinations are CD20/CD19 and CD20/PAX-5 or CD20/CD79. Generally, the expression of CD20 is restricted to B-lymphocytes, but rare cases of CD20 expression in peripheral T-cell lymphoma are reported. Another diagnostic pitfall is the interpretation of CD20 stain in patients after the specific CD20 immunotherapy (rituximab). Nuclear or nucleolar CD20 staining pattern are nonspecific.

CD23 (low-affinity IgE receptor)		
Expression pattern: membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
B-CLL, follicular dendritic cell tumors	Mediastinal large B-cell lymphoma, lymphoplasmacytic lymphoma, hairy cell leukemia, DLBCL	Follicular dendritic cells, EBV-transformed lymphoblasts, monocytes, platelets
Positive control: appendix/tonsil		

Diagnostic Approach CD23, also known as low-affinity IgE receptor, is a type II transmembrane glycoprotein involved in the regulation of IgE response. CD23 is expressed on mature B-lymphocytes, follicular dendritic cells, and activated macrophages. CD23 is an essential marker used to discriminate B-CLL from other lymphoma types with similar morphology (Fig. 16.5). CD23 also labels mediastinal large B-cell lymphoma and lymphoplasmacytic lymphoma. It is also an important marker for follicular dendritic cell tumors.

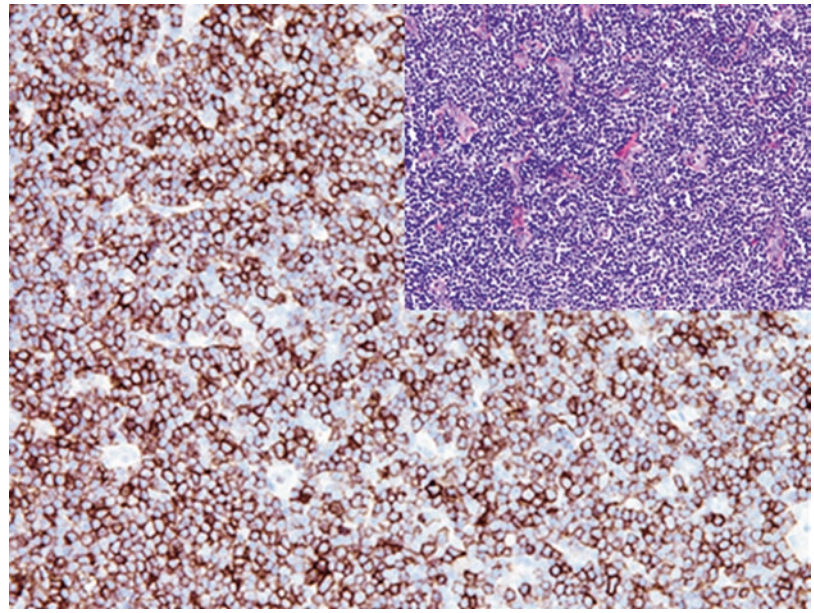


Fig. 16.5 Membranous CD23 expression in B-CLL

CD79a		
Expression pattern: membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
B-cell leukemia/ lymphomas	Acute promyelocytic leukemia (FAB-M3), multiple myeloma	B cells, small population of CD3+ T cells, subset of endothelial cells
Positive control: appendix/tonsil		

Diagnostic Approach CD79a is a disulfide-linked heterodimer associated with the membrane-bound immunoglobulin; it appears in the pre-B-lymphocyte stage and persists until the plasma cell development, rendering the majority of normal and neoplastic plasma cells positive for CD79a. CD79a exhibits a membranous stain, but plasma cells may also show a cytoplasmic staining pattern. The expression of CD79a is independent of the expression of CD20 and remains positive after the anti-CD20 immunotherapy.

Diagnostic Pitfalls CD79a is less reliable than CD20 for the diagnosis of B-cell lymphoma, as it is positive in a small fraction of T-ALL, AML (FAB-M3), and the majority of plasma cell neoplasms (see above).

PAX-5 (B-cell-specific activator protein, BSAP)		
Expression pattern: nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
B-cell lymphoma/ leukemia, Reed-Sternberg cells of classic Hodgkin's lymphoma	Merkel cell carcinoma, alveolar rhabdomyosarcoma, small cell carcinoma, Wilms' tumor, glioblastoma and neuroblastoma, mesonephric and Müllerian tumors	Pre-B to mature B cells
Positive control: appendix/tonsil		

Diagnostic Approach PAX-5 is a member of the PAX (**pa**ired bo**x**) family of transcription factors involved in tissue and organ differentiation. PAX-5 (also known as B-cell activator protein) is a B-cell-specific transcription factor encoded by the gene located at 9p13 and expressed in the early pro-B, pre-B, and naïve stages of B-cell development until the mature B cells [12]. The PAX-5 gene is involved in the t(9;14)(p13;q32) translocation associated with the plasmacytoid subtype of small lymphocytic lymphoma. PAX-5

is also expressed in the L&H cells of nodular lymphocyte-predominant Hodgkin's lymphoma. T-lymphocytes, plasma cells, and macrophages are constantly PAX-5 negative.

Diagnostic Pitfalls PAX-5 can be positive in some tumors resembling lymphoma such as Merkel cell carcinoma and small cell carcinoma and also rarely in acute lymphoblastic lymphoma of T-cell origin [13, 14]. PAX-5 maybe also expressed in acute myeloid leukemia, mainly the type associated with the t(8;21)(q22;q22) translocation. PAX-5 positivity is reported in rare cases of breast, endometrial, and transitional carcinomas in addition to alveolar rhabdomyosarcoma, but it is constantly negative in embryonal-type rhabdomyosarcoma [15, 16].

Cyclin D1 (bcl-1)		
Expression pattern: nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Mantle cell lymphoma	Inflammatory pseudotumor (myofibroblastic tumor), hairy cell leukemia, multiple myeloma, parathyroid adenoma/carcinoma, pulmonary adenocarcinoma, breast and prostate carcinoma, transitional cell carcinoma	Cells in the G_1 phase of cell cycle, histiocyts, endothelial cells
Positive control: mantle cell lymphoma		

Diagnostic Approach Cyclin D1 (also known as bcl-1) is a cell cycle protein involved in the regulation of cyclin-dependent kinases of the first gap phase (G_1) of the cell cycle. The expression of cyclin D1 is not restricted to lymphoid neoplasms and found in a number of nonlymphoid epithelial and mesenchymal tumors. The cyclin D1 overexpression—caused by the t(11;14) translocation associated with mantle cell lymphoma—makes it a characteristic marker for this lymphoma type (Fig. 16.6). In routine immunohistochemistry, cyclin D1 is usually used in combination with CD5, Sox-11, and other B-cell markers [8, 17].

A subset of multiple myeloma harbors also the t(11;14) translocation and is positive for cyclin D1; this myeloma type is usually associated with favorable prognosis.

Diagnostic Pitfalls Other lymphoma types exhibiting similar morphology such as hairy cell leukemia and B-CLL may be also positive for cyclin D1; however, the stain intensity is much less than that of mantle cell lymphoma [18]. A small subset of mantle cell lymphoma lacks the expression of cyclin D1; this subset is usually positive for Sox-11, which to consider in the differential diagnosis.

Sox-11		
Expression pattern: nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Mantle cell lymphoma	Hairy cell leukemia, Burkitt lymphoma, T- and B-ALL, prolymphocytic leukemia, ovarian carcinoma	Immature neurons
Positive control: mantel cell lymphoma		

Diagnostic Approach Sox-11 is a member of the Sox family of transcription factors (**s**ex-determining region Y-b**ox 11**), a transcription factor involved in embryogenesis and development of the central nervous system. Sox-11 strongly stains both cyclin D1 positive and negative mantle cell lymphoma (Fig. 16.7) in addition to other lymphoma types including hairy cell leukemia and ALL [19–21].

Sox-11 stains also a subset of ovarian carcinomas, generally associated with good prognosis.

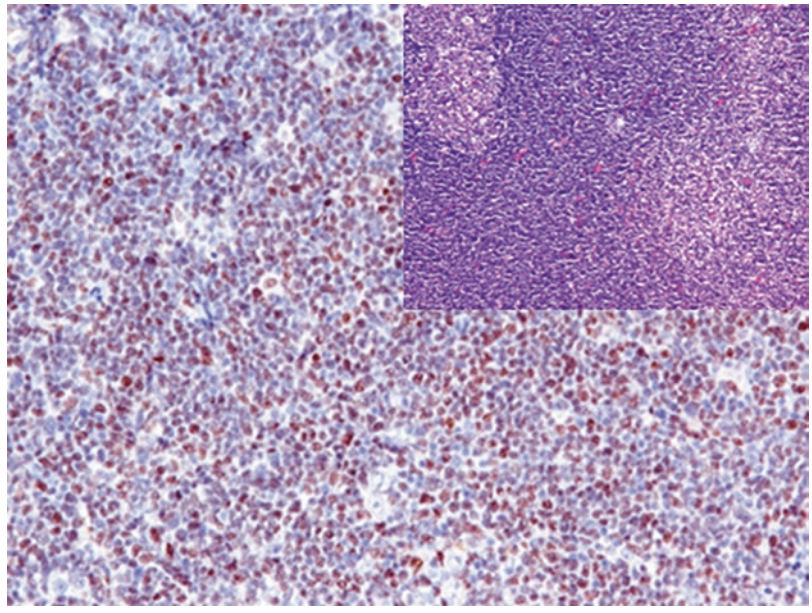


Fig. 16.6 Strong nuclear cyclin D1 expression in mantel cell lymphoma

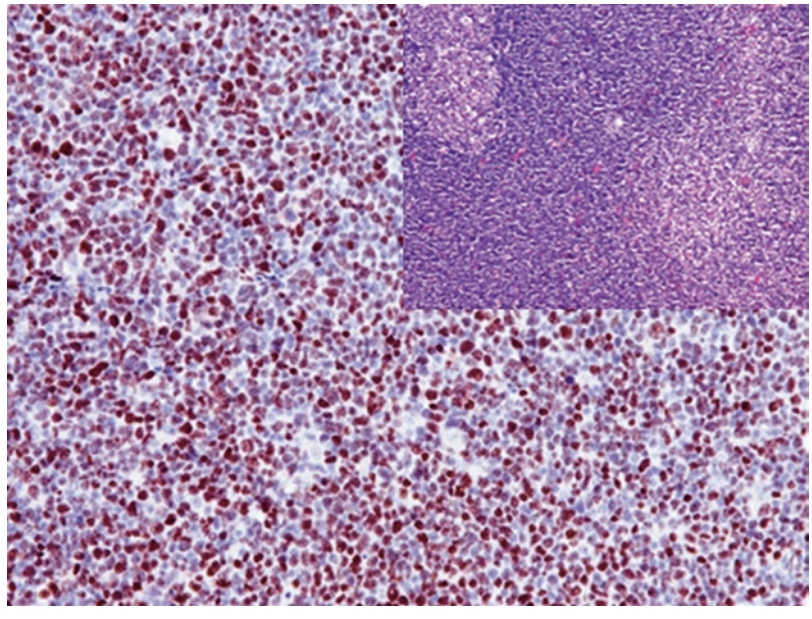


Fig. 16.7 Strong nuclear Sox-11 expression in mantel cell lymphoma

bcl-6		
Expression pattern: nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Follicular lymphoma (intra- and interfollicular cells), anaplastic CD30+ large cell lymphoma	Burkitt lymphoma, diffuse large B-cell lymphoma, mediastinal large B-cell lymphoma, L&H cells in nodular lymphocyte-predominant Hodgkin's lymphoma, ALK + anaplastic large cell lymphoma, angioimmunoblastic lymphoma, T-ALL	Germinal centers of lymph nodes, subset of intrafollicular CD4+ T-lymphocytes
Positive control: appendix/tonsil		

Diagnostic Approach bcl-6 (**B-c**ell **l**ymphoma **6** protein) is a sequence-specific transcriptional repressor protein expressed in normal germinal center B-lymphocytes with high proliferation rate and active somatic mutations. bcl-6 is a marker for lymphomas of germinal center origin such as follicular lymphoma (intra- and interfollicular cells), Burkett's lymphoma, majority of Hodgkin cells, and nodular lymphocyte-predominant Hodgkin's lymphoma [8]. Mutations within the bcl-6 gene are found in about 40% of diffuse large B-cell lymphoma and 15% of follicular lymphoma causing the overexpression of bcl-6 [22]. bcl-6 is also found in some NK-/T-cell lymphoma types such as angioimmunoblastic lymphoma and T-ALL. Mantle cell lymphoma, marginal zone lymphoma, and ALL are constantly bcl-6 negative.

bcl-2		
Expression pattern: cytoplasmic (mitochondrial membrane)		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Follicular lymphoma	Majority of B-cell lymphomas, subset of T-cell lymphoma, basal cell carcinoma, adrenocortical tumors, solitary fibrous tumor, synovial sarcoma, hemangiosarcoma, neurofibroma, schwannoma, nasopharyngeal carcinoma, dermatofibrosarcoma protuberans, spindle cell lipoma, rhabdomyosarcoma	Small B-lymphocytes in primary follicles and in the mantle and marginal zones, subset of T-lymphocytes, medullary cells in thymus, adrenal cortex, basal keratinocytes of the epidermis
Positive control: appendix/tonsil		

Diagnostic Approach bcl-2 (**B-c**ell **l**ymphoma **2** protein) is a family of regulator proteins involved in the regulation of programmed cell death divided into two main groups: the bcl-2 group as antiapoptotic and proapoptotic group (effectors and activators). The bcl-2 proteins are encoded by the bcl-2 gene on chromosome 18q21. The bcl-2 gene is transcribed into three mRNA variants, which are translated into two homologous integral cell and mitochondrial membrane proteins.

The t(14;18)(q32;q21) translocation characteristic for 90% follicular lymphoma juxtapose the bcl-2 gene to the Ig heavy chain gene resulting the deregulation of the bcl-2 gene and the overexpression of the bcl-2 protein giving a survival advantage for the lymphoma cells. One of the main diagnostic benefits of bcl-2 is to distinguish between reactive lymph nodes with follicular hyperplasia exhibiting bcl-2-negative germinal centers and grade 1 follicular lymphoma with bcl-2-positive neoplastic B cells in the follicles (Fig. 16.8) [8]. The bcl-2 expression is found in the majority of B-cell lymphomas and in a subset of T-cell lymphomas. It is also found in a large number of epithelial and mesenchymal tumors [8].

CD11c		
Expression pattern: membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Hairy cell leukemia	AML (M4 and M5), follicular lymphoma, Langerhans cell histiocytosis, lymphoplasmacytic lymphoma, B-CLL, splenic lymphoma, NK lymphoma	Myeloid hematopoietic cells, granulocytes, macrophages, NK cells, dendritic cells, subset of activated T-lymphocytes, histiocytes
Positive control:		

Diagnostic Approach CD11c (also known as integrin alpha X, CR4, LeuM5) is an integrin glycoprotein composed of alpha and beta chains involved in the adhesion and chemotaxis of monocytes, primarily expressed on myeloid hematopoietic cells. CD11c is a marker for different lymphoid and myeloid neoplasms. It is strongly expressed in hairy cell leukemia and

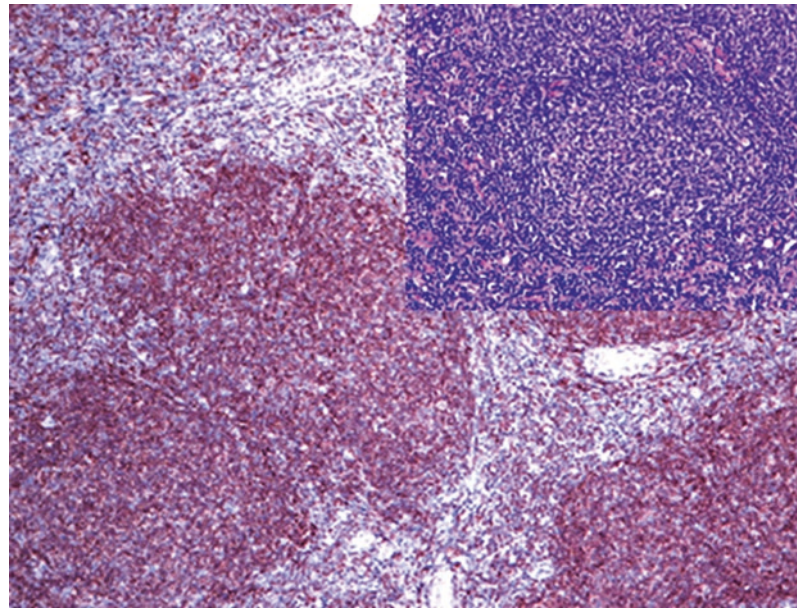


Fig. 16.8 Follicular lymphoma with strong diffuse bcl-2 expression in neoplastic follicles

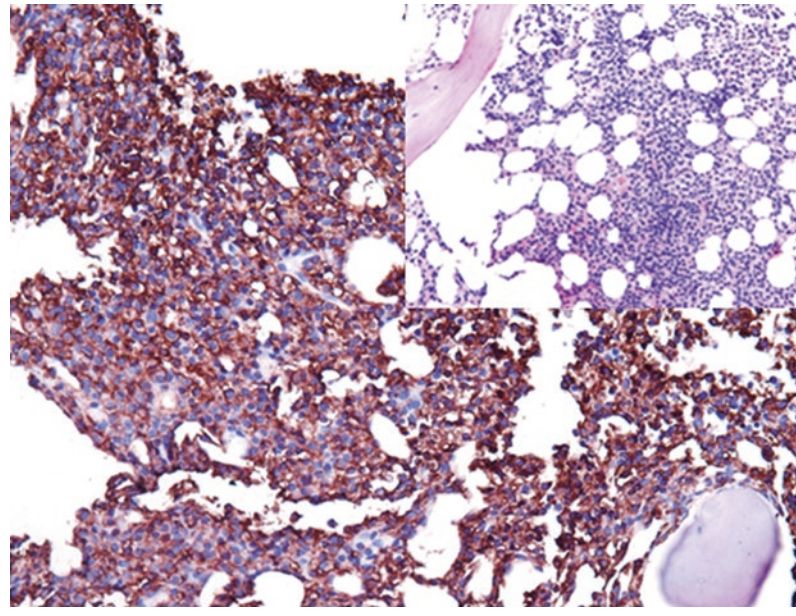


Fig. 16.9 Hairy cell leukemia, with CD11c-positive leukemia cells in the bone marrow

natural killer cell lymphoma (Fig. 16.9). CD11c is also found in about 50% of AML (M4 and M5) and in some cases of follicular lymphoma, Langerhans cell histiocytosis, lymphoplasmacytic lymphoma, splenic lymphoma with villous lymphocytes, and B-CLL. The expression of CD11c on B-CLL cells is usually associated with good prognosis.

Tartrate-resistant acid phosphatase (TRAP)		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Hairy cell leukemia, osteoclastoma (giant cell tumor)	Mantel cell lymphoma, mediastinal B-cell lymphoma, splenic marginal cell lymphoma	Osteoclasts, macrophages, lymphocytes of the marginal zone, neurons, decidual cells, prostatic glands, red blood cells
Positive control: osteoclasts, hairy cell leukemia		

Diagnostic Approach Tartrate-resistant acid phosphatase (TRAP) is a glycosylated iron-binding metalloprotein enzyme found in different tissue types and is highly expressed in osteoclasts and macrophages. TRAP is specific marker for hairy cell leukemia but should be used in combination with other markers such as CD11c and DBA.44 (Fig. 16.10) [23].

Diagnostic Pitfalls Other lymphoma type such as marginal zone B-cell lymphoma may reveal weak TRAP positivity. TRAP is also expressed in bone marrow marcophages.

Immunoglobulin Superfamily Receptor Translocation-1: IRTA-1 is a cell surface receptor involved in the lymphogenesis of B-lymphocytes in addition to intercellular communication. IRTA-1 is helpful marker to decimate between marginal zone lymphoma and other lymphoma types as it is expressed in more than 90% of extranodal marginal zone lymphoma and in about 75% of nodal marginal zone lymphoma but negative in splenic marginal zone lymphoma. Other lymphoma types including B-CLL, mantel cell lymphoma, follicular lymphoma, Burkitt lymphoma, hairy cell leukemia, and plasma cell neoplasms also lack the expression of IRTA-1 [24, 25]. IRTA-1 cannot distinguish between reactive and neoplastic marginal zone lymphocytes.

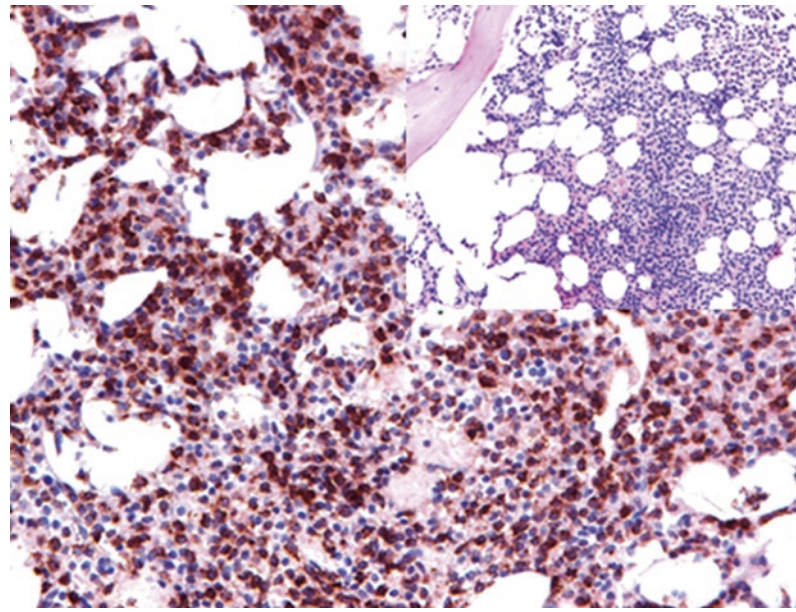


Fig. 16.10 Bone marrow trephine infiltrated by cells of hairy cell leukemia exhibiting strong cytoplasmic TRAP expression

LIM-only transcription factor 2 (LMO2)		
Expression pattern: nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Follicular lymphoma, mediastinal large B-cell lymphoma, Burkitt lymphoma	B- and T-ALL, endothelial tumors. GIST, myoepithelial tumors, juvenile xanthogranuloma	Germinal centers of lymph nodes, hematopoietic precursors, endothelium, breast myoepithelial cells, basal cells of prostatic gland, endometrial glands in secretory phase
Positive control: tonsil/lymph node		

LIM-Only Transcription Factor 2 LMO2 (also known as TTG2 or RBTN2) is a transcription factor regulating the yolk sac angiogenesis and erythropoiesis, normally expressed in erythroid and myeloid precursors as well as megakaryocytes and endothelial cells. The LMO2 protein is expressed in B-lymphocytes of germinal centers. LMO2 is a marker for several lymphoma types derived from germinal center cells. It is expressed in up to 70% of all grades of follicular lymphoma, mediastinal large B-cell lymphoma, Burkitt lymphoma and diffuse large B-cell lymphoma, and B- and T-ALL. CLL, mantel cell lymphoma, marginal zone lymphoma, lymphoplasmacytic lymphoma, and peripheral T-cell lymphomas usually lack the expression of LMO2. LMO2 is expressed in lymphocyte-predominant Hodgkin's lymphoma but not in classical Hodgkin's lymphoma. Furthermore LMO2 labels the myeloid blasts of acute myeloid leukemia [26, 27]. In addition to lymphoid and hematopoietic neoplasms, LMO2 labels normal endothelium of blood and lymph vessels and the majority of benign and malignant endothelial tumors [28].

Human Germinal Center-Associated Lymphoma HGAL: also known as germinal center B-cell-expressed transcript 2 (GCET-2) is exclusively expressed in the cytoplasm and on the membrane of germinal center B-lymphocytes and specially accentuated in the proliferating cells within the dark zone of germinal centers. HGAL is involved in the regulation of lymphocyte motility. Lymphocytes within the mantle and marginal zones as well as interfollicular and paracortical regions lack the expression of HGAL. HGAL is a marker for B-cell lymphomas derived from germinal center lymphocytes and expressed in 100% of Burkitt lymphoma, more than 90% of follicular lymphomas and mediastinal lymphoma, and about 70% of diffuse large B-cell lymphoma. The expression of HGLA is reported in less than 5% of marginal zone lymphoma whereas mantel cell lymphoma and B-CLL completely negative for HGAL [29, 30].

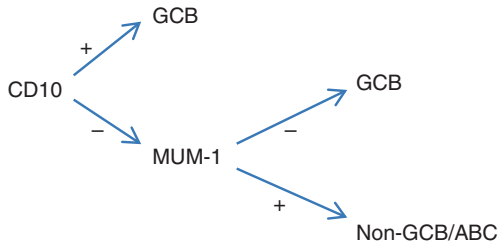
Lymphoid Enhancer-Binding Factor LEF-1: is a nuclear protein and a member of the T-cell-specific factor family that binds to the T-cell receptor playing a role in the regulation of cell proliferation and lymphopoieses. LEF-1 is normally expressed in pre-B- and T-lymphocytes but not in mature B cells. In lymphomas, LEF-1 labels the neoplastic small lymphocytes of chronic lymphocytic leukemia (CLL) but negative in other small B-cell lymphomas [31]. It is also found in about one third of diffuse large B-cell lymphoma. LEF-1 is not a specific lymphoma marker as it is also expressed in different carcinoma types such as colorectal adenocarcinoma [32].

Immunoprofile of B-cell neoplasms				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Precursor B-lymphoblastic leukemia/lymphoma	*TdT*, HLA-DR (CD74), *CD19*, CD79a, PAX-5 Proliferation index (Ki-67): 50–80%	*CD10*[a], CD22, CD24, CD45, CD99, CD34, FLI-1, LMO2	CD20, CD13	
B-cell chronic lymphocytic lymphoma (B-CLL)/small lymphocytic lymphoma	*CD5*, CD19, *CD20*, CD22, *CD23*, CD74, CD79a, *CD160*, *CD200*, *LEF-1*, PAX-5, p27, bcl-2, sIgM Proliferation index (Ki-67): ~ 5%	CD22, CD43, MUM-1, sIgD	CD11c, CD38[b]	CD10, Sox-11, bcl-6
Monoclonal B-cell lymphocytosis	See B-CLL immunoprofile (B-cell account in peripheral blood <5 x10 [9]/L with B-CLL phenotype with no signs of lymph node involvement)			
B-cell prolymphocytic leukemia	*CD19*, *CD20*, CD22, CD25, CD27, CD74, CD79a, PAX-5, bcl-2	sIgM, sIgD	CD5	CD10, CD23, CD43, CD138, cyclin D1
Lymphoplasmacytic lymphoma	*CD19*, *CD20*, CD22, CD43, CD74, CD79a, *CD200*, PAX-5, IgM Proliferation index (Ki-67): ~5–10%	CD38, CD138, MUM-1, bcl-2, MYD88	CD5	CD10, CD23, cyclin D1
Mantle cell lymphoma	*CD5*, CD19, CD20, CD22, CD37, CD43, CD74, CD79a, sIgM, sIgD, *cyclin D1*, *Sox-11*, PAX-5, FMC-7 Proliferation index (Ki-67): 5–50%	bcl-2		CD10, CD11c, CD23, bcl-6
Follicular lymphoma/in situ follicular neoplasia/ duodenal-type follicular lymphoma	CD19, *CD20*, CD22, CD74, CD79a, PAX-5, *HGAL*, sIg, bcl-2 Nodular meshwork of follicular dendritic cells positive for CD21 and CD23 *Proliferation index (Ki-67) in bcl-2-positive neoplastic follicles: < 20%* *Proliferation index (Ki-67) in bcl-2-negative reactive follicles: > 60%*	CD10, *bcl-6*, bcl-2 (in grade 3 follicular lymphoma), *LMO2* κ/λ light chain restriction	bcl-2 (in primary cutaneous follicular lymphoma)	CD5, CD23, CD43, Sox-11, cyclin D1
Pediatric-type follicular lymphoma	CD19, CD20, CD22, CD74, CD79a, PAX-5, CD10, *HGAL*, *LMO2*, sIg, Proliferation index (Ki-67): >30%	*bcl-6*, CD43		MUM-1, *bcl-2*[c]
Large B-cell lymphoma with IRF-4 rearrangement	CD19, CD20, CD22, *MUM-1*, *bcl-6*	CD10, bcl-2	CD5	
Primary cutaneous follicle center lymphoma	CD20, PAX-5, bcl-6	CD10, bcl-2	CD30, CD23	CD3, CD5, CD43, cyclinD1
Nodal marginal zone B-cell lymphoma	CD19, *CD20*, CD21, CD22, CD35, CD74, CD79a, PAX-5, sIgM	sIgA, sIgG, CD11c, bcl-2, *IRTA-1*	CD43, CD38, MUM-1, TRAP	sIgD, CD5, CD10, CD 23, bcl-6, Sox-11, cyclin D1
Extranodal marginal zone B-cell lymphoma of MALT type	CD19, *CD20*, CD21, CD22, CD35, CD74, CD79a, PAX-5, sIgM, *IRTA-1*	CD11c, MUM-1, sIgD, sIgA, sIgG, bcl-2	CD43	CD5, CD10, CD23, Sox-11, cyclin D1, bcl-6
Splenic marginal zone B-cell lymphoma	CD19, *CD20*, CD21, CD22, CD35, CD74, CD79a, PAX-5, bcl-2, sIgM, sIgD, Proliferation index (Ki-67): < 5%	sIgA, sIgG, CD11c	CD5	CD43, CD10, CD23, CD25, CD43, CD103, bcl-6, *cyclin D1*, annexin A1, IRTA-1

Immunoprofile of B-cell neoplasms				
Hairy cell leukemia	*CD11c*, CD19, *CD20*, CD22, CD25, CD74, CD79a, *CD103*, CD123, *annexin A1*, *TRAP*, *DBA.44*, *BRAF^{V600E}*, PAX-5, cyclin D1, sIgM, FMC7 Proliferation index (Ki-67): <5%	CD23, CD68 (cytoplasmic dots), PCA-1, HC1, HC2	CD5	*CD10*, *CD23*, *CD43*, bcl-6
Diffuse large B-cell lymphoma (DLBCL) - Germinal center cell type (GCB)[d] - Activated B-cell type (ABC)[d]	CD19, *CD20*, CD22, CD74, CD79a, CD45, PAX-5 Proliferation index (Ki-67): > 40%	bcl-6	bcl-2, CD5, CD30, fascin, MUM-1[e]	CD3, CD15, CD200
Primary cutaneous diffuse large B-cell lymphoma, leg type	CD20, CD70a, PAX-5, bcl-2, MUM-1			CD10
T-cell-/histiocyte-rich variant of diffuse large B-cell lymphoma	Neoplastic cells: CD19, *CD20*, CD22, CD74, CD79a, CD45, PAX-5, bcl-6, BOB 1, OCT-2 Nonneoplastic cells (>80% of cell population): positive for CD3, CD8, cytotoxic molecules, and CD68 in histiocytes		CD30, EMA	CD3, CD15, bcl-2, PU.1
Mediastinal (thymic) large B-cell lymphoma	CD19, *CD20*, CD45, CD74, CD79a, CD200, PAX-5	*CD23*, MUM-1, *CD30, HGAL, LMO2*	CD10	CD5, CD21
ALK-positive large B-cell lymphoma	*ALK*, EMA, CD138, VS38c	CD4, κ or λ Ig light chains	CD45, CD79a	CD3, CD20, CD30,
Plasmablastic lymphoma	CD38, CD138, VS38c, MUM-1, EBV (EBER), LCA Proliferation index (Ki-67): > 90%	CD79a, EMA, CD10	CD30	CD20, PAX-5, CD56
Intravascular large B-cell lymphoma	CD20, CD79a, PAX-5	Prostatic acid phosphatase	CD5, CD10	
Primary effusion lymphoma	CD45, *CD79a*, CD38, CD138, VS38c, *PAX-5*, *HHV-8*, MUM-1	CD30, EBV	CD20, CD19	CD43, bcl-6
Burkitt lymphoma	*CD10*, CD19, *CD20*, CD22, CD74, CD79a, PAX-5, sIgM, *c-myc*, *HGAL*, CD43, p53 Proliferation index (Ki-67): > 95%	bcl-6, EBV, LMO2, adipophilin		CD5, CD23, TdT, bcl-2
Burkitt-like lymphoma with 11q aberration	CD19, CD20, CD22, CD38, CD74, CD79a, PAX-5 Proliferation index (Ki-67): > 95%	CD43, bcl-6, sIgG, IgM	CD10	*c-myc*, bcl-2
EBV-positive mucocutaneous ulcer EBV-positive DLBCL	*EBV*[f], CD19, *CD30*, MUM-1, PAX-5	CD20, CD15, bcl-2		
Lymphomatoid granulomatosis	EBV, CD19, *CD20*	CD79a	CD30	CD15

[a]Negative in ALL with 11q23 translocation
[b]The expression of CD38 in B-CLL correlates with worse prognosis
[c]Pediatric-type follicular lymphoma lacks the t(14;18) translocation
[d]See modified Hans algorithm below [33]
[e]Positive in ABC (activated B-cell-like) subtype of DLCBL
[f]EBV antigens: EBER, LMP1, and EBNA2

Algorithm 16.1: Modified Hans Algorithm [33]



- GCB: Germinal center B-cell type
- ABC: Activated B-cell type

16.3 Markers and Immunoprofile of Plasma Cell Neoplasms

CD38, CD138, VS38c, MUM-1, CD56, and κ and λ light chains.

CD38		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Plasma cell neoplasms, plasmablastic lymphoma	Pre-T-ALL, primary effusion lymphoma, subtypes of B-cell lymphoma	Plasma cells, erythroid and myeloid precursors, early B and T cells, NK cells, pancreatic islets, neuronal tissue
Positive control: appendix		

Diagnostic Approach CD38 (also known as ADP-ribosyl cyclase) is a transmembrane glycoprotein expressed in the majority of CD34-positive pluripotent stem cells and in different maturation stages of B- and T-lymphocytes, plasma cells, and myeloid cells [34]. CD38 is commonly used in diagnostic panels of multiple myeloma. CD38 can be expressed on CLL cells and considered being an adverse prognostic factor.

Diagnostic Pitfalls CD38 has a wide expression spectrum and is found in different hematopoietic and non-hematopoietic cells; accordingly, the CD38 expression does not prove the plasma cell origin, and the plasma cell nature must be confirmed by other more specific markers.

CD138 (syndecan-1)		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Plasma cell tumors (myeloma, plasmacytoma)	Primary effusion lymphoma; multiple carcinomas including thyroid, breast, lung, head and neck, urothelium, prostatic, and liver; neuroendocrine tumors; thymoma; tumors of the adrenal cortex; keratoacanthoma; malignant melanoma; osteoid-forming tumors	B-cell precursors, plasma cells, stratified squamous epithelium, hepatocytes
Positive control: tonsil/squamous epithelium		

Diagnostic Approach CD138 (syndecan-1) is a transmembrane antigen and one of the four members of the syndecan family. The expression of CD138 is found in different maturation stages of B-lymphocytes and plasma cells and in different types of epithelial and mesenchymal cells; nevertheless, CD138 is one of the important markers for plasma cell neoplasms.

Diagnostic Pitfalls CD138 is widely used as a marker for plasma cells and plasma cell neoplasms. However, the expression of CD138 is found in a large number of epithelial tumors and some mesenchymal tumors. Among the epithelial tumors, CD138 is found in squamous cell carcinoma and adenocarcinomas of different origins including pulmonary and prostatic adenocarcinomas, which makes it necessary to consider these carcinomas in the differential diagnosis [35]. A particular pitfall is the plasmacytoid urothelial carcinoma, which

is often strongly CD138 positive and can be mistaken for a plasmacytoma. To differentiate between epithelial and plasma cell tumors, it is recommended to run a parallel reaction with a pan-cytokeratin antibody but not EMA as EMA may be also positive in plasma cell disorders as well [36]. The expression profile of κ and λ light chains is also important to confirm the diagnosis of plasma cell neoplasia and determine the clonality of the plasma cell population. CD138 is also expressed in other mesenchymal tumors such as alveolar soft part sarcoma, synovial sarcoma, and schwannoma in addition to malignant melanoma and bone-forming tumors including osteosarcoma [37].

MUM-1 (multiple myeloma oncogene 1/IRF4)		
Expression pattern: nuclear/cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Plasma cell neoplasms, diffuse large B-cell lymphoma ABC type	Hodgkin's lymphoma, CLL, marginal zone lymphoma, DLBCL, malignant melanoma	B cells (centrocytes), plasma cells, activated T cells
Positive control: appendix		

Diagnostic Approach The MUM-1 protein (**mu**ltiple **m**yeloma **1**) is a lymphocyte-specific transcriptional activator also known as interferon regulatory factor 4 expressed in the final differentiation stage of intra-germinal center B cells. MUM-1 is a marker for post-germinal center B cells, plasma cells, and subset of T cells and related lymphoma types in addition to Hodgkin cells. MUM-1 is usually negative in the cells of nodular lymphocyte-predominant Hodgkin's lymphoma.

Diagnostic Pitfalls MUM-1 stains also a subset of malignant melanoma, which can be also positive for other plasma cell markers such as CD138 and VS38c. Because of the multilineage expression of the MUM-1 protein, the immunostaining results must be carefully interpreted in combination with additional more specific markers to exclude other possible differential diagnoses [38, 39].

VS38c (plasma cell marker)		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Plasma cell neoplasms (myeloma, plasmacytoma), lymphoma with plasmacytic differentiation	Rare carcinoma types of different origin, malignant melanoma, clear cell sarcoma of soft tissue, neuroendocrine tumors	Plasma cells and plasmablasts, B-immunoblasts, epithelial cells (mucous glands, pancreatic epithelium, secretory breast cells, thyroid follicles), melanocytes, osteoblasts
Positive control: appendix		

Diagnostic Approach VS38c (rough endoplasmic reticulum-associated antigen) is a sensitive screening marker for plasma cells and cells with plasmacytoid differentiation. VS38c is expressed on the endoplasmic reticulum in the cell cytoplasm. The expression of VS38c is found in plasma cells, plasmablasts, lymphoplasmacytoid cells, and B-immunoblasts and related neoplasms.

Diagnostic Pitfalls Despite the specificity and high sensitivity of VS38c to normal and neoplastic plasma cell, it is always important to keep in mind that other tumor types such as melanocytic and neuroendocrine tumors may be positive for this marker [40]. Paratrabecular osteoblasts in trephine biopsies are also positive for VS38c.

Kappa and Lambda Light Chains: Each molecule of the five major classes of immunoglobulins is consisted of the combination of two identical heavy chain molecules and two identical light chain molecules. The light chain molecules are divided into two classes, kappa and lambda light chain; on the other hand, each B-lymphocyte

or plasma cell is able to produce either kappa or lambda light chain. In a polyclonal lymphocyte or plasma cell population, the kappa-to-lambda ratio is approximately 2:1. The clonal restriction of one of both chains indicates the monoclonal—neoplastic—nature of the lymphocyte or plasma cell population. In routine histopathology, the expression of the light chains can be indicated either by conventional immunohistochemistry or by in situ hybridization.

Immunoprofile of plasma cell neoplasms				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Plasma cell myeloma/plasmacytoma – Monoclonal gammopathy of undetermined significance (MGUS) – Heavy chain disease – Plasma cell myeloma – Solitary plasmacytoma of bone – Extraosseous plasmacytoma – Monoclonal immunoglobulin deposition disease	*CD38*, *VS38c*, *CD138*, PCA-1, MUM-1, vimentin *κ or λ Ig light chain restriction* Proliferation index (Ki-67):~50–60%	CD43, CD56, CD79a	CD45, EMA, cyclin D1, Steroid hormone receptors (ER)	CD19, CD20, CD22, PAX-5, E-cadherin

16.4 Markers and Immunoprofile of T-Cell Neoplasms

Immunohistochemical Markers for T-Cell Lymphoma CD2, CD3, CD4, CD7, CD8, CD30, ALK, TCL-1, CXCL13, and TdT [8, 10, 41].

CD2 (LFA-2)		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
T-cell lymphoma	Neoplastic mast cells (mastocytosis)	Thymocytes, mature peripheral T cells, NK cells
Positive control: appendix/tonsil		

Diagnostic Approach CD2 is a transmembrane glycoprotein (E rosette receptor) that mediates adhesion between T-lymphocytes and other cells. CD2 appears in the early stages of T-cell development. CD2 is an excellent marker for T-lymphocytes and NK cells and labels T-cell lymphomas and the majority of NK neoplasms. CD2 is negative in B-lymphocytes with the exception of a small subset of thymic B cells but negative in all B-cell lymphomas. CD2 is negative in normal mast cells, and the CD2 expression in mast cells is usually a criterion of malignancy.

CD3		
Expression pattern: membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
T-cell lymphomas	NK lymphoma (cytoplasmic stain)	Thymocytes, peripheral T cells, activated NK cells, Purkinje cells of cerebellum
Positive control: appendix/tonsil		

Diagnostic Approach CD3 is a complex structure composed of five polypeptide chains (γ, δ, ε, ζ, and η) forming three dimers. CD3 builds a complex with the T-cell receptor on the membrane of T-lymphocytes responsible for the recognition of antigens leading to the activation of immune response. In the early embryogenesis, CD3 is expressed in the cytoplasm of the prothymocytes and persists through all differentiation stages of T-lymphocytes until mature cells. CD3 is the most common used pan-T-cell marker expressed in the vast majority of T-cell lymphomas. CD3 labels also a subset of the NK lymphomas usually exhibiting a cytoplasmic staining pattern using CD3ε-specific antibody.

CD4		
Expression pattern: membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Mycosis fungoides, T-cell lymphomas	Histiocytic neoplasms, acute myeloid leukemia	Thymocytes, T-helper/T-inducer cells, macrophages, granulocytes, Langerhans cells, dendritic cells, hepatic sinusoidal cells
Positive control: appendix/tonsil		

Diagnostic Approach CD4 is a transmembrane glycoprotein and a member of the immunoglobulin family expressed on the surface of T-helper/T-inducer cells in addition to the majority of thymocytes and a subset of monocytes, macrophages, and dendritic cells. CD4 is a marker of lymphomas originated from these cells, which include the majority of peripheral T-cell lymphomas and cutaneous lymphomas, mainly mycosis fungoides.

Diagnostic Pitfalls In immunohistochemistry and flow cytometry, CD4 must be used in a panel including CD3 and CD8 and CD19. CD4 can be also positive in subtypes of acute myeloid leukemia and histiocytic neoplasms (Fig. 16.11).

CD7		
Expression pattern: membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
T-ALL and T-cell lymphomas	CML, immature myelomonocytic neoplasms, cholangiocarcinoma, pancreas carcinoma	Thymocytes, mature T cells and NK cells, pre-B cells, monocytes, early myeloid cells
Positive control: appendix/tonsil		

Diagnostic Approach CD7 is a membrane-bound protein and a member of the immunoglobulin family involved in T-cell/B-cell interaction. CD7 is expressed in early T-lymphocytes, thymocytes, NK cells, and subset of myeloid cells. The expression of CD7 persists in the majority of mature T-lymphocytes and T cell and NK lymphomas derived from these cells.

Diagnostic Pitfalls CD7 may be positive in a subset of AML and rarely in carcinomas such as pancreatic and bile duct carcinomas [36].

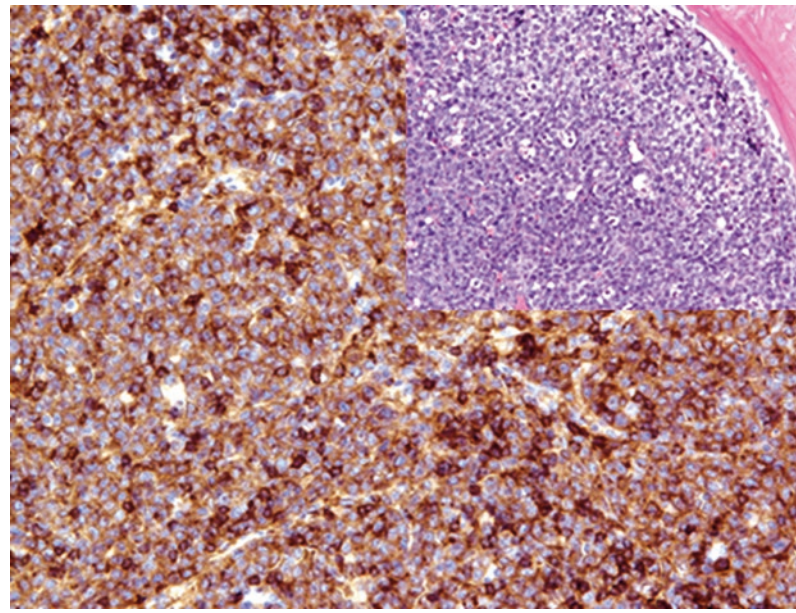


Fig. 16.11 Diffuse CD4 expression in myeloid blasts of AML (M5)

CD8		
Expression pattern: membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Subcutaneous panniculitis-like T-cell lymphoma	T-cell large granular lymphocytic leukemia, CLL, mantle cell lymphoma	Suppressor/ cytotoxic T cells and NK cells
Positive control: appendix/tonsil		

Diagnostic Approach CD8 is a transmembrane glycoprotein functioning as a co-receptor for the T-cell receptor, expressed in the suppressor/cytotoxic T-lymphocytes in addition to a subset of NK cells. CD8 is a marker of many types of T-/NK-cell lymphomas (Fig. 16.12).

Diagnostic Pitfalls CD8 is expressed in a small subset of B-cell lymphomas and generally should be a part of panel with CD3, CD4, and CD20 [36, 42]. The expansion of CD8-positive T-cell population is noted in lymph nodes-associated with acute infectious mononucleosis.

CD30: CD30 (Ki-1) is a transmembrane receptor participating in the regulation of cell transformation, antibody response, and apoptosis. CD30 is normally expressed in activated B, T, and NK cells. In addition to Hodgkin's lymphoma and some other lymphoma types, CD30 is a diagnostic marker for anaplastic large cell lymphoma (Fig. 16.13). CD30 is listed in details in the next chapter.

CD43		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
T-/NK-cell lymphomas	B-ALL, Burkitt lymphoma, mantle cell lymphoma, marginal zone lymphoma, granulocytic (myeloid) sarcoma, adenoid cystic carcinoma	Activated B cells, T cells, NK cells, plasma cells, granulocytes
Positive control: appendix/tonsil		

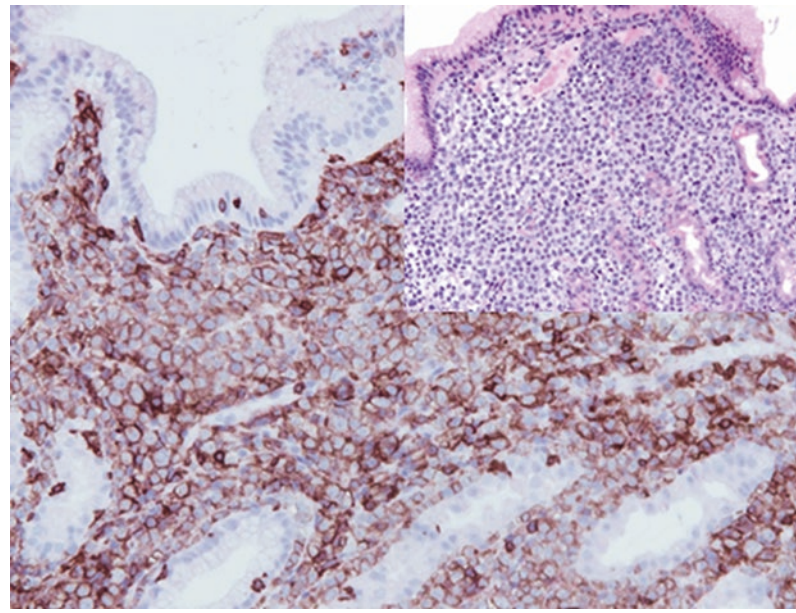


Fig. 16.12 Diffuse CD8 expression in enteropathy-type T-cell lymphoma (type II)

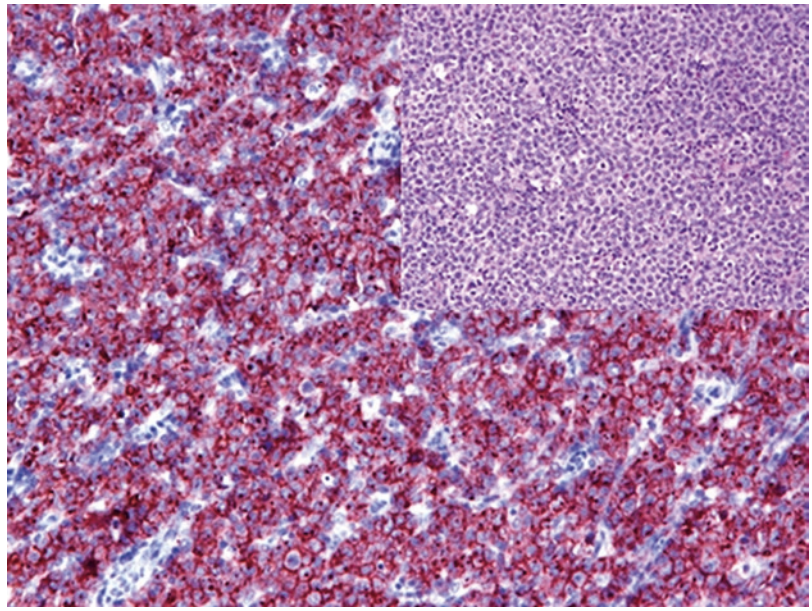


Fig. 16.13 Diffuse CD30 expression in anaplastic large cell lymphoma

Diagnostic Approach CD43 (also known as sialophorin) is expressed on the membrane and in the cytoplasm of the T-/NK-lymphocytes, cells of myeloid lineage, plasma cells, and tumors originating from these cells.

Noteworthy is the so-called "CD43 only pattern" characteristic for some rare tumors that express only CD43 in addition to vimentin. The CD43 only immunophenotype is characteristic for a subset of the following neoplasms, which to consider in the differential diagnosis:

- Myeloid sarcoma and subsets of AML
- Anaplastic large cell lymphoma and NK tumors
- Plasma cell neoplasms
- Langerhans cell histiocytosis

Diagnostic Pitfalls The expression of CD43 correlates with the expression of CD5 and is not restricted to T-cell lymphomas, but also found in many types of B-cell lymphomas such as chronic lymphocytic lymphoma (CLL and SLL), Burkitt lymphoma, mantle cell lymphoma, and nodal/extranodal marginal zone lymphoma [8]. Since normal B-lymphocytes lack the expression of CD43, CD43-positive B-lymphocytes are assumed to be neoplastic. Generally, CD43 must be used in a panel with other more specific lymphoma markers. Adenoid cystic carcinoma is one of the rare non-hematopoietic tumors that express CD43.

Anaplastic lymphoma kinase (ALK, CD246, p80)		
Expression pattern: cytoplasmic/nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Anaplastic large cell lymphoma, inflammatory myofibroblastic tumor	ALK-positive large cell lymphoma, malignant peripheral nerve sheath tumor, rhabdomyosarcoma, neuroblastoma, glioblastoma, Ewing's sarcoma/PNET, leiomyosarcoma, pulmonary non-small cell carcinoma	Glial cells, neurons, endothelial cells, T-lymphocytes
Positive control: anaplastic lymphoma/brain tissue/appendicular ganglion cells		

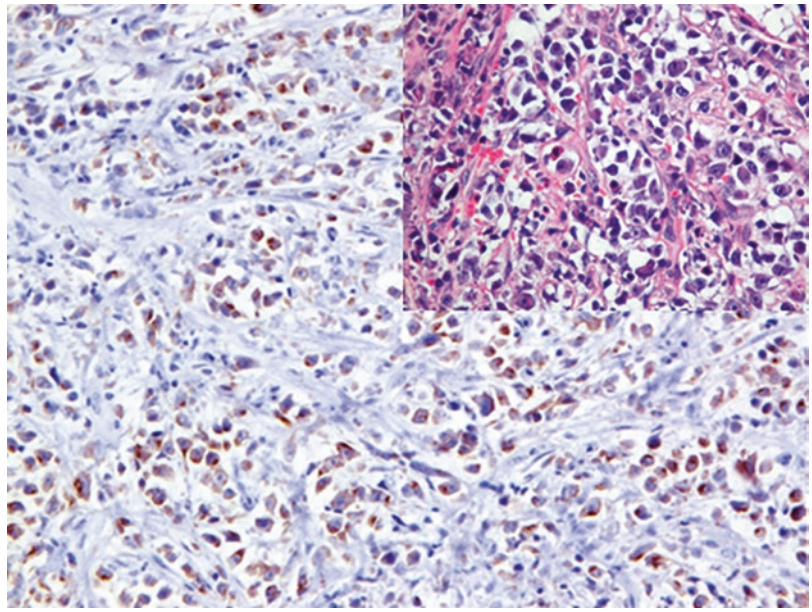


Fig. 16.14 Anaplastic large cell lymphoma with ALK -positive lymphoma cells

Diagnostic Approach Anaplastic lymphoma kinase (ALK) clustered as CD246 is a tyrosine kinase receptor expressed during the embryogenesis and remains positive in glial cells of CNS. ALK is negative in normal lymphoid tissue but expressed in some lymphoma types, namely, anaplastic large cell lymphoma, due to the activation of the ALK transcription caused by a potent promotor as a result of the t(2;5) translocation or another equivalent translocation [43]. ALK is also positive in the inflammatory myofibroblastic tumor also associated with the same translocation [44].

A strong ALK expression is also characteristic for the ALK-positive large B-cell lymphoma. This rare lymphoma type lacks the t(2;5) translocation and is consistently CD30 negative (Fig. 16.14).

T-Cell Leukemia Protein 1 (TCL-1): TCL-1 is an oncoprotein normally expressed in the early embryogenesis of lymphocytes. TCL-1 is overexpressed in the cells of T-cell prolymphocytic leukemia as a result of the t(14;14)(q11;q32) rearrangement specific for this leukemia type. Other T-cell lymphoma types usually lack the TCL-1 positivity. TCL-1 is expressed in different lymphoma types of B-cell origin including follicular lymphoma, Burkitt lymphoma, mantel cell lymphoma, CLL, hairy cell leukemia, and diffuse large cell lymphoma, whereas marginal zone lymphoma, CD30+ anaplastic lymphoma, and plasma cell tumors are constantly negative for TCL-1.

The expression of TCL-1 is also characteristic for testicular intratubular germ cell neoplasms and seminoma.

16.5 Markers and Immunoprofile of NK-Cell Neoplasms

Immunohistochemical Markers for NK-Cell Lymphoma CD2, CD3, CD56, cytotoxic molecules (TIA-1, granzyme B, perforin), and EMA [8, 10].

CD56 (N-CAM; NKH1)		
Expression pattern: membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
NK lymphomas, multiple myeloma, acute and chronic myeloid leukemia, neuro-endocrine tumors (small cell carcinoma, carcinoid and Merkel cell carcinoma), pheochromo-cytoma, neuroblastoma, ovarian sex cord-stromal tumors	Synovial sarcoma, embryonal and alveolar rhabdomyo-sarcoma, angiosarcoma, solitary fibrous tumor, chordoma, epithelioid sarcoma, Ewing's sarcoma/PNET, medulloblastoma, schwannoma and neurogenic sarcoma, astrocytomas, ependymoma, meningioma, retinoblastoma, paraganglioma, melanoma, mesothelioma, bile duct adenoma	NK cells, activated T cells, cerebellum and brain cortex, neuro-muscular junctions, neurons, intestinal ganglion cells, neuroendo-crine tissue, thyroid follicular, epithelium, hepatocytes, epithelium of renal tubules, osteoblasts
Positive control: brain tissue/intestinal ganglion cells		

Diagnostic Approach CD56 (neural cell adhesion molecule, N-CAM) is a transmembrane adhesion molecule and a member of the Ig superfamily involved in the development of neural cells and differentiation of neural tissue. Normally, CD56 is expressed on the membrane of neuroectodermal cells, NK cells, activated T cells, myoblasts, and skeletal muscle. CD56 is an important marker for NK-cell lymphoma and also a very helpful marker for the diagnosis of pulmonary and extrapulmonary small cell carcinomas. CD56 is also a sensitive but less specific marker for ovarian sex cord-stromal tumors (see related section).

Diagnostic Pitfalls CD56 is an unspecific marker with a very wide expression spectrum. It is found in a small subset of CD4- and CD8-positive T cells and plasma cells. CD56 is also expressed on the cells of multiple myeloma, whereas CD56-negative myeloma is found to have a poor prognosis. CD56 may be also expressed on other tumors with similar morphology such as embryonal rhabdomyosarcoma, neuroblastoma, malignant melanoma neurogenic sarcoma, and synovial sarcoma which to consider in the differential diagnosis [36, 45].

Cytotoxic Molecules (Granzyme B, Perforin, and TIA-1) Antibodies to the cytotoxic molecules are important markers for the diagnosis of T cell and NK lymphomas. Perforin granzyme B and TIA-1 are the most popular cytotoxic molecules used in routine immunohistochemistry.

Perforin: Perforin is a cytolytic pore-forming protein found in the granules of cytotoxic T-lymphocytes. It is able to perforate a pore in the membrane of targeted cells.

Granzyme B: Granzyme B is a serine protease stored in specialized lytic granules of cytotoxic T-lymphocytes and natural killer cells together with perforin. Granzyme B seems to enter the target cell through a perforin-caused transmembrane pore to induce DNA fragmentation initiating apoptosis of targeted cells.

TIA-1: TIA-1 (also known as nucleolysin) is a cytotoxic granule-associated protein expressed in natural killer cells and cytotoxic T-lymphocytes. TIA-1 has a nucleolytic activity against targeted cells initiating apoptosis. TIA-1 is also used to label tumor-infiltrating lymphocytes.

Immunoprofile of T-cell and NK-cell neoplasms				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Precursor T-cell lymphoblastic leukemia/lymphoma	*TdT*, CD7, *CD2* Proliferation index (Ki-67): 40–80%	CD3 (cytoplasmic), CD1a, *CD10*, CD4, CD5, CD8, CD33, CD34, CD99, Fli-1, LMO2	CD13, CD15	PAX-5, CD19, MPO
T-cell prolymphocytic leukemia	CD2, CD5, CD7, CD43, *TCL-1*	CD3, CD4	CD8	CD1a, CD10, CD25, CD28, CD56, TdT
T-cell large granular lymphocytic leukemia	CD2, *CD3*, CD5, CD8 (in the common type), CD16, cytotoxic molecules (TIA-1, perforin, granzyme B)	CD5, CD4, CD57 (in the common and NK-cell types)	CD56, CD56 (+ in NK-cell type), CD4 (+ in rare types)	CD7, CD10, CD25
Adult T-cell lymphoma (HTLV1+)	CD2, *CD3*, *CD4*, CD5, CD25			CD7, CD8
Extranodal NK-/T-cell lymphoma, nasal type	*CD2*, *CD3ε*, CD43, *CD56*, *cytotoxic molecules* (TIA-1, perforin, granzyme B), EBV	CD7		*CD3*, CD4, CD5, CD8, TdT
Peripheral T-cell lymphoma (NOS)	CD2, *CD3*, CD4, CD5	CD7	CD25, CD30, CD134	*ALK*, CD8, CD15[a], CD19, CD20[b]
Angioimmunoblastic T-cell lymphoma	CD2, *CD3*, CD4, CD5, CD7, *CD10*, CD28, *PD-1 (CD279)*, *bcl-6*, *CXCL13*[c] Expanded CD21- and CD23-positive meshwork of follicular dendritic cells		CD8, CD10, CD30 EBV+ B-cell blasts	CD15
Follicular T-cell lymphoma	CD3, CD4, *CD10*, *PD1 (CD279)*, *bcl-6*, *CXCL13*[c]			
Nodal peripheral T-cell lymphoma TFH phenotype	CD3, CD4, *CD10*, *bcl-6*, *PD-1 (CD279)*, *CXCL13*[c]			
Mycosis fungoides/ Sézary syndrome	CD2, *CD3*, CD5, CD4, CD45RO Proliferation index (Ki-67): <5%		CD7	CD8, CD25
Enteropathy-associated T-cell lymphoma	CD2, *CD3*, CD7, CD103	CD30, cytotoxic molecules (TIA-1, perforin, granzyme B)	CD8	CD4, CD5, CD56
Monomorphic epitheliotropic intestinal T-cell lymphoma	CD2, CD3, *CD8*, *CD56*			CD4
Indolent T-cell lymphoproliferative disorder of the GI tract	CD2, CD3, CD8	CD5, CD7, TIA-1		CD4, CD30, CD56
Hepatosplenic γδ T-cell lymphoma	CD2, *CD3*, CD43, CD45RO, TIA-1	CD7, CD56	CD8, CD16, CD5, CD11c, CD11b	CD4, CD5, perforin, granzyme
Anaplastic large cell lymphoma, ALK positive	*ALK*, *CD30*, clusterin[d], CD43, *cytotoxic molecules* (TIA-1, perforin, granzyme B)	CD2, CD4, CD25, CD45, EMA, galectin-3	CD3, CD5, CD7, CD15, fascin, bcl-6	CD8, CD20, CD28, PAX-5

Immunoprofile of T-cell and NK-cell neoplasms				
Anaplastic large cell lymphoma, ALK negative	*CD30*, clusterin[d], CD43, *cytotoxic molecules* (TIA-1, perforin, granzyme B)	CD2, CD4, CD25, CD45, EMA, galectin-3	CD3, CD5, CD7, CD15, fascin, bcl-6	*ALK*, CD8, CD20, CD28, PAX-5
Primary cutaneous anaplastic CD30-positive T-cell lymphoproliferative disorders – Lymphomatoid papulosis – Primary cutaneous anaplastic large cell lymphoma	*CD30*, CD4	CD45, CD25, cytotoxic molecules (TIA-1, perforin, granzyme B)	CD2, CD3, CD5, CD7	Clusterin, CD8, CD15, EMA, CD246 (ALK, p80), PAX-5
Subcutaneous (panniculitis-like) T-cell lymphoma	CD2, CD3, CD8, CD43, CD45, cytotoxic molecules (TIA-1, perforin, granzyme B)	CD5, CD7, CD25	CD30	CD4
Primary cutaneous gamma delta T-cell lymphoma	CD2, CD3, CD7, CD56, cytotoxic molecules (TIA-1, perforin, granzyme B)		CD8	CD4, CD5
Primary cutaneous CD8-positive aggressive epidermotropic cytotoxic T-cell lymphoma	CD3, CD8, cytotoxic molecules (TIA-1, perforin, granzyme B)	CD7	CD2	CD4, CD5
Primary cutaneous acral CD8-positive lymphoma	CD8	CD3, CD5, CD7, cytotoxic molecules (TIA-1, perforin, granzyme B)	CD4	CD30, CD56, EBV
Primary cutaneous CD4-positive small/medium T-cell lymphoproliferative disorder	CD3, CD4			CD8, CD30
Hydroa vacciniforme-like lymphoproliferative disorder	CD8, EBV	Cytotoxic molecules (TIA-1, perforin, granzyme B)		
Lymphomatoid papulosis	CD4, CD30[e]	CD2, CD3		CD8, ALK
Aggressive NK-cell leukemia	CD2, CD3ε, CD16, CD30 (only in large transformed cells), CD56 cytotoxic molecules (TIA-1, granzyme B)	CD8, EMA	CD7, CD16	CD3, CD4, CD5, CD8, CD57
Breast implant-associated anaplastic large cell lymphoma	CD2, CD4, CD5, CD30			CD10, ALK

[a]CD15 may be expressed in large cells of peripheral T-cell lymphoma
[b]B-cell antigens may be expressed in very rare cases (<5%) of peripheral T-cell lymphoma
[c]Chemokine (C-X-C motif) ligand 13 [46]
[d]Golgi staining pattern
[e]CD30 positive only in RS-like cells of type A lesion

16.6 Markers and Immunoprofile of Hodgkin's Lymphoma

16.6.1 Diagnostic Antibody Panel for Classical Hodgkin's Lymphoma

CD15, CD30, MUM-1, IMP3, fascin, and J-chain [47–49].

16.6.2 Diagnostic Antibody Panel for Nodular Lymphocyte-Predominant Hodgkin's Lymphoma

CD19, CD20, PAX-5, J-chain, BOB.1, Oct-2, and EMA [47].

CD15		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Hodgkin's lymphoma (Reed-Sternberg cells), myeloid leukemia	Adenocarcinoma, sweat and sebaceous gland tumors, thymoma, ovarian carcinoma, renal cell carcinoma, thyroid carcinoma, peripheral T-cell lymphoma, ALCL	Granulocytes and precursors (neutrophils and eosinophils), monocytes, activated B and T cells, proximal tubules of kidney, intestinal Paneth cells
Positive control: appendix		

Diagnostic Approach CD15 (X hapten) is a cell surface glycoprotein involved in the regulation of neutrophil functions. CD15 is frequently used as a marker for normal and neoplastic myeloid cells and monocytes. In combination with CD30, CD15 is commonly used as a marker for Reed-Sternberg cells in classical Hodgkin's lymphoma (Fig. 16.15). CD15 is also expressed on different carcinoma types but constantly negative in mesothelioma. Carcinomas positive for CD15 reported to have worse prognosis.

Diagnostic Pitfalls In view of the fact that CD15 is expressed in different hematopoietic

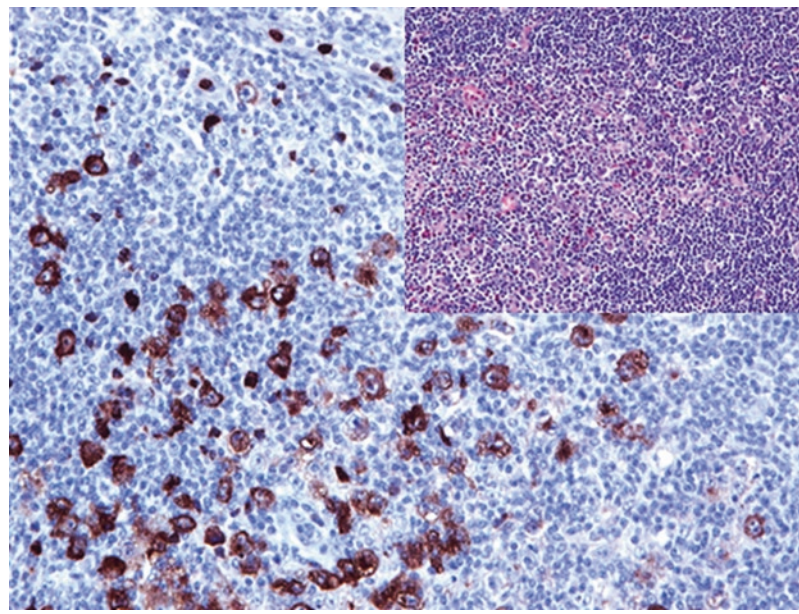


Fig. 16.15 CD15-positive Hodgkin cells in classical Hodgkin's lymphoma

and non-hematopoietic neoplasms including adenocarcinomas, it is important to keep in mind possible differential diagnoses and to support the final diagnosis by other more specific antibodies.

CD30		
Expression pattern: membranous/cytoplasmic paranuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Anaplastic large cell lymphoma, Reed-Sternberg cells in classic Hodgkin's lymphoma, primary mediastinal large B-cell lymphoma	Embryonal carcinoma, systemic mastocytosis, NK-/T-cell lymphoma, nasopharyngeal carcinoma, pancreatic adenocarcinoma melanoma, angiosarcoma, mesothelioma	Granulocytes, monocytes, activated B, T, and NK cells, small subset of plasma cells, exocrine pancreas glands, Purkinje cells of the cerebellum, cortical neurons, decidual cells
Positive control: embryonal carcinoma		

Diagnostic Approach CD30 (Ki-1)—also known as lymphocyte activation antigen—is a transmembrane glycoprotein receptor and member of the tumor necrosis factor superfamily participating in the regulation of cell transformation, antibody response, and apoptosis. CD30 is normally expressed in activated B, T, and NK cells. One of the major utilities of CD30 in routine immunohistochemistry is to highlight Hodgkin cells and multinucleated Reed-Sternberg cells in different types of classical Hodgkin's lymphoma (Fig. 16.16). CD30 is also a diagnostic marker for anaplastic large cell lymphoma and primary mediastinal large B-cell lymphoma as well as high malignant types of systemic mastocytosis [50].

The expression of CD30 is not restricted to lymphoid tissue and lymphoid neoplasms but also found in other different epithelial and mesenchymal tumors [51]. CD30 is a useful marker for the diagnosis of embryonal carcinoma. CD30 labels other carcinoma types such as nasopharyngeal carcinoma and pancreatic adenocarcinoma. In mesenchymal tumors, CD30 labels about 30% of angiosarcoma.

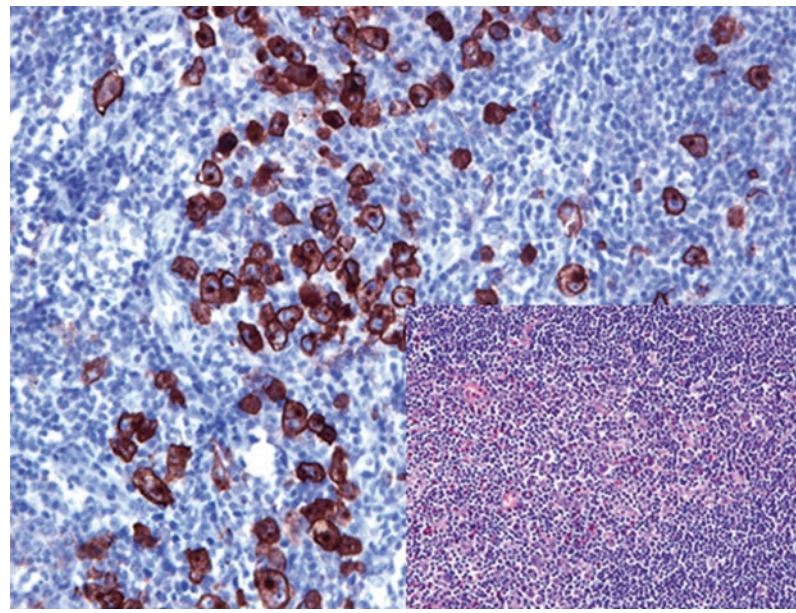


Fig. 16.16 CD30 expression in Hodgkin cells of classical Hodgkin's lymphoma

Diagnostic Pitfalls CD30-positive cells may be found in different T- and B-lymphoma types. CD30 stains also activated T and B cells in reactive lymph nodes, spleen, thymus, and tonsil; consequently, not all CD30-positive cells are Hodgkin cells.

Fascin (actin-bundling protein; p55)		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Reed-Sternberg cells in classic Hodgkin's lymphoma, anaplastic large cell lymphoma, follicular and interdigitating dendritic cell tumors	Adenocarcinomas of the breast, colon, biliary tract, pancreas, lung, ovary, and skin; papillary transitional cell carcinoma of the bladder; diffuse large B-cell lymphoma; synovial sarcoma	Interdigitating and follicular dendritic cells, endothelial cells, EBV infected B-lymphocytes
Positive control: lymph node		

Diagnostic Approach Fascin is an actin-binding protein involved in cell adhesion and motility. It is normally expressed in interdigitating and follicular dendritic cells and variably in endothelial cells but constantly negative in lymphocytes, plasma cells, and myeloid cells. Fascin is a good marker for Reed-Sternberg cells in classical Hodgkin's lymphoma. It is also expressed on the membrane of anaplastic large cell lymphoma and subtypes of diffuse large B-cell lymphoma.

Fascin is constantly negative in normal epithelium but positive in many types of transformed or neoplastic epithelium [52]. This phenomenon may be used for the differentiation between hyperplastic and neoplastic urothelium.

Diagnostic Pitfalls Because of the wide expression spectrum of fascin, many differential diagnoses must be considered in the interpretation of the fascin immunostain. In addition to Reed-Sternberg cells, fascin-positive cells in lymph nodes maybe activated B-lymphocytes, cells of diffuse large B-cell lymphoma, or even disseminated cells of metastatic adenocarcinoma.

Insulin-Like Growth Factor II mRNA-Binding Protein 3 (IMP3): IMP3 is a cytoplasmic protein mediating RNA trafficking and cell growth, highly expressed in the early embryogenesis. Benign adult tissue usually lacks the expression of IMP3 with the exception of fibroblasts, subset of lymphocytes (mainly germinal center lymphocytes), ovarian and testicular tissue, placenta, and brain. IMP3 is expressed in different premalignant and malignant lesions. IMP3 is positive in different carcinoma types including pulmonary carcinoma, esophageal and pancreatic carcinoma, cervical and endometrial carcinoma, transitional cell carcinoma, renal cell carcinoma, and neuroendocrine carcinoma.

In routine immunohistochemistry, IMP3 is used to discriminate between malignant and reactive proliferative lesions. It is a useful marker to discriminate between pancreatic adenocarcinoma positive for IMP3 and inflammatory pancreas lesions usually negative for IMP3. IMP3 selectively stains Hodgkin and Reed-Sternberg cells in both classical Hodgkin's lymphoma and nodular lymphocyte-predominant Hodgkin's lymphoma (Figs. 16.17 and 16.18).

Diagnostic Pitfalls IMP3 may be positive in other extrafollicular blasts and must be used with other more specific markers to label Hodgkin cells.

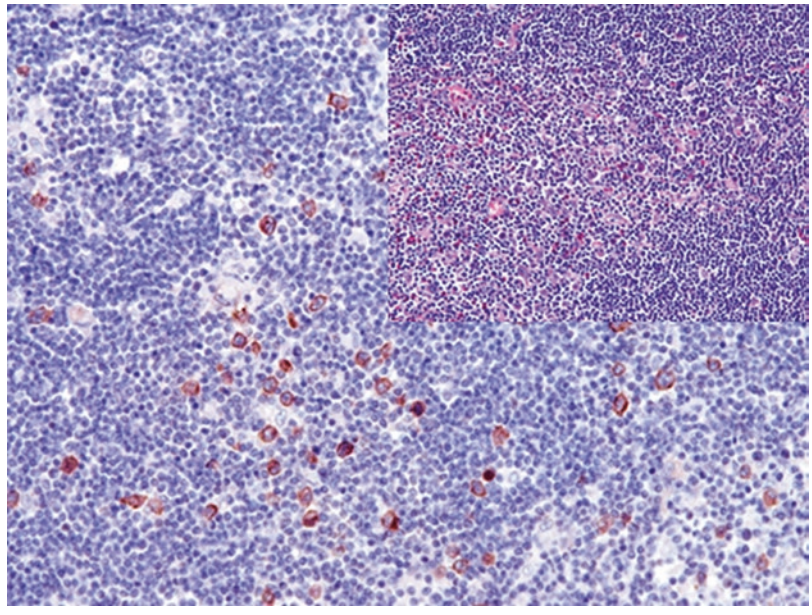


Fig. 16.17 IMP3 selectively labels in Hodgkin cells in classical Hodgkin's lymphoma

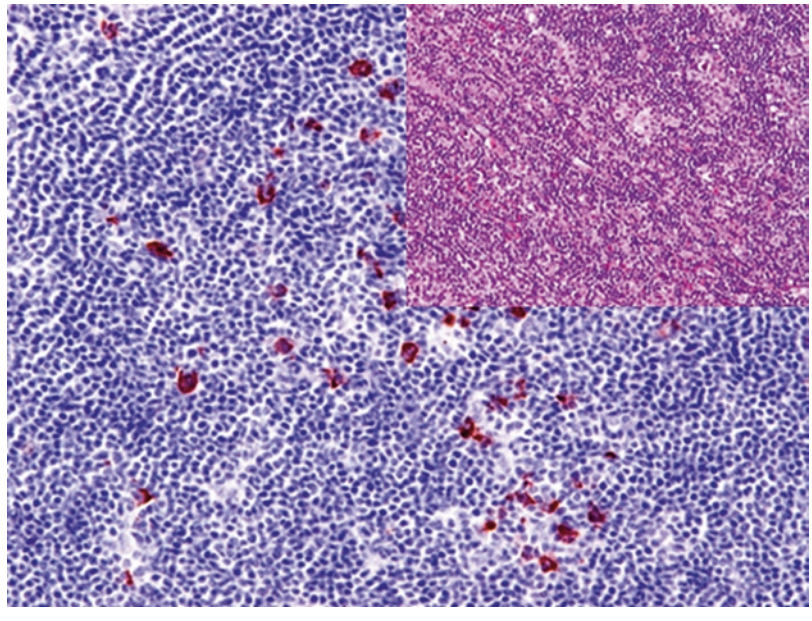


Fig. 16.18 IMP3 selectively labels in Hodgkin cells in nodular lymphocyte-predominant Hodgkin's lymphoma

Immunoprofile of Hodgkin's lymphoma				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Classical Hodgkin's lymphoma (Hodgkin and Reed-Sternberg cells[a]) in classical subtypes – Nodular sclerosis – Lymphocyte rich classic – Mixed cellularity – Lymphocyte depleted – Unclassifiable	*CD30*, IMP3, fascin	*CD15*, CD83, *PAX-5*, *MUM-1*, CD138, CD200, HLA-DR, EBV (LMP1)	CD20, CD79	CD45, Oct-2, BOB.1, J-chain, PU.1, EMA, bcl-6, CD22, ALK
Nodular lymphocyte-predominant Hodgkin's lymphoma (lymphocytic/histiocytic cells[a]) or popcorn cells (L&H cells)	CD19, CD20, CD22, CD45, CD86, PU.1, *Oct-2*, PAX-5, BOB.1, *J-chain*, IMP3	CD75, CD79a, CD40, bcl-6, *EMA*		CD10, CD15, fascin, MUM-1, CD30, CD138, CD200, ALK (p80), EBV

[a]Usually negative IgH and TCR gene rearrangements

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Markers and Immunoprofile of Myeloid Neoplasm

17

Contents

Diagnostic Antibody Panel for Myeloid Neoplasm CD13, CD14, CD15, CD33, and MPO [1]

Myeloperoxidase (MPO)		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
AML	Granulocytic sarcoma	Myeloid cells, monocytes
Positive control: bone marrow		

Diagnostic Approach Myeloperoxidase (MPO) is a heme protein and one of the main lysosomal enzymes in myeloid cells released during degranulation. MPO positivity is diagnostic for neoplasia of myeloid origin. MPO is constantly absent in normal and neoplastic lymphoid tissue.

CD15: CD15 is a further important marker for the myeloid lineage listed in details in the previous chapter. CD15 is expressed on the majority of granulocytes and monocytes and relates neoplasms.

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CD33		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
AML (M0-5), CML	B- and T-ALL, ALK+ anaplastic large cell lymphoma	Monocytes, premyelocytes, myeloid blasts, dendritic cells, mast cells
Positive control		

Diagnostic Approach CD33 is a transmembrane glycoprotein involved in cell-to-cell adhesion. CD33 is expressed in the early myeloid progenitor cells after CD34 but absent in stem cells [2]. The expression of CD33 persists during myelomonocytic differentiation and is weakly detectable on granulocytes, monocytes, mast cells, and dendritic cells. CD33 is an important marker for most types of acute myeloid leukemia (M0–M5) (Fig. 17.1), chronic myeloid leukemia (CML), and granulocytic sarcoma in addition to chronic myelomonocytic leukemia.

Diagnostic Pitfalls CD33 is a specific marker for myeloid cells and related leukemia; nevertheless, it may be detectable in a subset of nonmyeloid neoplasms such as ALK-positive anaplastic large cell lymphoma, Burkett's lymphoma, T- and B-ALL, and plasma cell neoplasia.

(Aminopeptidase N) CD13: CD13: is a transmembrane metalloproteases involved in cell surface antigen presentation. Similar to CD33, CD13 is also a myeloid-associated antigen expressed on myeloid cells and myeloid precursors in addition to other nonmyeloid cells such as fibroblasts, osteoclasts, endothelium, and various epithelial cells including cells of renal proximal tubules, bile canaliculi, and brush surface of enterocytes. Glands of acinar adenocarcinoma of the prostate often show a loss of CD13 expression in comparison to adjacent benign glands, which may be diagnostically utilized. CD13 is a marker for acute and chronic myeloid leukemia. CD13 is also detectable in a subset of ALL.

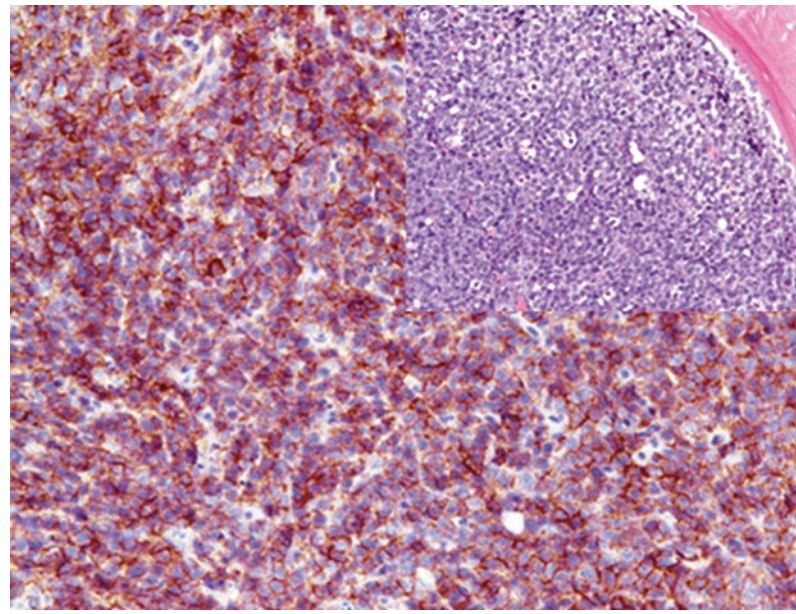

Fig. 17.1 CD33 expression in myeloid blasts of M5 AML

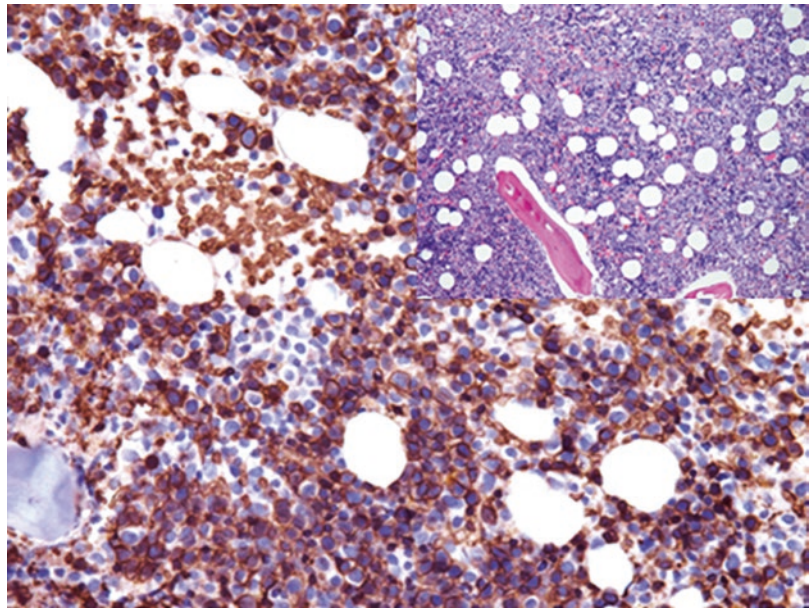

Fig. 17.2 Glycophorin expression in neoplastic erythroblasts of M6 AML

Glycophorins: Glycophorins are a group of sialoglycoproteins found in the membrane of erythrocytes. Glycophorin A and B are the main members of this group, clustered as CD235a and CD235b, and carry the antigenic determinants of the MN and Ss blood groups. Both glycophorins are found in erythroid precursors including erythroblasts and considered as specific markers for normal and neoplastic erythropoiesis including acute erythroid leukemia (M6) (Fig. 17.2). Other leukemia types lack the expression of glycophorins.

The following table includes the immunoprofile of myeloid leukemia of NOS type. The classification of those leukemia types with recurrent genetic abnormalities or with myelodysplastic-related changes depends on the molecular detection of associated genetic abnormalities.

Immunoprofile of myeloid neoplasm				
Tumor type	+ in >90% (+)	+ in 50–90% (±)	+ in 10–50% (∓)	+ in <10% (−)
A. Acute myeloid leukemia (NOS)				
Myeloblastic, minimally differentiated leukemia (M0)	*CD34*, CD13, CD117	CD33, CD11b, CD43, MPO, HLA-DR	TdT, CD2, CD7, CD19, CD65	CD14, CD15
Myeloblastic leukemia, without maturation (M1)	CD13, HLA-DR, *MPO*	CD15, CD19, CD33, CD34, CD43, CDw65, CD117	CD56	
Myeloblastic leukemia, with maturation (M2)	CD13, CD15, CD33, MPO, HLA-DR, CAE	CD43, CD117	CD56, CD34	

Immunoprofile of myeloid neoplasm				
Myelocytic leukemia (M3)	CD13, CD33, MPO, CAE	CD43, CD64	CD15, CD65, CD117, CD56	CD34, HLA-DR
Myelomonocytic leukemia (M4)	CD11b, CD13, CD33, CD64, CDw65, CD68, MPO, HLA-DR	CD4, CD14, CD15, CD36, CD43, CD117	CD7, CD34, CD56	
Monoblastic/monocytic leukemia (M5a/M5b)	CD4, CD15, CD33, CD56, CD64, CD68, HLA-DR, CAE	CD11c, CD13, CD14, CDw65	MPO	
Erythroblastic leukemia (M6)	Glycophorin, hemoglobin A	HLA-DR, CD33	CD71, CD117	CAE, CD13, MPO
Megakaryoblastic leukemia (M7)	CD61, CD41, CD42, spectrin	CD33	CDw65, CD13, HLA-DR	CD15, MPO, CAE
B. Chronic myeloid neoplasm				
Chronic myeloid leukemia	CD11b, CD11c, CD14, CD15		CD117, TdT	
Granulocytic sarcoma (myeloid sarcoma)[a]	CD43, vimentin, lysozyme	CD13, CD14, CD15, CD33, HLA-DR, MPO	CD34, CD68, CD117, CD5, CD7	CD3, CD20

[a]Myeloid sarcoma MPN/CML type

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Markers and Immunoprofile of Mastocytosis

18

Contents

Diagnostic Antibody Panel for Mast Cell Tumors Mast cell tryptase, CD117, CD2, CD25, CD30, and CD33 [1–6]

Mast cell tryptase		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Mast cell tumors		Mast cells
Positive control: appendix		

Diagnostic Approach Tryptase is a neutral serine protease and a member of the trypsin-like proteinases. It is one of the mediators of inflammation found in mast cells and basophiles and released in the extracellular matrix in response to activation. Antibodies to tryptase are used as specific markers for mast cells but cannot discriminate between normal and neoplastic mast cells. The aberrant tryptase expression is described in rare types of acute myeloid leukemia.

CD25: CD25 is a subunit of the interleukin-2 receptor, involved in the differentiation and activation of T lymphocytes, and is normally expressed in a subpopulation of T lymphocytes in addition to myeloid precursors and oligodendrocytes. It is also expressed in viral transformed T and B lymphocytes. CD25 labels the majority of T-cell lymphomas as well as hairy cell leukemia.

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In mast cell disorders, the expression of CD25 is restricted to neoplastic mast cells and is usually negative in reactive mast cells [6].

CD2: CD2 was listed in a previous chapter. CD2 is normally expressed in different stages of T-cell development and T-cell lymphomas but negative in B lymphocytes, B-cell lymphomas, and normal mast cells, whereas the expression of CD2 in mast cells indicates a neoplastic nature of these cells [3].

Immunoprofile of mastocytosis

Tumor type	+ in >90% (+)	+ in 50–90% (±)	+ in 10–50% (∓)	+ in <10% (−)
Mastocytosis: 1. Cutaneous mastocytosis 2. Systemic mastocytosis: a. Indolent systemic mastocytosis b. Smoldering systemic mastocytosis c. Systemic mastocytosis with associated hematological neoplasm d. Aggressive systemic mastocytosis e. Mast cell leukemia 3. Mast cell sarcoma 4. Extracutaneous mastocytoma	*Tryptase*, *CD117*, CD45, CD33, *CD25*[a], CD68	*CD2*[b], CD30[c], chymase	Calretinin	CD3, CD14, CD15, CD 20, MPO

[a]CD25 is usually negative in normal mast cells
[b]CD2 is usually negative in normal and reactive mast cells
[c]CD30 usually labels aggressive types of mastocytosis [7, 8]

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Markers and Immunoprofile of Histiocytic and Dendritic Cell Tumors

19

Contents

Diagnostic Antibody Panel for Histiocytic and Dendritic Cell Tumors CD1a, CD21, CD23, CD35, CD68, CD207, fascin, podoplanin, and S100 [1, 2]

CD1a		
Expression pattern: membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Langerhans cell histiocytosis	Myeloid leukemia, mycosis fungoides, cutaneous T-cell lymphomas, T-ALL	Cortical thymocytes, Langerhans cells, immature dendritic cells
Positive control: skin		

Diagnostic Approach CD1a is one of the four isoforms of CD1 (a, b, c, d) expressed on the antigen-presenting cells. CD1a is found on the surface of cortical thymocytes and dendritic cells in addition to Langerhans cells. CD1a is a specific marker for normal and neoplastic Langerhans cells but constantly negative in histiocytic, follicular dendritic, and interdigitating cell tumors. CD1a is also expressed in some types of T-cell lymphoma, chiefly cutaneous T-cell lymphoma.

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CD21		
Expression pattern: membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Follicular dendritic cell sarcoma	Hairy cell leukemia, mantle cell and marginal zone lymphoma	Follicular dendritic cells, mature B-cells, immature thymocytes, skin, pharyngeal and cervical epithelial cells, renal tubule, adrenal cortex, hepatocytes, capillary endothelial cells
Positive control: lymph node		

Diagnostic Approach CD21 is a C3d receptor on the membrane of the B lymphocytes that also acts as a receptor for EBV. CD21 is also expressed by follicular dendritic cells but constantly negative in monocytes, granulocytes, and T lymphocytes. CD21 is positive in a subset of B-cell lymphoma, namely, chronic lymphocytic lymphoma, and weakly in mantle cell lymphoma and follicular lymphoma. CD21 is rarely expressed in a small subset of T-cell lymphomas [3–6]. CD21, CD35, and podoplanin are very helpful markers for follicular dendritic cell tumors (sarcoma). CD21 is usually negative in histiocytic, Langerhans, and interdigitating cell tumors. The expression of CD21 in pharyngeal and cervical epithelial cells must be considered in the interpretation of the immunostain.

CD68		
Expression pattern: cytoplasmic/membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Histiocytic tumors, dendritic cell tumors, AML (FAB-M4/M5), giant cell tumors	Fibrous histiocytoma, nodular fasciitis, villonodular synovitis, granular cell tumor, inflammatory myofibroblastic tumor, mast cell disease, hairy cell leukemia	Macrophage, monocytes, osteoclasts, Kupffer cells, mast cells, synovial cells, microglia, dendritic cells, fibroblasts, Langerhans cells, myeloid cells, CD34+ progenitor cells, neutrophils, B and T cells
Positive control: appendix		

Diagnostic Approach CD68 is a glycoprotein found in the lysosomes and endosomes involved in the regulation of phagocytic activity of macrophages. CD68 is a widely used marker for histiocytes and histiocytic tumors but lacks specificity for these cells [7].

Diagnostic Pitfalls CD68 has a wide expression range and may be found in different hematologic diseases of B-cell, T-cell, NK, and myeloid lineage.

CD163		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Histiocytic sarcoma	Langerhans cell histiocytosis, AML, chronic myelomonocytic leukemia, myeloid sarcoma	Monocytes, macrophages
Positive control: skin		

Diagnostic Approach CD163 hemoglobin scavenger receptor (also known as Ber-Mac3) is a type 1 membrane glycoprotein expressed by tissue macrophages, monocytes, and their progenitor cells. CD163 is more specific than CD68 [8, 9].

CD207 (langerin)		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Tumors of Langerhans cell type		Langerhans cells, dermal and mucosal dendritic cells
Positive control: skin		

Diagnostic Approach CD207 (langerin) is a type II transmembrane cell glycoprotein involved in the formation of Birbeck granules in the cytoplasm of Langerhans cells [10]. CD207 is a specific marker for Langerhans cells and tumors arising from these cells including Langerhans cell histiocytosis (histiocytosis X) and Langerhans cell sarcoma.

Diagnostic Pitfalls CD207 is also expressed in subsets of dermal and mucosal dendritic cells and CD8-positive splenic dendritic cells.

Fascin: Fascin is an actin-binding protein listed previously as a marker for Reed-Sternberg cells. Fascin is strongly expressed in normal and neoplastic interdigitating and follicular dendritic cells [4].

Immunoprofile of histiocytic and dendritic cell tumors

Tumor type	+ in >90% (+)	+ in 50–90% (±)	+ in 10–50% (∓)	+ in <10% (−)
Histiocytic sarcoma	*CD68*, HLA-DR	CD163, CD11c, lysozyme, CD14, CD45	S100, CD4, CD15	CD1a, CD3, CD20, CD21, CD23, CD33, CD34, CD35, CD30, CD207, fascin, MPO
Tumors of Langerhans cell type: - Langerhans cell histiocytosis (histiocytosis X) - Langerhans cell sarcoma - Erdheim-Chester disease	*S100*, *CD1a*, *CD207*, CD86 Proliferation index (Ki-67): Langerhans cell histiocytosis: 2–25% (median 10%) Langerhans cell sarcoma: 10–60% (median 22%)	CD4, CD11c, CD163, CD45RB, HLA-DR, PLAP, CD68	CD45, $BRAF^{v600E}$	CD2, CD3, CD20, CD21, CD30, CD34, CD35, MPO, PAX-5, EMA
Follicular dendritic cell tumor/sarcoma	*CD21*, *CD23*, CD35, KiM4p, CXCL13, podoplanin, fascin, vimentin Proliferation index (Ki-67): 1–25% (median 13%)	Desmoplakin, EGFR, HLA-DR, S100, EMA	CD20, CD45, CD68, actin	CD1a, CD2, CD3, D30, CD34, CD35, CD79a, CD163, MPO, Pan-CK
Interdigitating dendritic cell tumor/sarcoma	*S100*, *CD4*, CD45RB, *fascin*, vimentin Proliferation index (Ki-67): 10–20% (median 11%)	CD68	CD45	CD1a, CD2, CD3, CD20, CD21, CD23, CD30, CD34, CD35, MPO, EMA, Pan-CK
Indeterminate dendritic cell tumor	CD1a, CD68, S100			CD21, CD23, CD30, CD35, CD207
Fibroblastic reticular cell tumor	Fascin, actin	CD31, tenascin-C	CD21, Pan-CK, EMA	CD1a, CD30, CD34, CD35, D2 40

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Markers and Immunoprofile of Skin Tumors

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Contents

Diagnostic Antibody Panel for Keratinocytic (Epidermal) Tumors: Cytokeratin profile, EMA, epithelial specific antigen (Ber-EP4), p16, p53, HPV, and Ki-67 (Fig. 20.1).

Diagnostic Antibody Panel for Sweat Gland Tumors (Apocrine and Eccrine Differentiation): Cytokeratin profile, p63, CEA, EMA, CD15, GATA-3, S100, ER, PgR, androgen receptors, and GCFP-15

Analogous to normal sweat glands, eccrine and apocrine gland tumors have the same cell components. Generally, they are composed of luminal cells and basal-type/myoepithelial cells but with disturbed distribution and morphology, which correlates with the differentiation grade of the tumor. The immunohistochemical expression profile of these tumors shows a mixture of both cell types with variable distribution and expression intensity in addition to the expression of CEA, steroid hormone receptors, and frequently GATA-3 [1–4]. Additionally, many sweat gland tumors have the same morphology and immunoprofile as salivary gland tumors such as adenoid cystic carcinoma.

Diagnostic Antibody Panel for Hair Follicle (Pilar) Tumors: Cytokeratin profile, p63, EMA, HKN, HHK, and Ber-EP4

The hair-specific keratins including the hair keratins (HKN) 5, 6, and 7 in addition to human hair keratin (HHK) are specific markers for pilar tumors.

M.S.A. Tuffaha et al., *Immunohistochemistry in Tumor Diagnostics*,
DOI 10.1007/978-3-319-53577-7_20

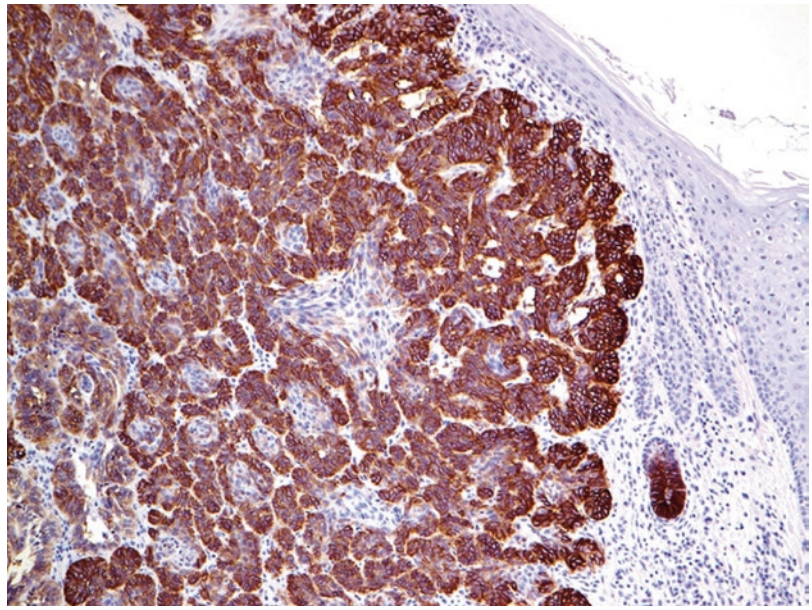

Fig. 20.1 Basal cell carcinoma with strong EPCAM (clone Ber-EP4) expression. Note negative stain of epidermal cells

Among the different cytokeratins, CK15 is the most specific cytokeratin for hair follicles, nails, and hair follicle tumors. CK15 is a marker of epidermal stem cells, and the expression of CK15 in stratified epithelium is restricted to the basal cell layer. Sebaceous tumors usually lack the expression of CK15.

Diagnostic Antibody Panel for Sebaceous Tumors: Cytokeratin profile, EMA, Ber-EP4, androgen receptors, adipophilin, and perilipin [5, 6]

Adipophilin		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Sebaceous neoplasia, xanthelasma	Burkitt lymphoma, renal cell carcinoma	Adrenal cortex, glands of lactating breast, Sertoli cells
Positive control: skin		

Diagnostic Approach Adipophilin is a lipid droplet-associated protein expressed on the surface of intracytoplasmic lipid droplets in various normal human cell types including acinar cells of lactating breast, zona fasciculate of adrenal glands, and Sertoli cells, whereas adipocytes lack the expression of adipophilin. Adipophilin labels lipid droplets containing neoplastic cells and is a specific marker for sebaceous neoplasia. Studies on the expression of adipophilin in sebaceous and other cutaneous tumors with clear cell histology mimicking sebaceous neoplasms reveal that adipophilin was positive in 92% of sebaceous carcinoma and all cases of sebaceous adenoma and xanthelasma and in 65% of metastatic renal cell carcinoma [7]. All other tumors with clear cell appearance including squamous cell carcinoma, basal cell carcinoma, trichilemmoma, and clear cell hidradenoma lack the expression of adipophilin [4]. Adipophilin is also a marker of

Burkitt lymphoma because of the presence of intracytoplasmic lipid vacuoles.

Lipid Droplet-Associated Protein (Perilipin): Perilipin is a further marker for sebaceous tumors. Perilipin is located on the surface of lipid droplets and plays a role of lipid metabolism. It is normally expressed in the cells of adrenal cortex, Leydig cells, and brown and adult fat. Perilipin is expressed in about one-third of sebaceous tumors but lacks the specificity as it can be also expressed in other tumors with clear cell morphology [8].

Diagnostic Antibody Panel for Melanocytic Tumors See next chapter.

Diagnostic Antibody Panel for Merkel Cell Carcinoma Cytokeratin profile, Merkel cell polyomavirus, EMA, CD56, and NSE

The exact histogenesis of Merkel cell carcinoma is not clarified, but the tumor could develop from skin-derived precursors or dermal stem cells. Recently, pro- or pre-B lymphocytes are discussed as the origin of Merkel cell carcinoma. Merkel cell carcinoma is generally associated/induced by the Merkel cell polyomavirus, which can be detected by immunohistochemistry or molecular biology.

Immunoprofile of skin tumors

Tumor type	+ in >90% (+)	+ in 50–90% (±)	+ in 10–50% (∓)	+ in <10% (−)
I. Keratinocytic (epidermal) tumors				
Squamous cell carcinoma in situ/Bowen's disease	CK5/6/14, p40, p63, EMA	p53		
Squamous cell carcinoma	CK5/6/, CK14, p40, p63, EMA			*Ber-EP4*, bcl-2, CK7, CK15, CK19, CK20
Basal cell carcinoma	*Epithelial cells*: *Ber-EP4*, CK5/6, CK14, p63, bcl-2 *Stroma cells*: CD10			*EMA*, CK7, CK19, CK15, CK20
II. Eccrine and apocrine sweat gland tumors	*Luminal (ductal) epithelial cells*: CK7, CK8, CD10, CK11, CK 13, CK14, CK18, CK19, EMA[a] *Myoepithelial (basal) cells*: CK5/6/14, p63, S100, calponin, actin	CD15, CEA		CK20
Immunoprofile of eccrine tumors	S100	ER, PgR		
Immunoprofile of apocrine tumors	GCFP-15, CK15	Androgen receptor, ER, EMA	CEA	S100
Tubular carcinoma	*Luminal (ductal) epithelial cells*: CK15, CK7	EMA	CEA	

Immunoprofile of skin tumors				
Microcystic adnexal carcinoma	*Luminal (ductal) epithelial cells*: CK15, CK7 *Myoepithelial (basal) cells*: CK5/6/14, p63		Ber-EP4, CD10, CK7	CD15, CK20
Malignant mixed tumor	*Epithelial cells*: CK15 *Myoepithelial cells*: CK5/6/14, p63, actin	EMA	CEA	CK20
Porocarcinoma	*Luminal (ductal) epithelial cells*: CK7, CK15	CK19, EMA	CEA	
Spiradenocarcinoma	CK15	EMA, GCFP-15		
Hidradenocarcinoma	*Luminal (ductal) epithelial cells*: CK15, CK7 *Myoepithelial (basal) cells*: CK5/6/14, p63	EMA		
Mucinous carcinoma	CK15, CK7 *Myoepithelial (basal) cells*: CK5/6/14, p63	ER, PgR		CK20,CDX-2
Digital papillary adenocarcinoma	*Luminal (ductal) epithelial cells*: CK7 *Myoepithelial (basal) cells*: CK5/6/14, p63	EMA, CEA		GCFP-15
Adenoid cystic adenocarcinoma	See profile of equivalent in salivary gland tumors			
Apocrine cribriform adenocarcinoma	*Luminal (ductal) epithelial cells*: CK7, CK15 *Myoepithelial (basal) cells*: CK5/6/14, p63	GCFP-15		
Extramammary Paget disease	CK7, EMA, BerEP-4, CEA	GCFP-15		CK5/6, CK20, ER, AR
III. Hair follicle (pilar) tumors	*HKN*, *HHK*, *CK15*, CK19, p63, Ber-EP4	CK14		CK7, CK20, EMA, S100, GCFP15, CEA, CD15
Trichilemmal carcinoma	CK10, CK15	CEA		EMA
Malignant proliferating trichilemmal tumor	CK10, CK15, CD34		CD34	

Immunoprofile of skin tumors				
IV. Sebaceous tumors (ocular and extraocular sebaceous carcinoma)	*Adipophilin*, CK8/18	EMA[a], CK5/6, androgen receptors, BerEP4, CD15	Perilipin	CK7, CK15, CK19, CK20, S100, GCFP-15
V. Merkel cell carcinoma	Pan-CK, *CK20*[b] (perinuclear), EMA, NSE, Merkel cell *polyomavirus*, E-cadherin[c]	*CD56*, Fli-1, chromogranin, CK8, CK18, TdT, Pax-5	Neurofilaments[b], CK7	S100, HMB45, CEA

[a]The expression of EMA is more characteristic for malignant tumors
[b]Perinuclear dot-like staining pattern
[c]Nuclear staining pattern

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Markers and Immunoprofile of Melanocytic Tumors

21

Contents

Melanoma is a high malignant tumor with exceptionally variable morphologic appearance that can mimic different epithelioid and sarcomatoid tumors. Generally, the diagnosis of malignant melanoma must be based on the morphology, immunoprofile, and clinical data. In metastatic tumors with ambiguous morphology, it is always advisable to rule out melanoma.

Diagnostic Antibody Panel for Malignant Melanoma HMB45, MART-1, tyrosinase, Sox-10, microphthalmia transcription factor (MITF), WT-1, S100, CD63 (NK-C3), PHH3, and Ki-67.

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HMB-45		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Malignant melanoma, Spitz and cellular blue nevi, clear cell sarcoma	PEComa (angiomyolipoma, sugar tumor of lung), lymphangioleiomyomatosis, pheochromocytoma, hepatoblastoma, ependymoma	Retinal pigmented cells, junctional-activated melanocytes and melanocytes of fetal skin, mononuclear cells
Diagnostic approach: melanoma		

Diagnostic Approach HMB45 (**h**uman **m**elanoma **b**lack **45**) also known as gp100 is a melanosomal glycoprotein involved in the maturation of melanosomes from stage I to II. In normal tissue, HMB45 is found in retinal pigment epithelium and fetal melanocytes but absent in mature melanocytes and intradermal nevi. HMB45 is a marker for melanocytic tumors and tumors with melanocytic differentiation including different types of malignant melanoma, dysplastic nevi, Spitz and blue nevi, as well as clear cell sarcoma (Fig. 21.1).

Diagnostic Pitfalls About 10% of malignant melanoma (more frequently amelanotic melanoma, desmoplastic and spindle cell melanomas) lacks the HMB45 expression. The use of an antibody cocktail containing different anti-melanoma markers (usually HMB45, MART-1, and tyrosinase) will markedly increase the sensitivity. Additionally, tumors with similar morphology such as pheochromocytoma and clear cell tumor of the lung (sugar tumor) may be positive for HMB45, but these are usually negative for tyrosinase or Sox-10.

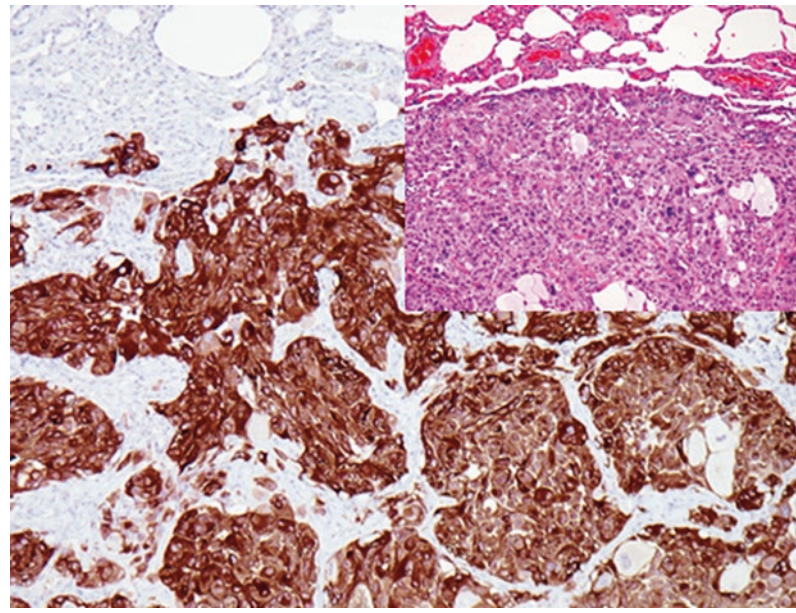

Fig. 21.1 Metastatic melanoma positive for HMB45

MART-1 (Melan A)		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Melanoma, adrenal cortical tumors, sex cord-stromal tumors	Angiomyolipoma, osteosarcoma	Adrenal cortex, melanocytes, brain tissue, granulosa and theca cells, Leydig cells
Positive control: adrenal cortex		

Diagnostic Approach MART-1 (also known as Melan A) is melanocyte antigen and member of the MAGE family involved in melanosomal maturation and regulation of pigmentation expressed in the endoplasmic reticulum of normal skin melanocytes and retinal cells and tumors derived from these cell types. The MART-1 antigen is recognized by cytotoxic T-lymphocytes.

Diagnostic Pitfalls MART-1 is one of the most common used melanoma markers expressed in more than 90% of melanomas. Nevertheless, MART-1 lacks the specificity for melanomas as it is found in other tumors such as adrenocortical and sex cord-stromal tumors. We recommend using MART-1 as a screening antibody and to confirm the diagnosis by further melanoma markers.

Tyrosinase		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Malignant melanoma	Clear cell sarcoma, benign melanocytic lesions	Melanocytes
Positive control: skin/melanoma		

Diagnostic Approach Tyrosinase is a copper-containing enzyme catalyzing the synthesis of melanin from tyrosine in melanocytes. Tyrosinase is a very specific melanoma marker expressed in more than 80% of melanomas, whereas the expression intensity correlates with the differentiation grade of the tumor. Because of its high specificity, tyrosinase is frequently used in a mixture with other melanoma markers as pan-melanoma cocktail. This pan-melanoma cocktail gives good results in the diagnosis of epithelioid, desmoplastic, and spindle cell melanomas and is effective in the detection of micrometastases in sentinel lymph nodes.

Sox-10		
Expression pattern: nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Melanoma	Clear cell sarcoma, schwannoma, neurofibroma, neuroblastoma, paraganglioma, MPNST, granular cell tumor, nerve sheath myxoma, triple-negative and metaplastic breast carcinoma, myoepithelial tumors, skin adnexal tumors, salivary gland tumors (acinic cell carcinoma, myoepithelial carcinoma, epithelial myoepithelial carcinoma), embryonal carcinoma	Epidermal melanocytes, Schwann cells, myoepithelial cells, autonomic ganglia
Positive control: skin, melanoma		

Diagnostic Approach Sox-10 is a member of the Sox family of transcription factors (**s**ex determining region Y-b**ox 10**); a neural crest transcription factor involved in the maturation and differentiation of melanocytes and Schwann cells. Sox-10 is normally expressed in melanocytes, Schwann cells, and myoepithelial cells. Sox-10 is a sensitive marker for different types of malignant melanoma including desmoplastic melanoma (Fig. 21.2) [1].

Diagnostic Pitfalls Sox-10 is an excellent melanoma maker but lacks the specificity. It stains other tumors such as schwannoma, neurofibroma, and granular cell tumor and is found in up to 60% of malignant peripheral nerve sheath tumors [2]. Sox-10 is also a marker for triple-negative and metaplastic breast carcinomas (Fig. 21.3) [3, 4]. Strong Sox-10 expression is found in myoepithelial cells and myoepithelial tumors including different types of salivary gland tumors [5]. In doubtful cases, other more specific melanoma markers should be used to confirm the diagnosis.

Wilms Tumor Protein (WT-1): WT-1 is another marker for malignant melanoma already listed in the mesothelioma chapter [6]. Similar to HMB45, WT-1 is a helpful marker to discriminate between malignant and benign melanocytic lesions.

Immunoprofile of melanocytic tumors

Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in < 10% (−)
Melanoma:	*HMB45*, *Melan A*, *Sox-10*, *tyrosinase*, S100, MAGE1, MITF, CD63 (NK1-C3), PNL2, WT-1, bcl-2[a], vimentin High proliferation index (Ki-67) in melanoma but very low in nevus cells	Nestin, p16, CD10	CD68, CD117, MUM-1, CD30	Pan-CK[b], EMA

[a]Usually negative in benign nevi
[b]Diagnostic pitfall: A weak focal cytokeratin expression maybe found in a small subset of malignant melanoma

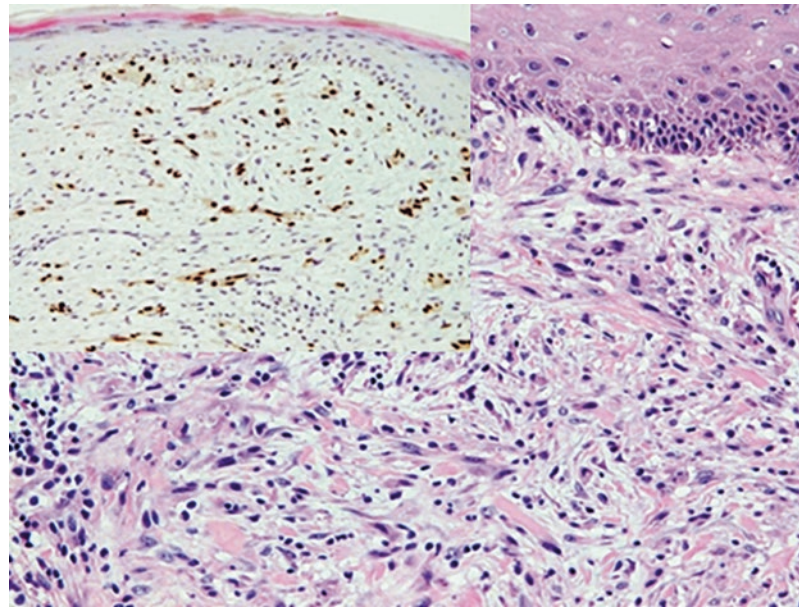

Fig. 21.2 Desmoplastic melanoma exhibiting strong nuclear Sox-10 expression

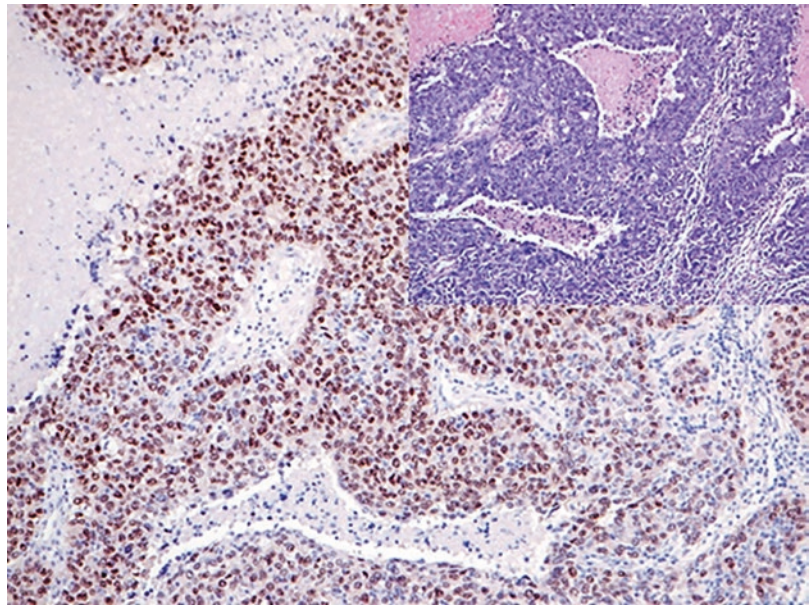

Fig. 21.3 Moderate nuclear Sox-10 expression in neoplastic cells of triple-negative breast carcinoma

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Markers and Immunoprofile of Fibroblastic, Myofibroblastic, and Fibrohistiocytic Tumors

22

Contents

Diagnostic Antibody Panel for Fibroblastic, Myofibroblastic, and Fibrohistiocytic Tumors
Vimentin, actin, desmin, CD34, and CD68

Vimentin		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Mesenchymal tumors	Metaplastic carcinoma, endometrioid carcinoma, carcinomas of salivary glands, follicular thyroid carcinoma, clear cell renal cell carcinoma, hepatocellular carcinoma, poorly differentiated carcinomas of different origin	Cells of mesenchymal origin: fibrocytes and fibroblasts, lipocytes, smooth muscle cells, endothelium, macrophages, myoepithelial cells, thyroid follicular cells, adrenal cortex, renal tubules, mesangial cells of renal glomerulus, pancreatic acinar cells, melanocytes, lymphocytes, astrocytes, Schwann cells
Positive control: appendix		

Diagnostic Approach Vimentin is a 57-kDa protein, a member of the type III family of intermediate filaments, expressed in all mesenchymal cells forming an important part of the cytoskeleton of these cells. The type III family of intermediate filaments includes vimentin, desmin, GFAP, and

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peripherin. Vimentin is generally expressed in all primitive cells in the early embryogenesis and be replaced by other intermediate filaments during maturation and differentiation.

Diagnostic Pitfalls The use of vimentin as a single marker is of limited diagnostic value as the co-expression of vimentin with other different cytokeratins has been demonstrated in many types of epithelial cells and tumors such as carcinomas of the lung, salivary glands, liver and biliary tract, thyroid gland, adrenal cortex, kidney, endometrium, gonads and meningioma (Fig. 22.1). Generally, poorly differentiated carcinomas may acquire vimentin expression with loss of keratins and finally resulting a sarcomatoid phenotype. For diagnostic purposes, vimentin can be only used as a part of diagnostic antibody panel.

STAT-6: STAT-6 is a member of the STAT family of cytoplasmic transcription factors involved in the modulation of signal transmission between DNA promoters and cell receptors. The inv. (12)(q13;q13) is a chromosomal aberration characteristic for solitary fibrous tumor generating the NAB2-STAT6 fusion transcript causing the overexpression of the STAT-6 protein, which is a characteristic immunohistochemical marker for solitary fibrous tumor (Fig. 22.2). This chromosomal abnormality affects also the promoter of the ERG-1 gene causing the overexpression of the ERG-1 transcription factor, which can be a further marker for this tumor identity [1, 2].

Diagnostic Pitfalls The overexpression of STAT-6 is also found in a limited number of other mesenchymal tumors including meningeal hemangiopericytoma that carries the same genetic abnormality, subset of dedifferentiated liposarcoma, and desmoid tumor [3–5].

Mucin-4: MUC-4 is a transmembrane mucoprotein mentioned in a previous chapter with other mucins. In addition to glandular epithelial tumors, the expression of MUC-4 is also a characteristic marker for low-grade fibromyxoid sarcoma, sclerosing epithelioid fibrosarcoma, and glandular components in biphasic synovial sarcoma [6, 7].

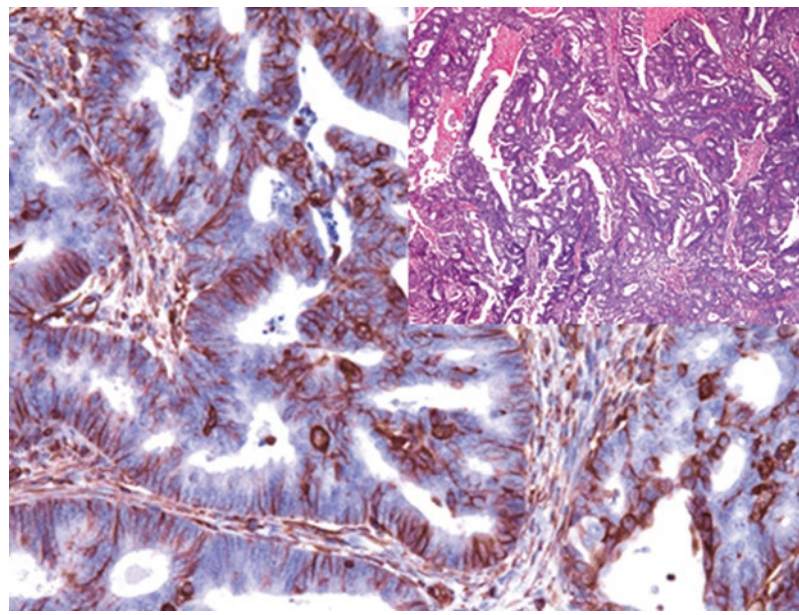

Fig. 22.1 Neoplastic glands of endometrioid carcinoma exhibiting strong vimentin expression

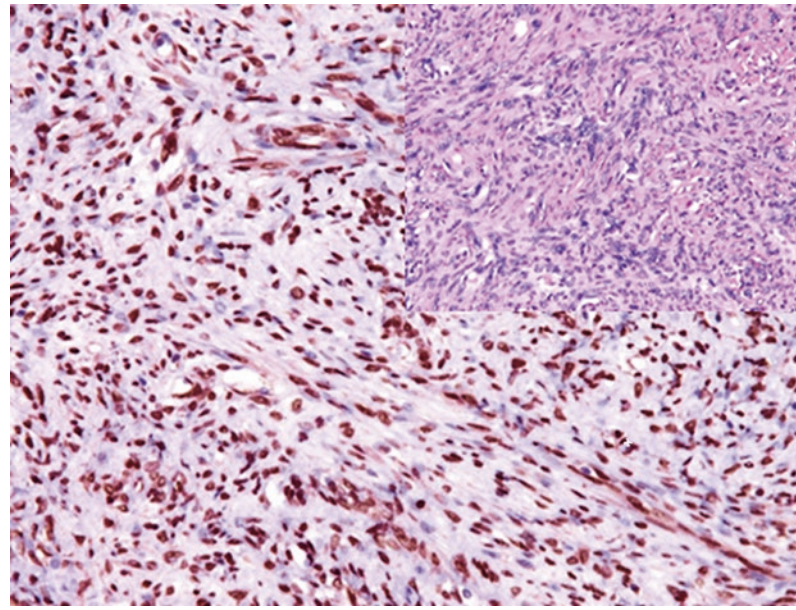

Fig. 22.2 Strong nuclear STAT-6 expression in the cells of solitary fibrous tumor

Immunoprofile of fibroblastic and myofibroblastic tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in < 10% (−)
Nodular fasciitis:	Vimentin	Actin, CD68		Desmin, S100, CD34, pan-CK, EMA
Proliferative fasciitis:	Vimentin, myoglobin	Actin		Desmin, S100, pan-CK
Myofibroblastoma:	Vimentin, actin, desmin			Pan-CK
Angiomyxoid fibroma:	Vimentin, sm-actin			Pan-CK
Giant cell angiofibroma:	Vimentin, CD34			CD31, S100
Calcifying aponeurotic fibroma:	Vimentin	CD68, CD99, S100		Actin, pan-CK
Angiomyofibroblastoma:	Vimentin, desmin	CD34, ER	Actin	Pan-CK, S100
Desmoid fibromatosis (abdominal and extraabdominal fibromatosis including desmoids tumor of the pleura):	Vimentin, β-catenin	Actin	Desmin, S100	CD34, CD117, EMA, pan-CK
Cellular angiofibroma:	Vimentin		CD34, actin, desmin	
Dermatomyofibroma:	Vimentin, actin		Calponin	h-Caldesmon, desmin, CD34, S100
Superficial acral fibromyxoma:	Vimentin, CD99, CD34	CD117, EMA		Pan-CK, S100, desmin
Solitary myofibroma (myofibromatosis):	Vimentin, actin, desmin			Pan-CK, S100

Immunoprofile of fibroblastic and myofibroblastic tumors				
Intranodal myofibroblastoma:	Vimentin, actin			S100
Infantile myofibromatosis:	Vimentin, actin		Desmin	
Solitary fibrous tumor (pleural and extrapleural):	Vimentin, CD34, *STAT-6*, F XIIIa	bcl-2, CD99	Actin, TLE-1, CD10, β-catenin[a]	Desmin, S100, pan-CK, EMA, CD56, CD68, CD117
Inflammatory myofibroblastic tumor (inflammatory pseudotumor):	Vimentin	ALK (p80), cyclin D1, actin	Desmin, CD68, bcl-2, pan-CK	EMA, CD34, CD117
Low grade fibromyxoid sarcoma:	*MUC-4*, vimentin	EMA	Actin, desmin, bcl-2, CD34, pan-CK	S100, EMA
Congenital and infantile fibrosarcoma:	Vimentin		Actin, desmin, S100, CD34	Myoglobin
Acral myxoinflammatory fibroblastic sarcoma (inflammatory myxohyaline tumor):	Vimentin		CD34, CD68	EMA
Infantile and congenital fibrosarcoma:	Vimentin	CD34	Actin, desmin	
Fibrosarcoma (adult):	Vimentin			
Sclerosing epithelioid fibrosarcoma:	Vimentin	*MUC-4*, EMA	Pan-CK, S100	

[a]Nuclear stain

Immunoprofile of fibrohistiocytic tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in < 10% (−)
Fibrous histiocytoma (dermatofibroma):	FXIIIa, α-1 antitrypsin, vimentin	Actin	Desmin, CD34	S100
Giant cell tumor of soft tissue:	CD68, actin[a], vimentin			
Dermatofibrosarcoma protuberans:	CD34, PDGF, p53, vimentin,	Nestin, bcl-2, CD63	Calponin	Actin, desmin, h-caldesmon, CD31, CD56, FVIII, pan-CK, EMA
Giant cell fibroblastoma:	*CD34*, PDGF vimentin		Actin	Desmin, FVIII, CD31, S100, pan-CK
Atypical fibroxanthoma (pleomorphic undifferentiated sarcoma of skin):	CD10, S100A6, -procollagen-1 vimentin	CD68, actin	TLE-1	Pan-CK, desmin
Localized giant cell tumor of tendon sheath:	CD68[b], CD45, vimentin			
Tenosynovial giant cell tumor:	CD68[b], CD45, vimentin	CD31, CD34	Desmin	Actin, S100, h-caldesmon, F VIII

[a]Giant cell lack actin expression
[b]CD68 and CD45 expression only in multinucleated cells

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Markers and Immunoprofile of Muscle Tumors

23

Contents

23.1 Diagnostic Antibody Panel for Skeletal Muscle Tumors

Desmin, myoglobin, myogenin, myosin, MyoD1, EGFR, fibrillin-2, and p-cadherin [1, 2].

Desmin		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Rhabdomyosarcoma and rhabdomyoma, smooth muscle tumors	Desmoplastic small round cell tumor, alveolar soft part sarcoma, malignant rhabdoid tumor, myofibroblastoma, tenosynovial giant cell tumor	Smooth and striated muscle, myoblasts and myofibroblasts, mesothelial cells, endometrium
Positive control: appendix		

Diagnostic Approach Desmin is a type III intermediate filament protein present in intercalated disks and Z-lines of cardiac muscle and Z-line of skeletal muscle. Desmin stains cardiac, skeletal, and smooth muscle cells and tumors derived from these cells. The intensity of desmin expression correlates with the differentiation grade of muscle or muscle tumor. Desmin is an important diagnostic marker for all myogenic tumors and tumors with myogenic differentiation, whereas myoepithelial cells are negative (Fig. 23.1).

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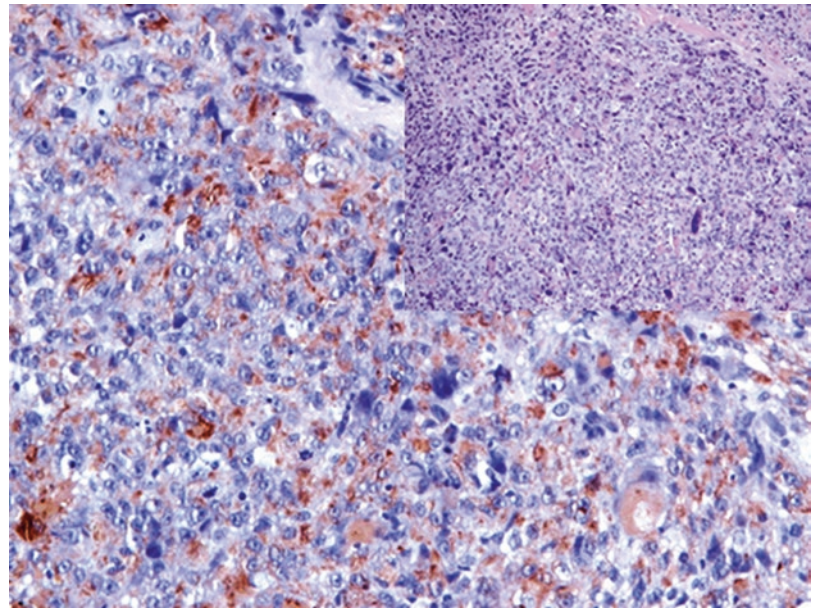

Fig. 23.1 Cells of pleomorphic rhabdomyosarcoma exhibiting marked cytoplasmic expression of desmin

Diagnostic Pitfalls Desmin is found in other tumors with similar morphology to rhabdomyosarcoma such as desmoplastic small round cell tumor and alveolar soft part sarcoma; hence, the diagnostic panel for rhabdomyosarcoma must include at least one of the antibodies to myogenic transcriptional regulatory proteins (myogenin, Myo D-1, or Myf-3). Markers for smooth muscle differentiation can be also included. It is noteworthy that mesotheliomas (mainly sarcomatous type) and very rarely carcinomas can show focal positivity to desmin; this makes it necessary to determine the cytokeratin profile in doubtful cases.

Myoglobin		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Tumors with skeletal muscle differentiation/ rhabdomyosarcoma	Various carcinomas, e.g., breast, prostate, colorectal, head, and neck (see below)	Striated muscle, secretory epithelium, goblet cells
Positive control: skeletal muscle		

Diagnostic Approach Myoglobin is an iron- and oxygen-binding single chain polypeptide that appears in the early stages of muscle differentiation. Myoglobin is expressed in skeletal muscle, cardiac muscle, rhabdomyoblasts and adult-type skeletal muscle tumors. Embryonal muscle tumors and smooth muscle tumors as well as other sarcoma types lack the expression of myoglobin.

Diagnostic Pitfalls Weak to moderate expression is reported in various carcinomas (e.g., breast, prostate, colon, head and neck) associated with hypoxia and steroid hormone receptor positivity.

Myogenin and MyoD1		
Expression pattern: nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Rhabdomyosarcoma	Wilms' tumor	Fetal muscle, myoblasts
Positive control: rhabdomyosarcoma/fetal muscle		

Diagnostic Approach The Myo D family of myogenic transcriptional regulatory factors includes MyoD1 (Myf-3), myogenin (Myf-4) myf-5, and MRF-4 (Myf-6). These transcriptional factors participate in the activation of muscle stem cells and take a part in the regulation of skeletal muscle differentiation in early embryonal stages, maintenance of myogenic program, and repair. The expression of MyoD1 and myogenin

is downregulated in mature skeletal muscle, and the expression of both markers is specific for all rhabdomyosarcoma types (Figs. 23.2 and 23.3) [3, 4].

Diagnostic Pitfalls Both myogenic transcriptional factors can be positive in nonneoplastic myoblasts found within regenerative and atrophic muscle lesions [5]. The expression myogenin and MyoD1 is also reported in some cases of desmoid tumors, infantile fibrosarcoma, mesenchymoma, and Wilms' tumor. In the interpretation of myogenin and MyoD1 stains, only nuclear stain can be considered as positive; other stain types (cytoplasmic or membranous) are nondiagnostic artifacts.

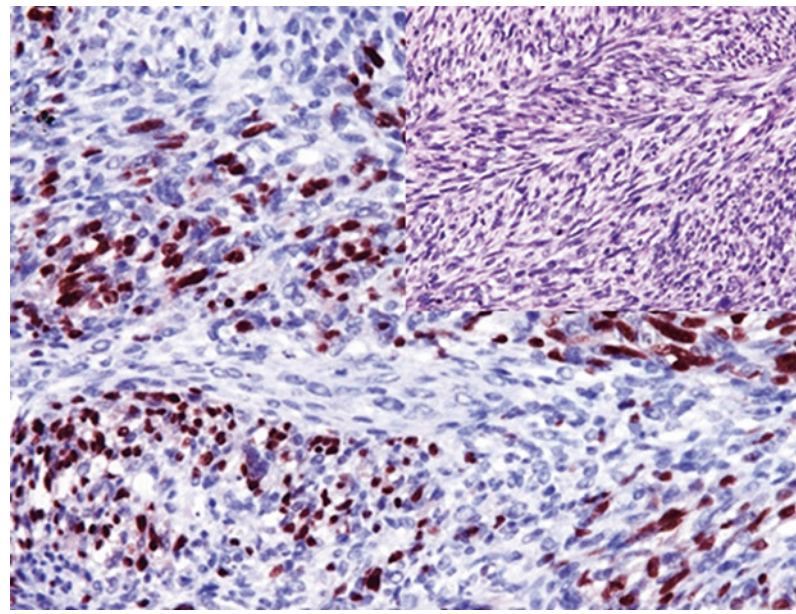

Fig. 23.2 Strong nuclear myogenin expression in rhabdomyosarcoma

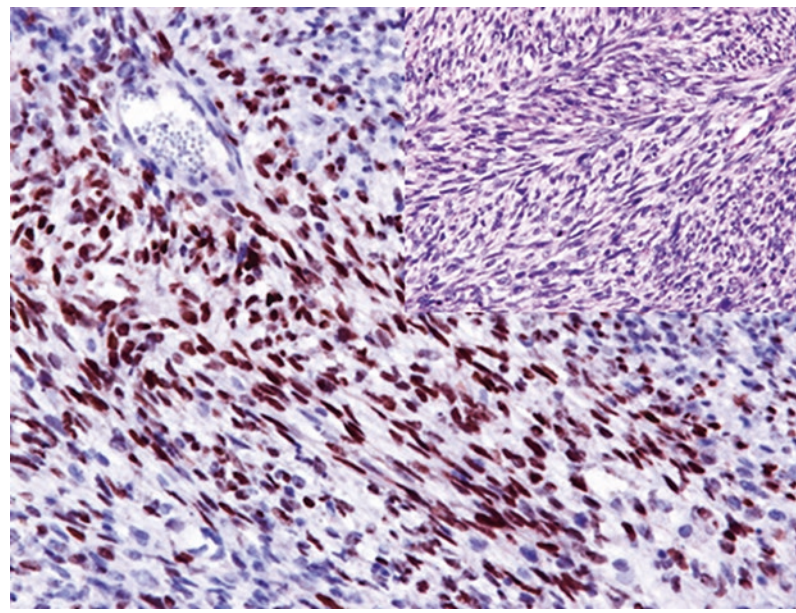

Fig. 23.3 Strong MyoD1 nuclear expression in cells of rhabdomyosarcoma

PAX-5: PAX-5 is a member of the PAX family of transcription factors was mentioned as a B-lymphocyte marker and a marker for some neuroendocrine carcinomas. In non-lymphoid neoplasms, PAX-5 stains alveolar rhabdomyosarcoma, but it is constantly negative in embryonal-type rhabdomyosarcoma [6].

Epidermal Growth Factor Receptor-1: EGFR is a member of type 1 receptor tyrosine kinase family described in a previous chapter (see Chap. 2). EGFR is a transmembrane glycoprotein normally expressed on the membrane of various types of normal epithelial and non-epithelial cells. The expression of EGFR is a characteristic marker for many epithelial and non-epithelial tumors and is diagnostic marker for embryonal rhabdomyosarcoma discriminating it from other rhabdomyosarcoma types (Fig. 23.4) [7].

Immunoprofile of skeletal muscle tumors

Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Fetal rhabdomyoma:	*Desmin*, sr-actin, myosin, myoglobin	MyoD1, vimentin	GFAP	Pan-CK
Adult rhabdomyoma:	*Desmin*, sr-actin, myoglobin	Myosin, myotilin, vimentin		Pan-CK, sm-actin, S100, GFAP
Genital rhabdomyoma:	*Desmin*, sr-actin, myoglobin			sm-actin, pan-CK
Embryonal rhabdomyosarcoma:	*MyoD1, desmin, EGFR, fibrillin-2*	*Myogenin, Myf-5*, CD56		Pan-CK[a], p-cadherin, AP2β,
Alveolar rhabdomyosarcoma:	*Desmin, myogenin*, Myf-5, *AP2β*, *p-cadherin*	*MyoD1*, myosin, myotilin, myoglobin, sr-actin, PAX-5, CD56, bcl-2	PLAP, NSE	Pan-CK[a], fibrillin-2, EGFR
Pleomorphic rhabdomyosarcoma:	*Desmin*	*MyoD1*, *myogenin*, Myf-5,	Pan-CK	
Spindle cell/sclerosing rhabdomyosarcoma:	*Desmin, myogenin*	*MyoD1*, Myf-5	Pan-CK, S100	

[a]Variable degree of cytokeratin expression is noted in a small percentage of different types of rhabdomyosarcoma, which may be the cause of misdiagnosis

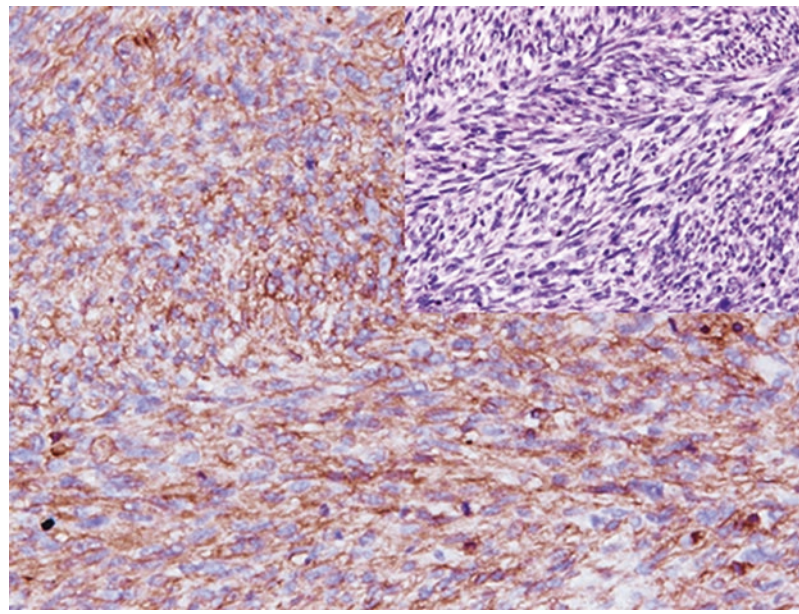

Fig. 23.4 Strong EGFR expression in embryonal rhabdomyosarcoma

23.2 Diagnostic Antibody Panel for Smooth Muscle Tumors

Desmin, sm-actin, h-caldesmon, calponin, smoothelin, transgelin, and steroid hormone receptors [8].

Smooth muscle actin (sm-actin, SMA)		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Smooth muscle tumors	Myoepithelial and myofibroblastic tumors GIST, endometrial stromal sarcoma	Smooth muscle cells, myoepithelial cells, myofibroblasts, capillary endothelia
Positive control: appendix		

Diagnostic Approach Actins are a major cytoskeletal protein, which are a group of contractile microfilaments that include α, β, and γ subtypes. α-Actin is composed of three isoforms: α-actin-1, a cardiac muscle actin; α-actin-2, a smooth muscle actin; and α-actin-3, a skeletal muscle actin. Antibodies to α-actin-2 (sm-actin) label smooth muscle cells, myoepithelial cells, and myofibroblasts. The actin clone 1A4 is a widely used antibody to sm-actin, effective for the diagnosis of smooth muscle, myoepithelial, and myofibroblastic lesions [9]. Another widely used actin clone is HHF-35 reacting with both skeletal and smooth muscle actins and accordingly stains both smooth muscle and skeletal muscle tumors (Fig. 23.5).

Diagnostic Pitfalls The expression of sm-actin can be found in some tumors with a similar morphology other than smooth muscle tumors, including endometrial stromal tumors, synovial sarcoma, GISTs, and sarcomatous mesothelioma.

h-Caldesmon		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Smooth muscle tumors	Glomus tumors, GIST, myoepithelial tumors, inflammatory myofibroblastic tumor, epithelioid mesothelioma	Visceral and vascular smooth muscle cells, myoepithelial cells
Positive control: appendix		

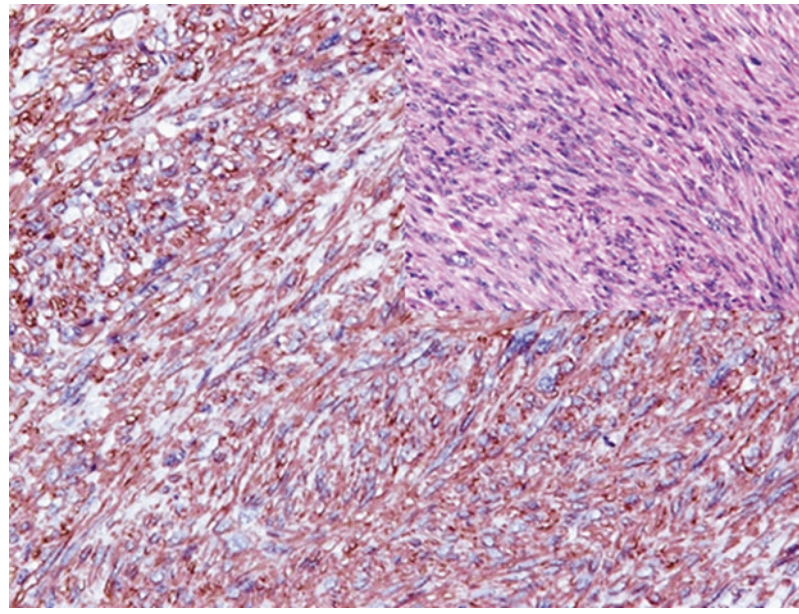

Fig. 23.5 Strong cytoplasmic expression of sm-Actin in leiomyosarcoma

Diagnostic Approach Caldesmon is a cytoplasmic calcium- and calmodulin-binding protein taking part in the regulation of smooth muscle contraction. Caldesmon has two isoforms, a low molecular weight isoform (l-caldesmon) taking part in the modulation of cytoskeleton and cell shape and regulation of cell proliferation and a high molecular weight isoform (h-caldesmon) mainly expressed in visceral and vascular smooth muscle cells in addition to myoepithelial cells. In routine histopathology, h-caldesmon is used as a specific marker for smooth muscle tumors considering that the expression spectrum of h-caldesmon in non-smooth muscle tumors is narrower than that of sm-actin (Fig. 23.6). In contrast to actin, myofibroblasts lack the expression of h-caldesmon [10].

Diagnostic Pitfalls h-Caldesmon can be positive in non-smooth muscle lesions such as gastrointestinal stroma tumor and inflammatory myofibroblastic tumor in addition to pleural and peritoneal epithelioid mesothelioma, which to consider in the differential diagnosis.

Calponin (basic)		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Smooth muscle tumors	Myoepithelial and myofibroblastic tumors	Smooth muscle, myoepithelial cells
Positive control: appendix		

Diagnostic Approach Calponin is a cytoskeleton-associated actin-, tropomyosin-, and calmodulin-binding protein involved in the regulation of smooth muscle contraction. The expression spectrum of calponin is similar to that of h-caldesmon. GIST lacks usually the expression of calponin.

Transgelin: Transgelin is an actin-binding gelling protein of the calponin family found on the membrane and in the cytoplasm of smooth muscle cells. Transgelin is one of the earliest markers of smooth muscle differentiation and stains visceral and vascular smooth muscle cells in

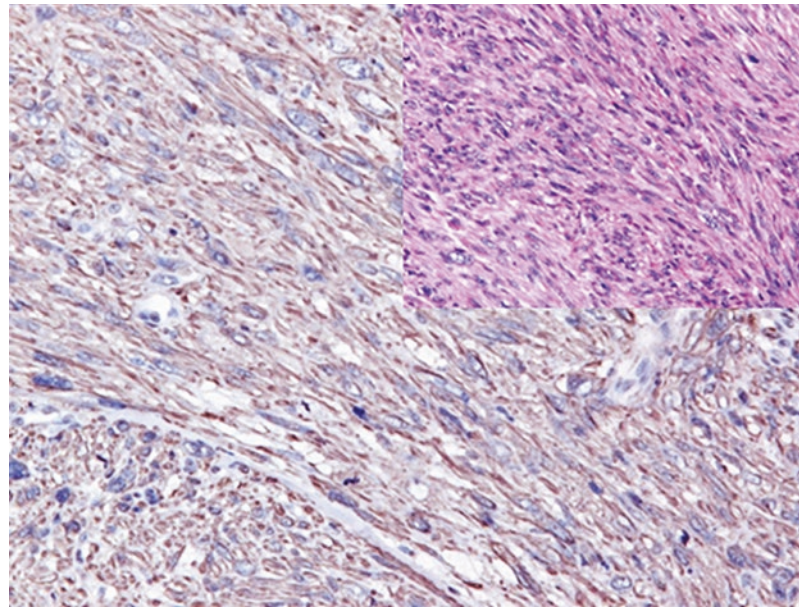

Fig. 23.6 Leiomyosarcoma exhibiting strong cytoplasmic h-caldesmon expression

addition to myofibroblasts and related benign and malignant tumors [11, 12]. Transgelin labels also the epithelial tumor cells of triple-negative breast carcinoma of basal type and a subset of malignant nerve sheath tumor [13]. Rhabdomyosarcoma, GISTs, and endometrial stromal tumors lack the expression of transgelin [14].

Diagnostic Pitfalls The expression of transgelin is also found in fibroblasts, myofibroblasts, and some epithelial cells.

Smoothelin: Smoothelin is a component of the cytoskeleton of differentiated smooth muscle cells and presents into two isoforms: type A composed of a short chain found in visceral smooth muscle and type B composed of a long chain distinctive for vascular smooth muscle [15]. Myoepithelial cells, myofibroblasts, and skeletal and cardiac muscles lack the expression of smoothelin. Smoothelin is a specific marker of smooth muscle tumors, and the expression of smoothelin correlates with the differentiation grade of these tumors (Fig. 23.7) [16]. Smoothelin is also a useful marker to highlight the muscularis propria and muscularis mucosae for the interpretation of bladder and intestinal tumors. For the latter, the comparative use with sm-actin is recommended.

Immunoprofile of smooth muscle tumors

Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Angioleiomyoma:	*sm-actin*, desmin, collagen IV, vimentin			
Leiomyoma:	*sm-actin*, h-caldesmon, vimentin	Desmin, calponin, smoothelin, transgelin	bcl-2	
Leiomyosarcoma:	*sm-actin*, h-caldesmon, vimentin	Desmin, calponin, smoothelin, transgelin, CD146, D2-40	Pan-CK, CK8, CK18, CD34, bcl-2	CD117

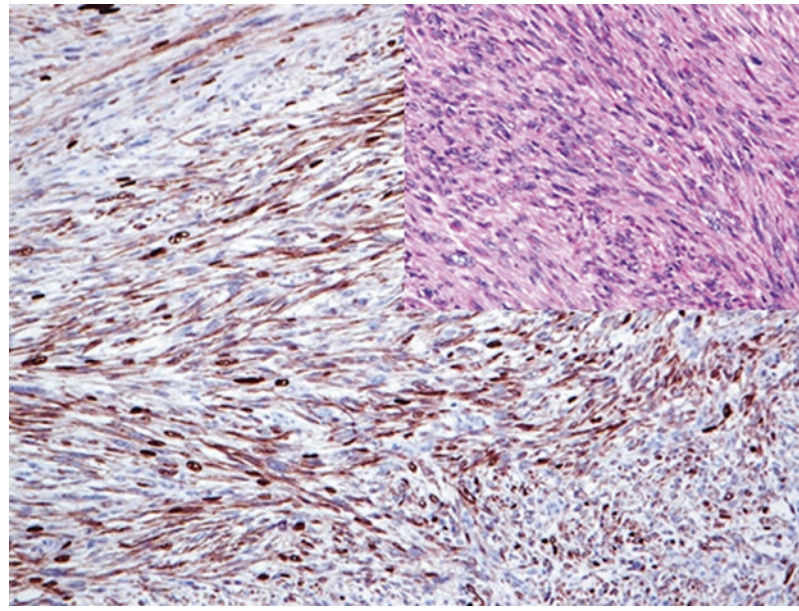

Fig. 23.7 Smoothelin highlighting the cells of leiomyosarcoma

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Markers and Immunoprofile of Vascular and Perivascular Tumors

24

Contents

Diagnostic Antibody Panel for Vascular Tumors CD31, CD34, factor VIII, CD105, ERG, podoplanin, thrombomodulin (CD141), and Fli-1 [1].

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CD31 (PECAM-1)		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Vascular tumors	Plasmacytoma, Langerhans cell histiocytosis and Langerhans sarcoma, granulocytic sarcoma, Ewing's sarcoma, rare carcinoma types	Endothelial cells, megakaryocytes/platelets, macrophages/monocytes, Kupffer cells, osteoclasts, myoblasts, granulocytes, mantle zone B cells, T/NK cells and plasma cells
Positive control: appendix		

Diagnostic Approach CD31, also known as PECAM-1 (**p**latelet **e**ndothelial **c**ell **a**dhesion **m**olecule-**1**), is a transmembrane glycoprotein and member of the immunoglobulin family normally expressed on endothelial cell junctions and on the surface of platelets, monocytes, granulocytes, and B-lymphocytes. CD31 is a sensitive and specific marker for blood vessels and vascular tumors [1, 2].

Diagnostic Pitfalls Low expression levels of CD31 are reported in rare nonvascular tumors such as chronic lymphocytic lymphoma, plasmacytoma, Langerhans cell neoplasia, leiomyosarcoma, mesothelioma, and glioma in addition to few carcinoma types such as carcinoma in situ and invasive breast carcinoma and papillary thyroid carcinoma.

CD34		
Expression pattern: membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Vascular tumors, Kaposi's sarcoma, GIST, dermatofibrosarcoma protuberans, solitary fibrous tumor, epithelioid sarcoma, AML (M0), granulocytic sarcoma, neurofibroma, liposarcoma	Pre-B-ALL, AML (M7), alveolar soft part sarcoma, congenital and infantile fibrosarcoma, inflammatory fibrous polyp of gastrointestinal tract, breast fibroadenoma, giant cell fibroblastoma, juxtaglomerular cell tumor, superficial acral fibromyxoma	Hematopoietic progenitor cells (myeloid, B- and T-lymphocyte precursors), endothelial cells, hepatic sinusoidal cells, interstitial cells of Cajal, endometrial stroma, fibroblasts
Positive control: appendix		

Diagnostic Approach CD34 is a cell surface adhesion glycoprotein expressed on the surface of precursor hematopoietic cells of myeloid and lymphoid lineage, a subset of mesenchymal stem cells and endothelial cells, and a large number of tumors originated from these cells. CD34 is a widely used marker to highlight blood vessels and vascular tumors, but it is less specific than CD31 (Fig. 24.1) [1, 2]. CD34 is also an important marker for other tumors such as dermatofibrosarcoma protuberans and GIST. Furthermore, CD34 is one of the essential markers for hematopoietic and mesenchymal stem cells that also label myeloid blast in AML.

Diagnostic Pitfalls Because of its broad expression spectrum, CD34 must be used as a screening marker supported by a panel of more specific antibodies [3].

Factor VIII (von Willebrand factor)		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Vascular tumors		Endothelial cells and endocardium, platelets and megakaryocytes, mast cells
Positive control: appendix		

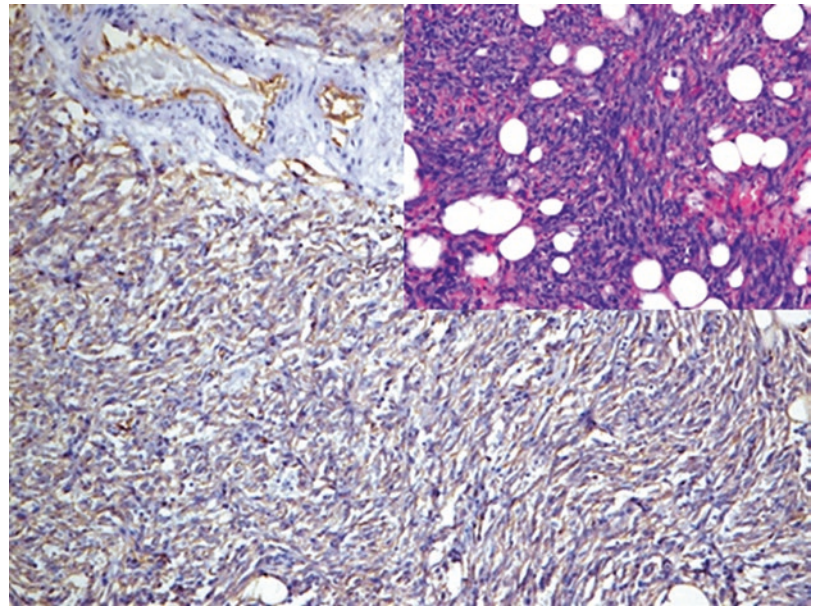

Fig. 24.1 CD34 labeling neoplastic endothelium in angiosarcoma

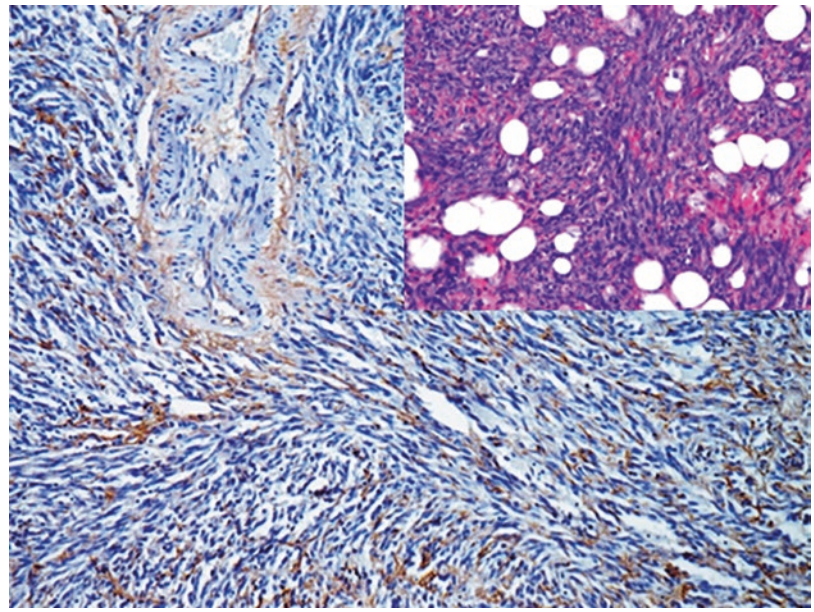

Fig. 24.2 Angiosarcoma with diffuse expression of factor VIII

Diagnostic Approach Factor VIII (von Willebrand factor) is a glycoprotein complex composed of three subunits with functional binding domains to platelet glycoproteins, collagen, and heparin. Factor VIII is synthesized by endothelial cells and megakaryocytes and stored in the Weibel-Palade bodies of endothelial cells. Factor VIII is a specific marker for blood vessels and vascular tumors. The intensity of factor VIII expression correlates with the differentiation grade of the vascular tumors and is very low in poorly differentiated vascular tumors such as angiosarcoma (Fig. 24.2).

Podoplanin (D2-40)		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Lymphangioma and other tumors of lymphatic vessels, mesothelioma, adenomatoid tumor	Vascular tumors, skin adnexal carcinomas, germ cell tumors (dysgerminoma and seminoma), Kaposi's sarcomas, follicular dendritic cells tumors, dermatofibroma, schwannoma, meningioma, glial tumors, GIST, synovial sarcoma, leiomyosarcoma, desmoid, malignant peripheral nerve sheath tumor, epithelioid sarcoma, chondrosarcoma	Lymphatic endothelium, mesothelial cells, adrenal cortex, follicular dendritic cells, renal podocytes, granulosa cells, testicular germ cells, myoepithelial cells and cells of Cajal, glial and Schwann cells, ependymal cells, fibroblasts and osteocytes, smooth and striated muscle cells
Positive control: appendix		

Diagnostic Approach Podoplanin (also known as D2-40) is a type I transmembrane mucoprotein expressed in fetal germ cells and on the membrane of several mature cell types, mainly lymphatic endothelium and mesothelial cells [1, 2]. In routine immunohistochemistry, podoplanin is widely used as a marker to highlight lymphatic vessels and as a marker for tumors of lymphatic endothelium and mesothelioma (Fig. 24.3). Furthermore, it is one of the important seminoma makers [4].

Diagnostic Pitfalls Podoplanin has a broad expression spectrum as it is expressed in various tumors with ambiguous morphology such as leiomyosarcoma and desmoid and peripheral nerve sheath tumors accordingly must be used in a panel with other more specific antibodies [5].

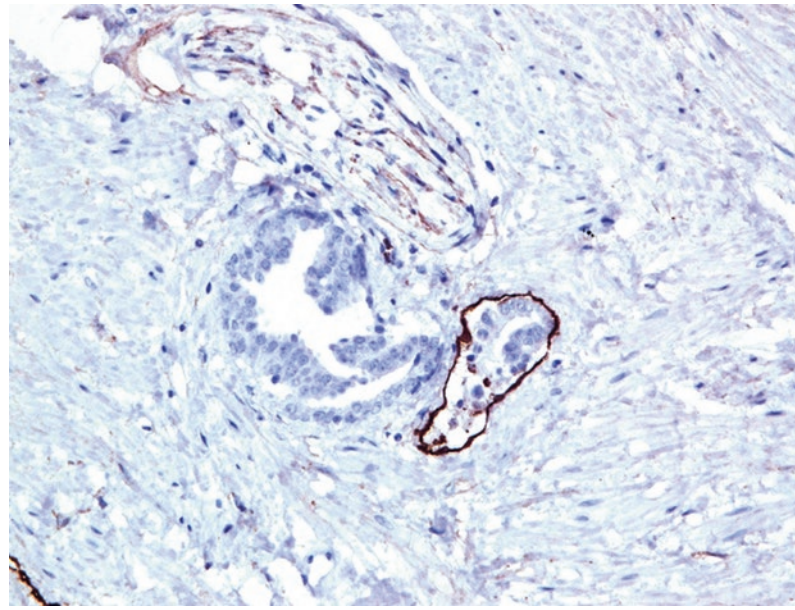

Fig. 24.3 D2-40 highlighting endothelial cell of lymphatic vessel with lymphangitic carcinomatosis. D2-40 is also staining the Schwann cells appearing in the upper part of the section

ERG		
Expression pattern: nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Endothelial/vascular tumors, prostatic adenocarcinoma	Acute myeloid leukemia, solitary fibrous tumor, epithelioid sarcoma, meningioma	Endothelial cells
Positive control: blood vessels		

V-ETS avian erythroblastosis virus oncogene homolog (ERG) is a member of the ETS family transcription factors listed in a previous section (see markers for prostatic carcinoma). ERG is normally expressed in endothelial cells and involved in the regulation of angiogenesis and endothelial apoptosis. The expression of ERG is also found in a subset of immature hematopoietic cells. ERG is a very sensitive and specific marker for endothelial neoplasia (Fig. 24.4) [6].

Diagnostic Pitfalls ERG is also positive in prostate carcinomas harboring the TMPRSS2-ERG translocation. In mesenchymal tumors, the expression of ERG is reported in some other mesenchymal tumors with morphology resembling vascular tumors including solitary fibrous tumor due to other genetic anomalies associated with this tumor, fibrous meningioma, and epithelioid sarcoma [7, 8]. The expression of ERG is also found in a small subset of some lymphoma types.

Human Herpesvirus Type 8 HHV-8 is a DNA virus suggested as the etiological agent of Kaposi's sarcoma, primary effusion lymphoma, and multicentric Castleman's disease. The demonstration of latent nuclear antigen is a diagnostic marker for Kaposi's sarcoma (Fig. 24.5) [9].

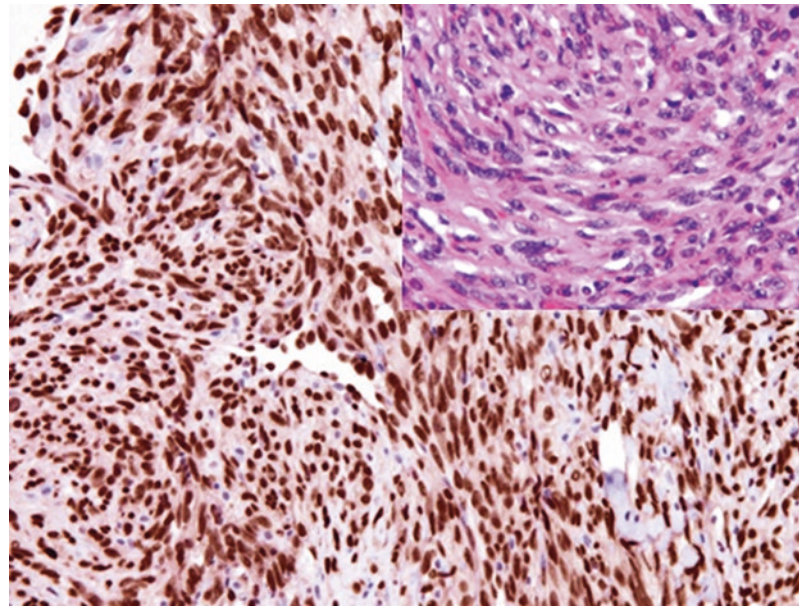

Fig. 24.4 Angiosarcoma cells exhibiting strong nuclear ERG expression

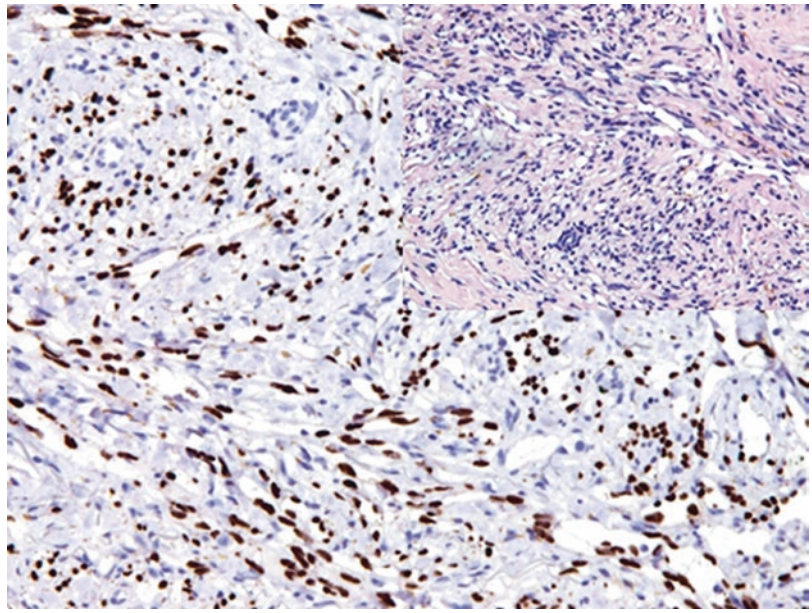

Fig. 24.5 Nuclear expression of HHV-8 (LNA) in neoplastic cells of Kaposi's sarcoma

Immunoprofile of vascular tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Epithelioid hemangioma:	*CD31*, *F VIII*, *CD34*, vimentin	Glut-1[a]	Pan-CK	
Lymphangioma:	*Podoplanin* (D2-40), CD31, F VIII, CD34, vimentin			Pan-CK
Retiform hemangioendothelioma:	*CD31*, *CD34*, *FVIII*, vimentin			
Kaposiform hemangioendothelioma:	*CD31*, *CD34*, vimentin			HHV-8, F VIII
Epithelioid hemangioendothelioma:	CD31, CD34, F VIII, vimentin	Fli-1	Actin, pan-CK, CK8/18, ER, Melan A, HMB-45	EMA, S100
Angiosarcoma:	CD31, CD34, CD105 (endoglin), F VIII, CD141 (thrombomodulin), *ERG*, *Fli-1*, vimentin	Laminin, CK1	Pan-CK, MDM2, CD117, inhibin A	D2-40, CD56, CDK4, HHV-8
Epithelioid angiosarcoma:	*CD31*, *CD34*, *F VIII*, Fli-1, vimentin	Pan-CK		EMA, S100
Kaposi's sarcoma:	*CD31*, *CD34*, CD105, *HHV-8*, D2-40, Fli-1, vimentin	bcl-2	MDM2	F VIII, CDK4
Malignant endovascular papillary angioendothelioma (papillary intra-lymphatic angioendothelioma; Dabska tumor):	*CD31*, *CD34*, F VIII, VEGFR-3, D2-40, vimentin			Pan-CK, EMA, S100

Immunoprofile of perivascular tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Solid glomus tumor/ glomangiosarcoma:	*sm-actin*, myosin, calponin, laminin collagen IV, vimentin	h-Caldesmon	CD34	CD56, desmin, S100, F VIII, EMA, pan-CK
Myopericytoma:	*sm-actin*, vimentin	h-Caldesmon	Desmin	S100, pan-CK, EMA
Sinonasal hemangiopericytoma:	VEGF, vimentin	actin	F VIII, CD34	Desmin, CD31, F VIII, EMA, pan-CK

[a]Glut-1 usually positive in hemangioma but negative in vascular malformation pyogenic granuloma and granulation tissue

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Markers and Immunoprofile of Adipocytic Tumors

25

Contents

Diagnostic Antibody Panel for Adipocytic Tumors S100, CD34, MDM2, CDK4, and p16 [1, 2].

MDM2		
Expression pattern: nuclear/cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Liposarcoma	Clear cell sarcoma, desmoplastic small round cell tumor, angiosarcoma, Kaposi's sarcoma, epithelioid sarcoma, embryonal rhabdomyo-sarcoma, leiomyosarcoma, MPNST, adrenal oncocytoma, osteosarcoma, various carcinomas	Wide variety of epithelia, spermatogenesis, lymphocytes
Positive control: liposarcoma		

Diagnostic Approach MDM2 (**m**urine **d**ouble **m**inute **2**, also known as E3 ubiquitin-protein ligase) is a nuclear phosphoprotein enzyme that interacts with p53 affecting the cell cycle and apoptosis. MDM2 is overexpressed in many tumors, while the main diagnostic use

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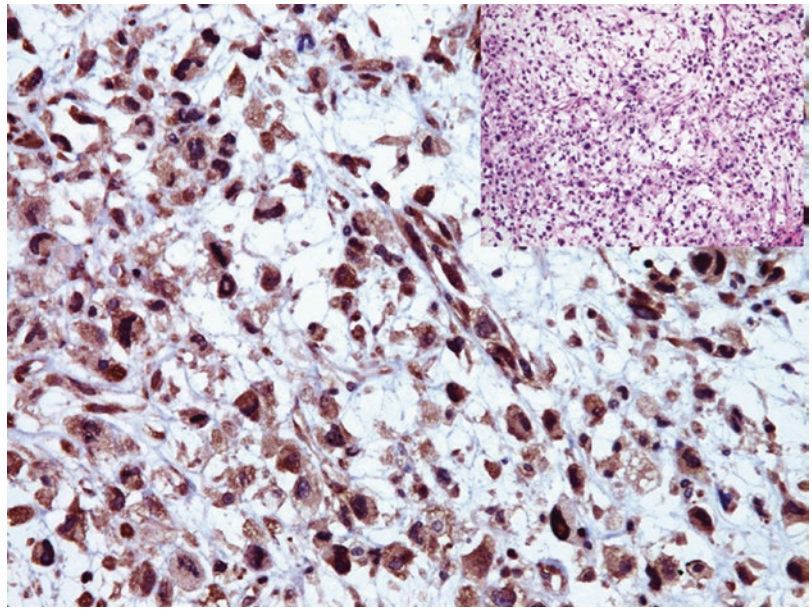

Fig. 25.1 MDM2 expression in neoplastic cells of dedifferentiated liposarcoma

is to differentiate between benign adipocytic tumors and well-differentiated liposarcoma (Fig. 25.1) [3–5].

The overexpression of MDM2 is also noted in osteosarcoma but absent in benign fibro-osseous lesions, which can be helpful to discriminate between the two identities.

Diagnostic Pitfalls As abovementioned, the expression or overexpression of MDM2 might be found in many sarcoma types, which must be considered in the differential diagnosis. It is also important to mention that the clone SMP14 of the MDM2 antibody shows cross-reactivity with some cytokeratins including the cytokeratins 6, 14, and 16, which label squamous epithelium and squamous cell carcinoma.

CDK4: CDK4 (**c**yclin-**d**ependent **k**inase **4**) is nuclear enzyme involved in the regulation of the cell cycle. CDK4 is normally expressed in different types of normal and neoplastic cells but overexpressed in some epithelial and mesenchymal tumors. The overexpression of CDK4 is found in liposarcoma, osteosarcoma, and a subset of malignant peripheral nerve sheath tumor in addition to rhabdomyosarcoma; accordingly, CDK4 can be used to discriminate these malignant tumors from benign lesions with similar morphology such as benign lipomatous tumors, benign fibro-osseous lesions, schwannoma, and neurofibromas. CDK4 is also markedly expressed in malignant melanomas, gliomas, and different gastrointestinal, lung, ovarian, and breast carcinomas.

p16: p16 (cyclin-dependent kinase inhibitor 2A) is a tumor suppressor protein expressed in few carcinoma types and HPV-associated squamous cell carcinoma of different origin. P16 is a helpful marker to distinguish between well-differentiated and dedifferentiated liposarcoma positive for p16 and benign lipoma and normal fatty tissue negative for p16 (Figs. 25.2 and 25.3) [6, 7].

Diagnostic Pitfalls p16 is not a specific liposarcoma marker as it is reported to stain other malignant mesenchymal tumors. p16 positivity can be also found in areas with liponecrosis [8].

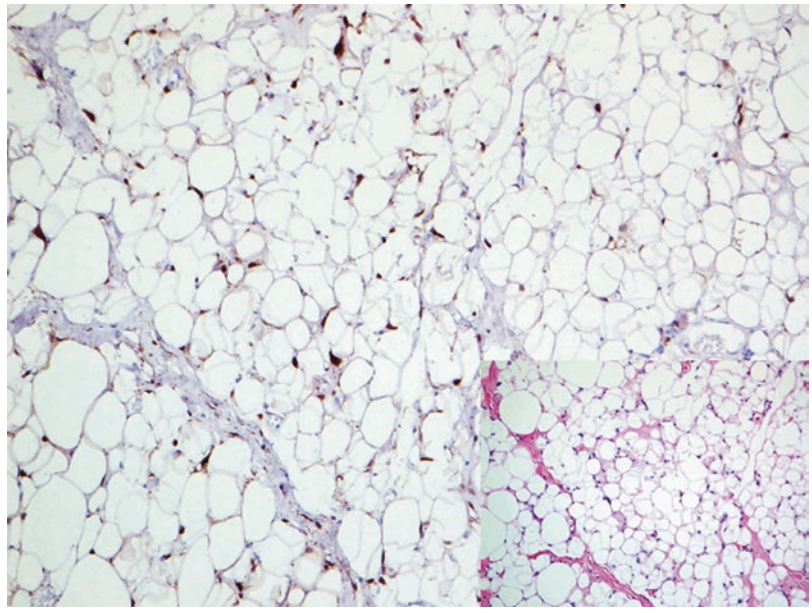

Fig. 25.2 Strong nuclear p16 expression in cells of atypical lipomatous tumor

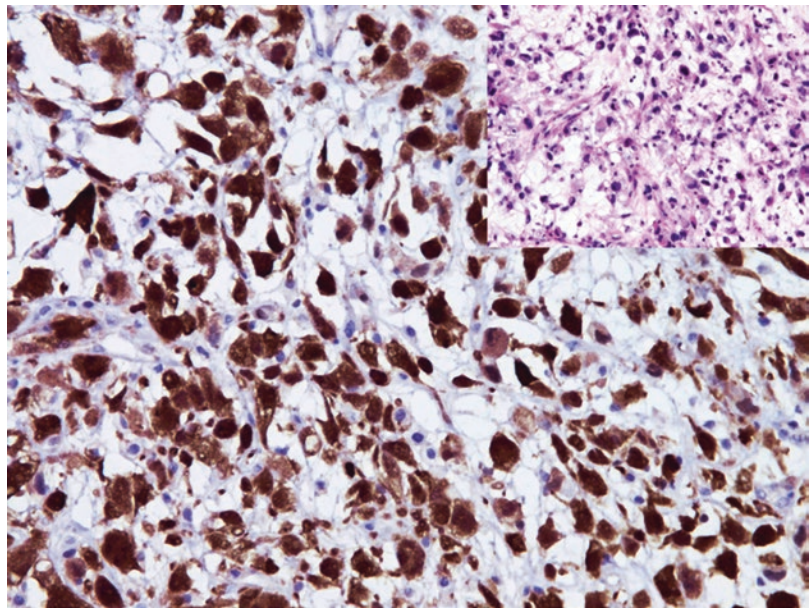

Fig. 25.3 Strong p16 expression in neoplastic cells of dedifferentiated liposarcoma

Immunoprofile of adipocytic tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Hibernoma:	Estrogen receptors, aP2 (P422), vimentin			
Lipoma:	Vimentin, *S100*	Calretinin		*p16*, *MDM2*, aP2

Immunoprofile of adipocytic tumors				
Lipoblastoma:	*S100*, *CD34*, vimentin Proliferation index (Ki-67): 0–5%		p16	
Spindle cell lipoma:	CD34, bcl-2, S100, vimentin			MDM2, p16, aP2
Chondroid lipoma:	S100, vimentin		CD68	MDM2, p16, aP2
Atypical lipomatous tumor (well-differentiated liposarcoma):	*CDK4*, *MDM2*, *p16*, aP2, Ki-67 (clone K-2), vimentin	Calretinin	S100	
Myxoid liposarcoma:	*CDK4*, *MDM2*, *p16*, aP2, Ki-67 (clone K-2), vimentin	Calretinin	S100	
Dedifferentiated liposarcoma:	CDK4, MDM2, p16, aP2, Ki-67 (clone K-2), vimentin	S100		
Pleomorphic liposarcoma:	*S100*, aP2, Ki-67 (clone K-2), vimentin		MDM2	

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Markers and Immunoprofile of Peripheral Nerve and Nerve Sheath Tumors

26

Content

Diagnostic Antibody Panel for Peripheral Nerve and Nerve Sheath Tumors S100, CD56, PGP 9.5, myelin basic protein, glial fibrillary acidic protein (GFAP), and neurofilaments

Myelin basic protein		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Neurogenic sarcoma, neuroma, neurofibroma, ganglioneuroma	Granular cell tumor	Cells of white matter of central and peripheral nervous systems
Positive control: brain tissue		

Diagnostic Approach Myelin basic protein (MBP) is a major component of the myelin sheath produced by oligodendrocytes and Schwann cells. It is localized in myelin surrounding nerve fibers in both the central and the peripheral nervous systems and takes a part in formation and stabilization of neuronal structures. Antibodies to MBP are used as a marker for neuroma, neurofibroma, and neurogenic sarcoma but are negative in other spindle cell tumors.

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Neurofilaments		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Medulloblastoma, retinoblastoma, neuroblastoma, ganglioglioma, paraganglioma, neurofibroma	Merkel cell tumor, pancreatic endocrine neoplasms, carcinoid, small cell carcinoma, parathyroid tumors, pheochromocytoma	Neuronal cells
Positive control: brain tissue		

Diagnostic Approach Neurofilaments are intermediate filament proteins, heteropolymers composed of four subunits (light, medium, high, and internexin or peripherin). They are the main cytoskeletal element in nerve axons and dendrites of both central and peripheral nervous systems providing neuronal structural support and regulate the axon diameter and the transmission of electrical impulses. Neurofilaments are good markers for tumors derived from neurons and ganglion cells and label tumors with neuronal differentiation.

Diagnostic Pitfalls The expression of the neurofilaments is reported in rare cases of non-neurogenic tumors such as rhabdomyosarcoma and epithelioid sarcoma and rare carcinoma types.

Protein gene product 9.5 (PGP 9.5)		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Malignant nerve sheath tumor, neuroblastoma, paraganglioma	Neuroendocrine tumors, Merkel cell carcinoma, granular cell tumor, atrial myxoma	Neurons and nerve fibers, neuroendocrine cells, melanocytes, distal renal tubular epithelium, spermatogonia, Leydig cells
Positive control: brain tissue		

Diagnostic Approach Protein gene product 9.5 (known as ubiquitin carboxyl-terminal hydrolase-1, PGP 9.5) is an enzyme involved in the breakdown of cytoplasmic and nuclear proteins. PGP 9.5 is a neuron-specific protein expressed in the central and peripheral nervous systems and in neuroendocrine tissue. Antibodies to PGP 9.5 are good markers to highlight neuronal and neuroendocrine tumors (Fig. 26.1).

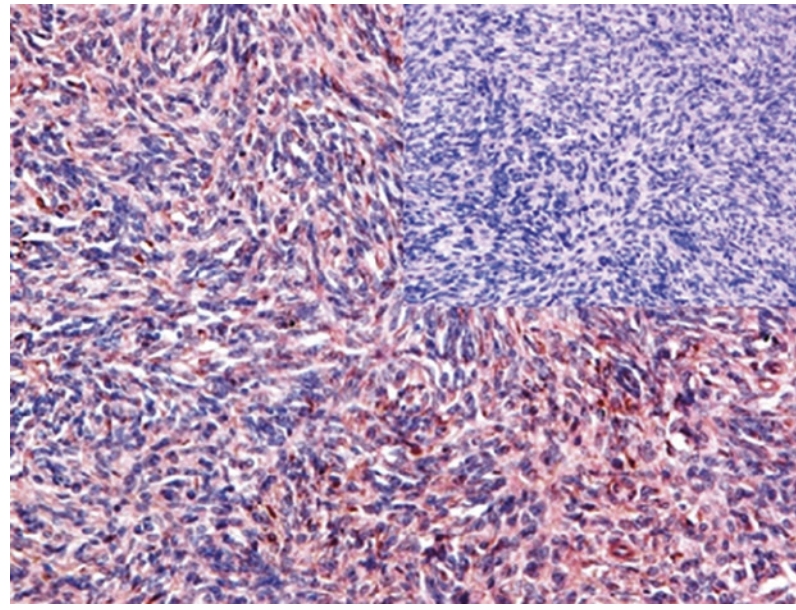

Fig. 26.1 PGP 9.5 labeling cells of neurogenic sarcoma

Diagnostic Pitfall PGP 9.5 has a low specificity and found to be expressed in a number of non-neuronal tumors [1].

Sox-10: Sox-10 is a neural crest transcription factor involved in the maturation and differentiation of melanocytes and Schwann cells (see Chap. 19). Sox-10 is normally expressed in melanocytes, Schwann cells, and myoepithelial cells. Besides melanocytic tumors, Sox-10 stains also schwannomas, neurofibromas (Fig. 26.2), granular cell tumors (Fig. 26.3), and clear cell sarcoma and is found in up to 60% of malignant peripheral nerve sheath tumors [2, 3].

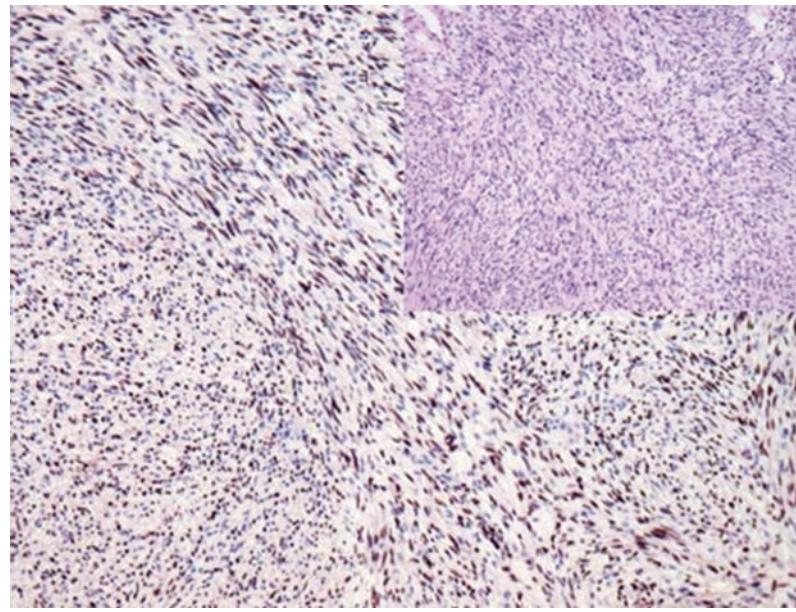

Fig. 26.2 Nuclear Sox-10 expression in cells of neurofibroma

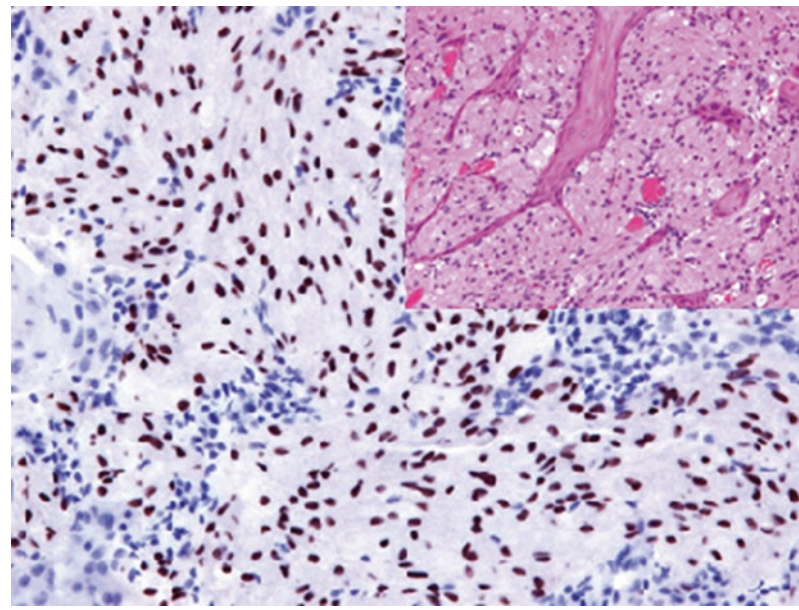

Fig. 26.3 Strong nuclear Sox-10 expression in cells of granular cell tumor

Immunoprofile of peripheral, cranial, and paraspinal nerve tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Neurofibroma:	*CD34*, *Sox-10*, claudin-1, collagen IV, vimentin Proliferation index (Ki-67): Benign: ~5%, Atypical: >8%	S100, neurofilaments, bcl-2	GFAP	EMA, CD56, calretinin
Neurilemmoma (schwannoma):	*S100*, *calretinin*, D2-40	Sox-10, CD56, leu7 (CD57), CK1, NGFR (gp75), TLE1, bcl-2	GFAP, CD34 (in Antoni B areas)	Neurofilaments, CK5/6, CK7, CK, CK18, CK20
Perineurioma:	Claudin-1, Glut-1, EMA	CD56		CD34, CD117, S100, actin, desmin, GFAP
Paraganglioma:	*Chief cells:* NSE, CD56, synaptophysin	PGP9.5, chromogranin, VIP, serotonin, somatostatin, bombesin	GFAP	S100, Pan-CK, EMA
	Sustentacular cells: CD56, S100 (in benign paraganglioma)	GFAP, S100 (in malignant paraganglioma)		Synaptophysin
Neurothekeoma (dermal nerve sheet myxoma):	S100, NGRF, GFAP, Col IV	CD34	EMA, CD57 (leu7), calponin	Actin neurofilaments, Pan-CK, NSE
Cellular neurothekeoma:	CD63 (NK1-C3), PGP9.5	Actin, NSE, desmin, CD10		S100
Granular cell tumor:	S100, *Sox-10*, CD56, NSE, laminin, nestin	PGP 9.5, calretinin, CD68	CD56	GFAP, neurofilaments, EMA, Pan-CK
Pigmented (melanotic) neuroectodermal tumor of infancy:	Pan-CK, HMB45	Sox-10, NSE	Synaptophysin	
Malignant nerve sheet tumor:	Myelin basic protein, PGP9.5 Proliferation index (Ki-67): 5–38% (main ~18%)	CD57 (leu7), NGFR (gp75), CD99, S100, bcl-2, c-MET	Sox-10, CD34, GFAP, EMA, EGFR, bcl-2, CD56	Pan-CK, HMB45, Melan A

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Markers and Immunoprofile of Central Nervous System Tumors

27

Contents

27.1 Diagnostic Antibody Panel for Tumors of the Central Nervous System

GFAP, MAP2, NeuN, Olig-2, neurofilaments, synaptophysin, pan-cytokeratin, Ki-67.

Glial fibrillary acidic protein (GFAP)		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
CNS tumors (astrocytoma, glioblastoma, oligodendroglioma, medulloblastoma, ependymoma), retinoblastoma, neurilemoma, neurothekeoma, MPNST	Salivary gland tumors (myoepithelial tumors, basal cell adenoma/carcinoma, pleomorphic adenoma), neuroblastoma, osteosarcoma, chondrosarcoma	Astrocytes, subset of CNS ependymal cells, cells of choroid plexus, Schwann cells, Kupffer cells, myoepithelial cells, chondrocytes
Positive control: brain tissue		

Diagnostic Approach Glial fibrillary acidic protein (GFAP) is a member of class III of intermediate filament proteins. GFAP is mainly expressed in neuroglia including astrocytes and ependymal cells. Lower expression levels are found in Schwann cells, paraganglial cells, enteric glial cells, Kupffer cells of the liver, osteocytes, chondrocytes, and myoepithelial cells. GFAP is a

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marker of neoplastic glial cells and glial differentiation. Lower GFAP expression level is also found in neurilemoma and neuroblastoma.

Diagnostic Pitfalls GFAP is an important marker to discriminate between primary brain and metastatic tumors; however, it can be expressed in non-glial tumors such as myoepithelioma and myoepithelial component of different types of salivary gland tumors, osteosarcoma, chondrosarcoma, and angiosarcoma.

Microtubule-Associated Protein 2 (MAP2): MAP2 is one of the five members of the microtubule-associated protein family. This protein is a neuron-specific cytoskeletal protein found in three isoforms a, b, and c expressed in neurons and reactive astrocytes. MAP2 labels the cytoplasm of the neuronal cell body and basal dendrites and is considered as an early marker for neuronal differentiation. In immunohistochemistry, MAP2 is used as a marker of neuronal differentiation. Positive stain is found in glial tumors, medulloblastoma, neuroblastoma, pulmonary neuroendocrine tumors, a subset of melanomas, and some carcinoma types (mainly thyroid and prostate).

Neuronal Nuclear Antigen (NeuN): NeuN (also known as FOX-3 protein) is a low molecular weight protein localized in the nuclei and cytoplasm of most neuronal cells of the central and peripheral nervous system and tumors derived from these cells. NeuN is a marker for central neurocytoma and gangliogliomas. The majority of PNETs of the CNS and medulloblastoma are also NeuN positive. Less than 5% of astrocytic and oligodendroglial tumors show NeuN expression.

Oligodendrocyte Lineage Transcription Factor 2 (Olig-2): Olig-2 is a transcription factor involved in the regulation of neuroectodermal progenitor cells and development of oligodendrocytes and motoneurons. Normally, Olig-2 is strongly expressed in oligodendroglial cells and oligodendroglioma. Weak to moderate Olig-2 expression is also found in all other gliomas including glioblastoma. Olig-2 expression is also reported in neuroendocrine carcinomas and in a small subset of central neurocytoma and supratentorial ependymoma.

27.2 Diagnostic Antibody Panel for Meningeal Tumors

S100, podoplanin, nestin, claudin-1, pan-cytokeratin, EMA, CEA, vimentin, Ki-67.

Characteristic for meningeal tumors is the co-expression of EMA, pan-cytokeratin, and S100. Other markers such as podoplanin (D2 40) are useful to confirm the diagnosis mainly in aggressive tumor types such as atypical and anaplastic meningioma (Fig. 27.1). For the assessment of tumor grade, the estimation of Ki-67 proliferation index is essential. The CEA expression is characteristic for the pseudopsammoma bodies found in secretory meningioma (Fig. 27.2).

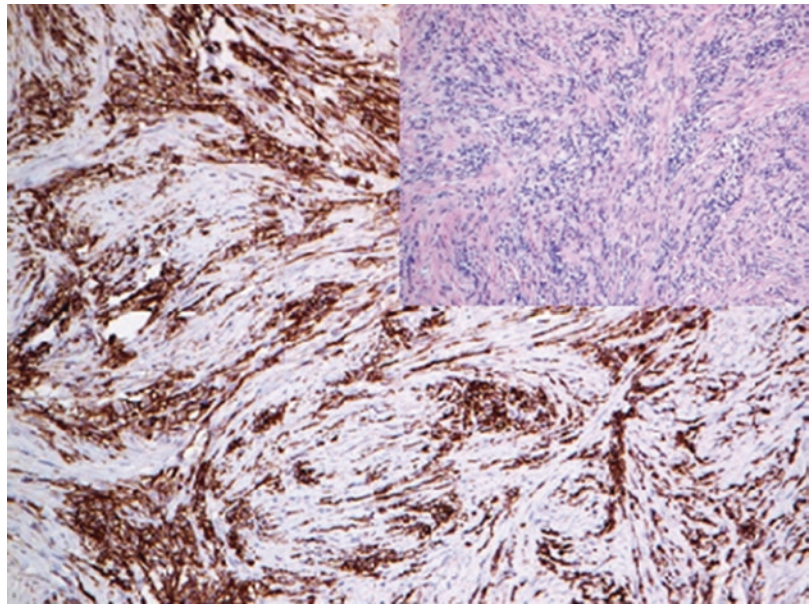

Fig. 27.1 Strong podoplanin (D2-40) expression in anaplastic meningioma

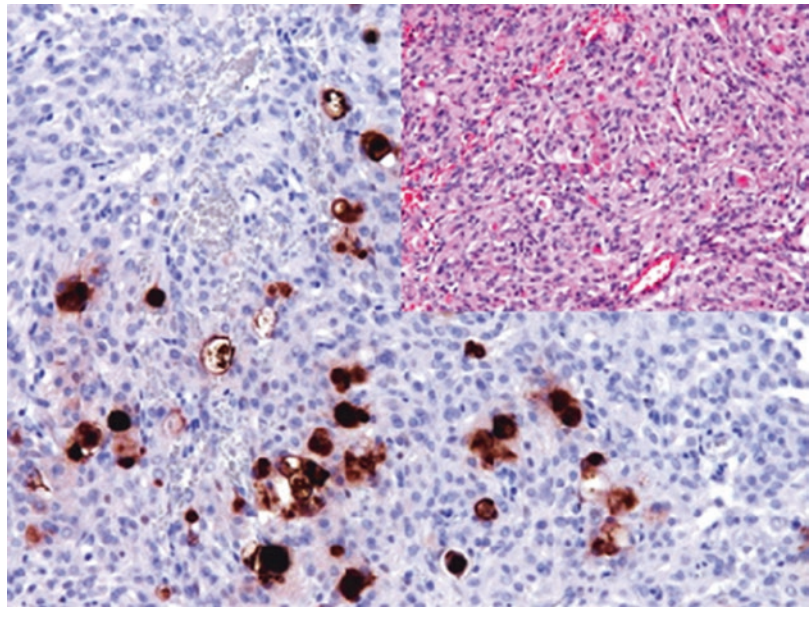

Fig. 27.2 Secretory meningioma with CEA-positive pseudopsammoma bodies

Immunoprofile of central nervous system tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (±)	+ in 10–50% (∓)	+ in <10% (−)
A. Astrocytic tumors				
– Pilocytic astrocytoma (grade I) – Diffuse astrocytoma – (grade II) – Anaplastic astrocytoma (grade III) – Glioblastoma (grade IV)	*GFAP*, S100, NSE, Olig-2, bcl-2 (only in gemistocytic astrocytoma) Proliferation index (Ki-67): Diffuse astrocytoma: <5% Anaplastic astrocytoma: 5–10% Glioblastoma: >15% (5–40%)	CD56, CD99, HER-2	Synaptophysin, pan-CK[a]	Chromogranin, CK7, CK20, neurofilaments
Diffuse midline glioma (grade IV)	CD56, Olig-2, S100	GFAP, MAP2	Synaptophysin	Chromogranin
Subependymal giant cell astrocytoma (grade I)	GFAP, S100, NSE, Olig-2			
– Pleomorphic xanthoastrocytoma (grade II) – Anaplastic pleomorphic xanthoastrocytoma (grade III)	GFAP, S100 Proliferation index (Ki-67) in grade II: <1%	Synaptophysin, neurofilaments, CD34, MAP2		
Astroblastoma	S100, vimentin	*GFAP*	EMA	
Chordoid glioma of the third ventricle (grade II)	*GFAP*, *TTF-1*, CD34	S100	EMA	
Angiocentric glioma (grade I)	*GFAP*, S100 Proliferation index (Ki-67): <5%		EMA	Synaptophysin, chromogranin
B. Oligodendroglial tumors				
Oligodendroglioma (grade II)	S100, NSE, synaptophysin, MAP-2, Olig-2, SOX-10 Proliferation index (Ki-67): <5%			Pan-CK, EMA
Anaplastic oligodendroglioma (grade III)	S100, NSE, synaptophysin, MAP-2, CD57 Proliferation index (Ki-67): >10%	GFAP, CD56, vimentin	Chromogranin, pan-CK	EMA, neurofilaments
C. Ependymal tumors				
Subependymoma (grade I)	*GFAP* Proliferation index (Ki-67): <1%	NSE, CD56, STAT-3		
Myxopapillary ependymoma (grade I)	*GFAP*, S100, vimentin	CD56, CD99	Pan-CK	

Immunoprofile of central nervous system tumors				
Ependymoma/anaplastic ependymoma (grade II/III):	*Podoplanin*, *GFAP*, S100, nestin	EMA, TTF-1[b]	Synaptophysin, CD99, pan-CK	Chromogranin
D. Tumors of the choroid plexus				
– Choroid plexus papilloma (grade I) – Atypical choroid plexus papilloma: (grade II) – Choroid plexus carcinoma (grade III)	*Podoplanin* (D2-40), pan-CK, stanniocalcin-1, Kir7.1 Proliferation index (Ki-67): Choroid plexus papilloma: <6% Choroid plexus carcinoma: >6%	Transthyretin, S100, CK7, CD44, vimentin	GFAP, EMA, synaptophysin	Chromogranin, CD56, SOX10
E. Neuronal and mixed neuronal glial tumors				
Desmoplastic infantile astrocytoma and ganglioglioma: (grade I) – Leptomeningeal component – Poorly differentiated neuroepithelial component	Vimentin *GFAP*, MAP2, vimentin Proliferation index (Ki-67): <5%	GFAP	Actin	
Dysembryoplastic neuroepithelial tumor (grade I)	*Oligodendroglia-like cells*: S100, Olig-2 Proliferation index (Ki-67): <8%	Neurofilaments, MAP2, β-tubulin	Synaptophysin	GFAP
Ganglioglioma and gangliocytoma (grade I)	*Neuronal/ganglion cells*: neurofilaments, synaptophysin, MAP2 *Astrocytic cells*: S100, GFAP Proliferation index (Ki-67): <3%	CD34	S100	GFAP, pan-CK
Central and extraventricular neurocytoma (grade II)	*Synaptophysin*, NeuN		S100, GFAP	Pan-CK, chromogranin, neurofilaments
Cerebellar liponeurocytoma (grade II) neuronal component	Synaptophysin, MAP2, NSE		GFAP	
Papillary glioneuronal tumor (grade I) perivascular cells neuronal cell component	GFAP synaptophysin, NeuN			Chromogranin
Rosette-forming glioneuronal tumor of the fourth ventricle (grade I)	*Neurocytic perivascular cells*: synaptophysin, NSE *Glial cells:* GFAP, S100	MAP-2		GFAP, S100

Immunoprofile of central nervous system tumors				
Spinal paraganglioma (grade I)	*Chief cells*: synaptophysin, chromogranin, NSE, NF *Sustentacular cells*: S100	S100 GFAP	Pan-CK	
F. Tumors of the pineal region				
Pineocytoma (grade I) and pineoblastoma (grade IV)	Synaptophysin, neurofilaments, NSE	β-tubulin, PGP9.5, chromogranin, serotonin	S100	GFAP, pan-CK
Pineal parenchyma tumor of intermediate differentiation (grade II–III)	Synaptophysin, NSE	Neurofilaments, chromogranin, S100		
Papillary tumor of the pineal region (grade II–III)	*Pan-CK*, NSE, S100, MAP2, vimentin	GFAP	Synaptophysin, chromogranin, EMA	Stanniocalcin-1, Kir7.1, neurofilaments
G. Embryonal tumors				
Medulloblastoma (grade IV)	S100, *CD56*, nestin, β-tubulin, vimentin	MAP2, NSE, synaptophysin, PGP9.5, neurofilaments, PAX-8	GFAP, bcl-2, chromogranin	CD99, Sox-2, PAX-2
Neuroblastoma (grade IV)	Synaptophysin, neurofilaments, S100, NSE, β-tubulin, vimentin	GFAP		
Embryonal tumor with multilayered rosettes (grade IV)	*Neuroepithelial cells*: nestin, vimentin *Neuropil-like areas*: synaptophysin, NeuN, neurofilaments		Pan-CK, EMA, CD99	
Medulloepithelioma (grade IV)	*Neuroepithelial neoplastic cells*: synaptophysin, neurofilaments, nestin, vimentin	Neurofilaments	Pan-CK, EMA	GFAP, NSE
Ependymoblastoma (grade (IV)	S100, vimentin	Pan-CK, GFAP		
Atypical teratoid/rhabdoid tumor (grade IV)	EMA, vimentin	sm-Actin, GFAP, neurofilaments, pan-CK		Desmin, AFP, PLAP

Immunoprofile of central nervous system tumors				
H. Meningeal tumors				
Meningioma (intra- and extracranial)	*S100*, vimentin Proliferation index (Ki-67): – Meningioma (grade I): >4% – Atypical meningioma (grade II): 6–10% – Anaplastic meningioma (grade III): >10% Progesterone receptor expression: – Meningioma (grade I): ~ 60–90% – Atypical meningioma (grade II): ~20–40% – Anaplastic meningioma (grade III): <20%	Podoplanin, nestin, claudin-1, NSE, CD141, *EMA*, *pan-CK*, CK8/18, CD99, PgR, CEA[c], ERG[d]	Osteonectin, CD34, CK7, bcl-2	*GFAP*, synaptophysin, chromogranin, CD56, CK5/6, CK20, neurofilaments

[a]Mainly found in epithelioid glioblastoma
[b]In ependymoma of the third ventricle
[c]Characteristic for secretory type meningioma
[d]Characteristic for fibrous meningioma

Markers and Immunoprofile of Ewing's Sarcoma/Primitive Neuroectodermal Tumors (PNETs)

28

Content

Diagnostic Antibody Panel for Ewing's Sarcoma/Primitive Neuroectodermal Tumors CD99, Fli-1, NKX2.2, DAX-1, CD56, chromogranin, and synaptophysin.

CD99 (MIC2)		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Ewing's sarcoma/PNET, T- and B-ALL, solitary fibrous tumor	T-cell lymphoma, anaplastic large cell lymphoma, AML, GIST, various carcinomas including the breast and prostate and hepatocellular carcinoma, thymoma, ovarian sex cord-stromal tumors, synovial sarcoma, rhabdomyosarcoma, osteosarcoma, atypical fibroxanthoma, Merkel cell carcinoma, endocrine and neuroendocrine tumors, desmoplastic small round cell tumor, Wilms' tumor, melanoma, nephroblastoma, ependymoma, mesenchymal chondrosarcoma, extrarenal malignant rhabdoid tumor, meningeal hemangiopericytoma	Cortical thymic lymphocytes, T cells and activated B cells, ovarian granulosa cells, Sertoli cells, pancreatic islet cells, endothelial cells, fibroblasts, ependymal cells, urothelium
Positive control: PNET		

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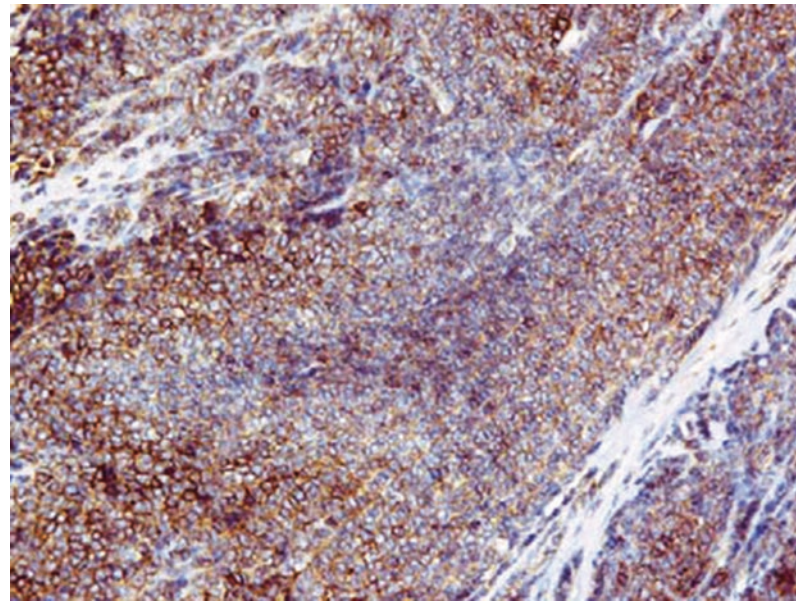

Fig. 28.1 Ewing's sarcoma with strong membranous CD99 expression

Diagnostic Approach CD99 (known as MIC2 or E2 antigen) is a cell surface glycoprotein expressed on the surface of cortical thymocytes and subset of mature T- and B-lymphocytes. CD99 plays a role in T-cell adhesion and leukocyte migration and extravasation. CD99 has a broad expression spectrum and found in a large number of normal and neoplastic cells. CD99 is widely used as a marker for Ewing's sarcoma/PNET family exhibiting a membranous stain, while a cytoplasmic stain can be noted in other tumor types. CD99 is negative in neuroblastoma (Fig. 28.1).

Diagnostic Pitfalls As listed in the table above, CD99 has a very wide expression spectrum and low specificity; consequently CD99 should never be used as a single marker for tumor diagnosis, especially in tumors with similar morphology such as PNET and ALL [1, 2]. A panel of more specific antibodies must be always used to confirm the diagnosis.

Fli-1		
Expression pattern: nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Ewing's sarcoma, vascular tumors	Lymphoblastic lymphoma, anaplastic large cell lymphoma, angioimmunoblastic lymphoma, desmoplastic small round cell tumor, Merkel cell carcinoma, melanoma	Endothelial cells, T-lymphocytes
Positive control: endothelial cells		

Diagnostic Approach Fli-1 gene (**f**riend **l**eukemia virus **i**ntegration site 1, also known as transcription factor ERGB) is a member of the ETS proto-oncogene family functioning as a transcriptional activator highly expressed during embryogenesis. The Fli-1 gene is the translocation partner in the t(11;22)(q24;q12) translocation, the most

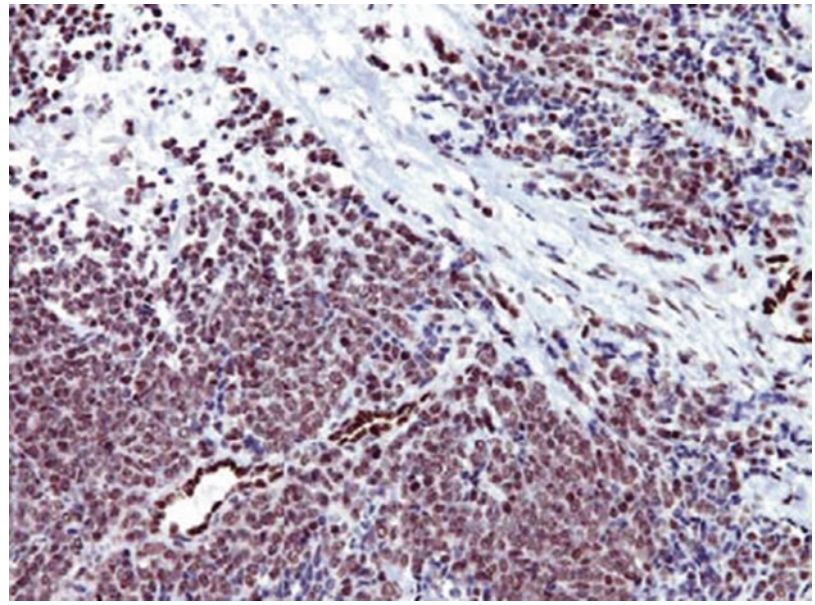

Fig. 28.2 Ewing's sarcoma showing strong nuclear Fli-1 expression. FLi-1 labels also the endothelial cells

common and the most specific molecular marker for Ewing's sarcoma/PNET family that is found in more than 90% of the cases. Available antibodies to Fli-1 gene product found to be of high specificity for the PNET family (Fig. 28.2).

Diagnostic Pitfalls The expression of the Fli-1 transcription factor is not restricted to the PNET family. Fli-1 is a good marker for vascular tumors; it is also expressed in a subset of melanoma, mainly aggressive types [3, 4]. A diagnostic pitfall is the expression of Fli-1 in the blasts of acute lymphoblastic leukemia, which are also positive for CD99 and may have a similar PNET morphology. In such cases, the expression of TdT is essential for the assessment of correct diagnosis [5].

NKX2.2: NKX2.2 is a member of the NK family of transcription factors involved in the differentiation of the ventral region of the CNS and endocrine cells of the pancreas and the gastrointestinal tract. Molecular studies demonstrate that NKX2.2 acts as a mediator for the EWS/Fli-1 translocation specific for Ewing's sarcoma. The expression of NKX2.2 was reported in more than 80% of this Ewing's sarcoma/PNET family [6–8]. NKX2.2 is normally expressed in pancreas islet cells and intestinal endocrine cells as well tumors derived from these cells.

DAX-1: DAX-1 is a nuclear receptor protein and a member of the orphan nuclear receptor family encoded by the NR0B1 gene, regulating the synthesis of steroid hormones listed in the previous chapter as a marker for adrenocortical tumors. Due to the genetic alteration caused by the EWS/Fli-1 translocation that induce the expression of DAX-1, DAX-1 is overexpressed in Ewing's sarcomas bearing this translocation [9, 10].

Neural Cell Adhesion Molecule (CD56): CD56 is a member of the immunoglobulin superfamily clustered as CD56 functioning as a mediator of cell-to-cell adhesion and cell-to-matrix

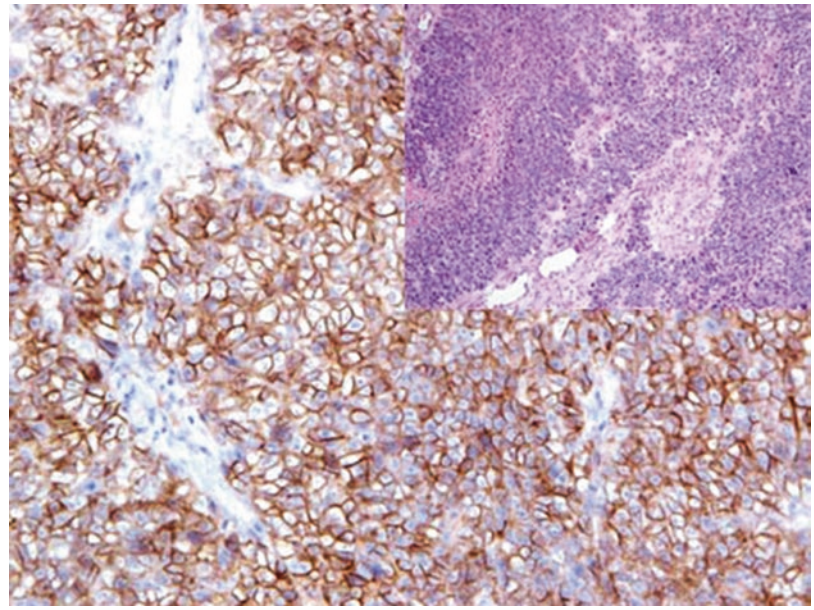

Fig. 28.3 CD56 staining the membrane of olfactory neuroblastoma cells

interaction and involved in the regulation of cell adhesion, synaptic plasticity, migration, proliferation, differentiation, and apoptosis. CD56 is an important molecule for the development and differentiation of the nervous system. Normally, CD56 is expressed on neuroectodermal cells, glial cells, myoblasts, skeletal muscle, neuromuscular junctions, and tumors derived from these cell types (Fig. 28.3). Furthermore, it is also expressed on the NK cells and activated T cells playing an important role in the immune reaction. In routine immunohistochemistry, CD56 is used as a marker for NK neoplasms as mentioned in a previous chapter, however it is also a useful marker for wide spectrum of neural and neuroendocrine tumors.

Immunoprofile of primitive neuroectodermal tumors and related lesions

Tumor type	+ in >90% (+)	+ in 50–90% (±)	+ in 10–50% (∓)	+ in <10% (−)
PNET	*CD99*, synaptophysin, vimentin	*Fli-1, NKX2.2*, NSE, CD56, S100, chromogranin, bombesin	Pan-CK	WT-1
Ewing's sarcoma	*CD99*, Vimentin	*Fli-1, NKX2.2*, vimentin	NSE, pan-CK, CD117,	Synaptophysin, CD56, WT-1
Neuroblastoma/olfactory neuroblastoma (esthesioneuroblastoma)	*CD56*, CD57, NSE, PGP9.5, neurofilaments, NB84, GATA-3, *S100*	Bombesin, synaptophysin, chromogranin, Fli-1	pan-CK	EMA, WT-1, CD99
Merkel cell carcinoma	Pan-CK, *CK20*[a] (perinuclear), EMA, NSE, Merkel cell *polyomavirus*, E-cadherin[b]	*CD56*, Fli-1, chromogranin, CK8, CK18, TdT, Pax-5	Neurofilaments, CK7[a]	S100, HMB45, CEA

[a]Perinuclear staining pattern
[b]Nuclear staining pattern

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Markers and Immunoprofile of Extraskeletal Osseous and Cartilaginous Tumors

29

Content

Diagnostic Antibody Panel for Osseous and Cartilaginous Tumors S100, osteocalcin, osteonectin, androgen receptors, SATB-2, pancytokeratin [1, 2].

Osteocalcin: Osteocalcin is a non-collagenous calcium-binding protein synthesized by osteoblasts involved in the mineralization of bone tissue and dentin. It is expressed by osteoblasts in the bone and dentin. Osteocalcin is a specific marker for bone and osteogenic tumors.

Osteonectin		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Bone tumors	Sarcomatoid renal cell carcinoma, cartilaginous tumors	Osteocytes, fibroblasts, endothelium, subset of epithelial cells
Positive control: bone tissue		

Diagnostic Approach Osteonectin is a calcium-binding bone matrix glycoprotein involved in the early mineralization steps of bone tissue. It is highly expressed in activated osteocytes. It is also expressed to a lesser degree in other cell types such as fibroblasts, endothelial cells, chondrocytes, and some epithelial types; consequently, osteonectin has a high sensitivity but low

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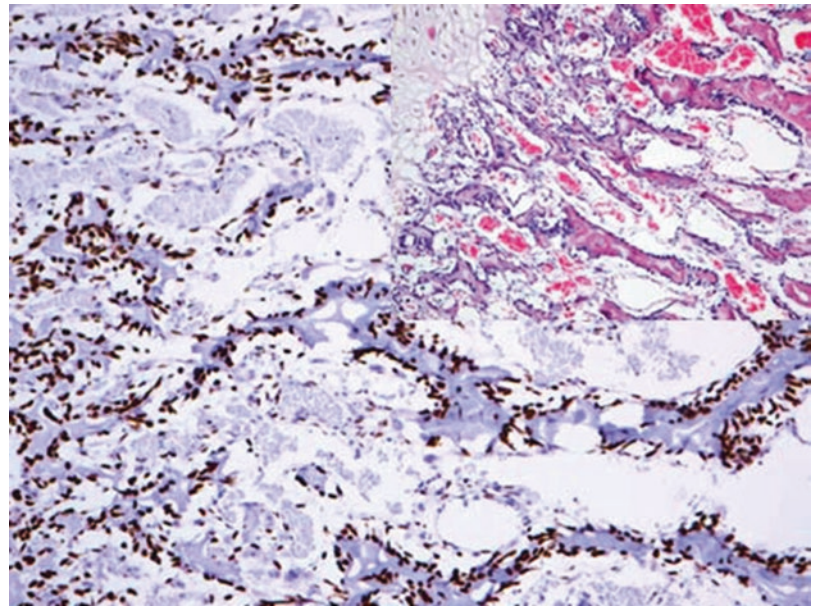

Fig. 29.1 Section of fetal bone showing osteoblasts exhibiting strong SATB-2 expression

specificity for bone tissue and bone tumors and must be a part of antibody panel.

Special AT-Rich Sequence-Binding Protein 2 (SATB-2): SATB-2 is a transcription factor and DNA-binding nuclear protein involved in the differentiation of osteoblasts. SATB-2 is normally expressed in osteoblasts (Fig. 29.1), brain, liver, kidney, and colorectal epithelium (see also Chap. 7.1.1, markers for colorectal carcinoma). SATB-2 labels neoplastic osteoblasts in both skeletal and extraskeletal osteosarcomas [3].

Immunoprofile of extraskeletal osseous and cartilaginous tumors

Tumor type	+ in >90% (+)	+ in 50–90% (±)	+ in 10–50% (∓)	+ in <10% (−)
Soft tissue chondroma	S100, vimentin			
Chondroblastoma	S100, NSE, EMA, vimentin	Pan-CK, CK8, CK18, CK19		
Mesenchymal chondrosarcoma	S100, vimentin	CD99[a], CD57	Actin	Desmin, chromogranin, pan-CK, EMA, osteonectin
Extraskeletal osteosarcoma	*Osteonectin*, vimentin	*SATB-2*, osteocalcin, androgen receptors, sm-actin, CD99	EMA, desmin, CD117	S100

[a]CD99 positive only in the small cell undifferentiated components

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Markers and Immunoprofile of Miscellaneous Tumors and Tumors of Uncertain Differentiation

30

Content

Diagnostic Antibody Panel Vimentin, pan-cytokeratin, actin, desmin, HMB45, S100, CD34, CD99, TLE-1.

Transducer-Like Enhancer of Split 1 (TLE-1): TLE-1 is one of the four transcriptional repressors expressed during the embryogenesis involved in the regulation of hematopoiesis and epithelial and neuronal differentiation [1–4]. TLE-1 is normally expressed in acinar cells of salivary glands. In routine immunohistochemistry, the expression of TLE-1 is mostly characteristic for synovial sarcoma; however, the overexpression of TLE-1 is also reported in different soft tissue tumors including endometrial stromal sarcoma, acral myxoinflammatory fibroblastic sarcoma, solitary fibrous tumor, epithelioid sarcoma, lipoma and liposarcoma, leiomyosarcoma, neurofibroma, malignant nerve sheet tumor, chordoma, mesothelioma, and undifferentiated pleomorphic sarcoma.

Transcription Factor-E3 (TFE-3): TFE-3 a transcription factor encoded by a gene located on Xp11.2. This gene is the fusion partner of the ASPL gene in the t(X;17) translocation associated with alveolar soft part sarcoma. The generated fusion transcript ASPL-TFE3 causes the activation of the TFE3 gene and the overexpression of the TFE-3 protein. The expression of TFE-3 is characteristic for alveolar soft part

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sarcoma as well as for the Xp11.2 translocation-associated renal cell carcinoma mentioned in the previous chapter [5].

Brachyury: Brachyury is a nuclear transcription factor involved in epithelial-mesenchymal transition, normally expressed in notochord and plays a role in the development of posterior and caudal body parts. In adult tissue, brachyury is expressed in the cells of spermatogenesis. In neoplastic tissue, it is a sensitive and specific marker for chordoma expressed in more than 95% of the cases. Brachyury is negative in other tumors with chordoid or myxoid differentiation that mimics chordoma such as chondrosarcoma, chordoid meningioma, and clear cell and epithelioid sarcoma. Brachyury expression is also found in a subset of pulmonary adenocarcinoma, squamous cell carcinoma, and small cell carcinoma and in different germ cell tumors including embryonal carcinoma, seminoma, and yolk sac tumor [6, 7].

Immunophenotype of miscellaneous tumors and tumors of uncertain differentiation

Tumor type	+ in >90% (+)	+ in 50–90% (±)	+ in 10–50% (∓)	+ in <10% (−)
Synovial sarcoma[a]	*Epithelioid cell components*: pan-CK, TLE-1[b], bcl-2 *Sarcomatous spindle cell components*: *TLE-1*, vimentin, *SYT*, bcl-2, calponin	SYT, CK7, CK19, EMA, HER-2 calretinin, CD99, CD56, CD57	CEA, vimentin, calponin E-cadherin, CD34, S100, CD117, pan-CK, EMA, actin	CD34 desmin, caldesmon
Clear cell sarcoma	*HMB45*, S100, MITF, vimentin	*Sox-10*, NSE, Melan A, Leu-7	MDM2, tyrosinase	Desmin, actin, pan-CK, EMA, CDK4
Epithelioid sarcoma	*Pan-CK*, EMA, vimentin	CK1, CK8, CK18, CK19, CD34, podoplanin	Actin, NSE, CK7, ERG, S100	CK5/6, CD31, FVIII, CK20, Fli-1, ERG, CEA
Desmoplastic small round cell tumor	Pan-CK, CK 8/18, EMA, WT-1, vimentin	NSE, desmin[c], CK19, CD15, CD57	CD99	CD34, CD117, actin, h-caldesmon, MyoD1, myoglobin, S100, CK5/6, CK20
Extraskeletal myxoid chondrosarcoma	Vimentin	S100, Leu 7, NSE	Synaptophysin, EMA	Chromogranin, CD68, pan-CK, CEA, actin, desmin
Rhabdoid tumor	Pan-CK, vimentin	CK8, EMA, CD99, NSE	Synaptophysin, actin	Desmin, myoglobin, CD34, S100
Alveolar soft part sarcoma	Vimentin	*TFE-3*, desmin	sm-Actin, S100, NSE, pan-CK, CD34	Synaptophysin, chromogranin, myoglobin, myogenin, MyoD1, HMB45, EMA, CD 31, CD117
Pleomorphic hyalinizing angiectatic tumor	Vimentin	CD34	EMA	CD31, F VIII, actin, desmin, pan-CK, S100
Myxoma (cutaneous, intramuscular and juxta-articular)	Vimentin, pan-CK[d]	Calretinin, CD34	Desmin, actin, CD68	S100
Myxoma of the jaw	S100, vimentin			Pan-CK, desmin

Immunophenotype of miscellaneous tumors and tumors of uncertain differentiation				
Chordoma	*Brachyury*, NSE, pan-CK, CK8/18, CK19[e] EMA, S100, vimentin	CK5, CK10, CK14, CK18, E-cadherin, β-catenin	CK7, CEA	Desmin, CK20, GFAP, D2-40
Aggressive angiomyxoma	Desmin, vimentin	Actin, CD34, ER	PgR	S100, pan-CK
Myoepithelioma (mixed tumor of soft tissue, parachordoma)	Pan-CK, vimentin	CK8/18, S100, calponin, CK5/14	EMA, desmin, sm-actin, GFAP	CK19
Angiomatoid fibrous histiocytoma	Vimentin	Desmin	CD99, EMA, CD68	CD31, CD34, pan-CK
Ossifying fibromyxoid tumor	Vimentin	S100, desmin	Actin, GFAP	Pan-CK, EMA
Intimal sarcoma	Osteopontin, vimentin	Actin, MDM2	Desmin	CD31, CD34, F VIII

[a]Demonstration of specific t(X; 18) translocation is recommended to confirm the diagnosis
[b]Not specific for synovial sarcoma
[c]Perinuclear stain
[d]Only in epithelioid components if present
[e]Negative in parachordoma

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Markers to Assist the Diagnosis of Dysplasia and Malignant Transformation

31

Ki-67: Ki-67 is a nonhistone nuclear protein expressed in active cell cycles. The expression of Ki-67 begins in the G1 phase and persists during the active phases of cell cycle throughout the S, G2, and M phases. Ki-67 is undetectable in the G0 phase or in the initial stage of the G1 phase and during DNA repair. The expression of Ki-67 strongly correlates with the intensity of cell proliferation and tumor grade. In routine histopathology, Ki-67 is an important marker for the assessment of cell proliferation. The Ki-67 index is an important criterion for tumor diagnosis (benign, borderline, malignant, low- or high-grade tumor). Furthermore, it is a helpful marker to differentiate between atrophy, thermal alterations, and dysplasia. The irregular accumulation of Ki-67-positive cells in different tissue types would suggest a tendency of the cells to escape the regulation mechanisms. In stratified squamous epithelium, the expression of Ki-67 in more than 30% of the full thickness of the epithelium above the suprabasal layers signifies an abnormal or dysplastic behavior of the epithelium. The Ki-67 index is also an important parameter to distinguish between high-grade and low-grade lymphoma.

p53: p53 is a nuclear phosphoprotein encoded by the TP53 gene located on chromosome 17p13, which in turn encodes several isoforms of the p53 protein. p53 is a tumor-suppressor protein that binds to DNA inducing the synthesis of the p21 protein, which regulates the genomic stability and binds to the cell division-stimulating protein cdk2. The p21-cdk3 complex hinders the cells to pass through to the next phase of cell division, which can activate the transcription of different preapoptotic genes and initiate the apoptosis.

Mutations within the TP53 cause the overexpression and accumulation of mutated p53 protein not able to bind DNA to stimulate the p21 synthesis acting as a stop signal in the cell cycle, consequently causing an uncontrolled proliferation of involved cells.

The overexpression of p53 is associated with different neoplastic and preneoplastic lesions. The detection of p53 by immunohistochemistry can be useful to differentiate between dysplastic or neoplastic changes usually positive for p53 and reactive changes negative for p53.

The examples listed below demonstrate the role of p53 overexpression as a criterion for the diagnosis of malignant and premalignant lesions:

- Reactive urothelium vs. urothelial carcinoma in situ and transitional cell carcinoma (Fig. 31.1).
- Flat dysplasia and DALM of colonic mucosa vs. reactive hyperplasia.
- Reactive squamous epithelium vs. cervical/vulvar intraepithelial neoplasia (CIN/VIN).
- Normal ductal mucosa of the pancreas vs. mucinous cystic neoplasia.

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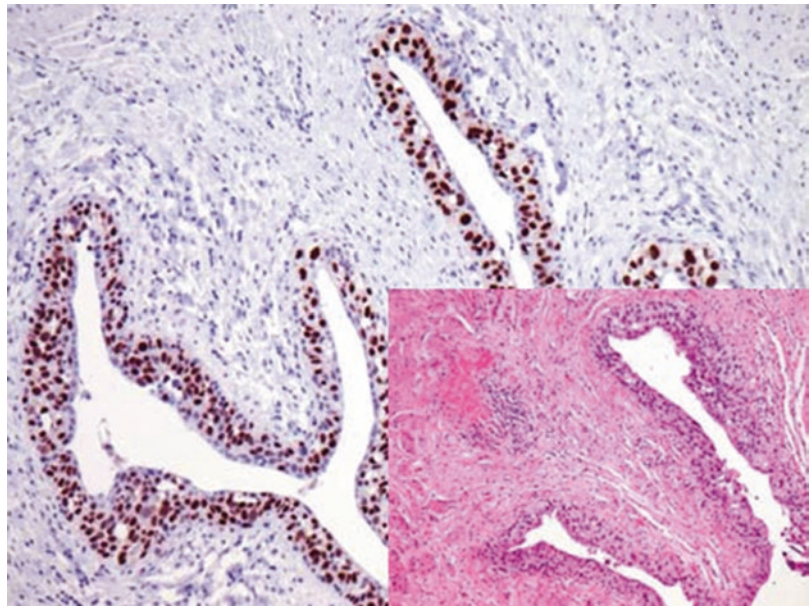

Fig. 31.1 High-grade dysplasia with carcinoma in situ of the ureter with strong p53 expression

- Dysplasia in esophageal columnar mucosa.
- Transformation of B-CLL/SLL to high-grade lymphoma (Richter syndrome).
- Secondary glioblastoma.
- p53 expression is a characteristic marker for serous uterine carcinoma.

IMP3: IMP3 is a cytoplasmic oncofetal protein listed in the mesothelioma chapter. Benign adult tissue usually lacks the expression of IMP3 with the exception of ovarian and testicular tissue, placenta, endocrine cells, and brain. In routine immunohistochemistry, IMP3 is used to discriminate between malignant and reactive proliferative lesions. Similar to GLUT1 and BAP-1, IMP3 is a helpful marker to discriminate between mesothelioma and reactive mesothelial proliferation, as the majority of benign mesothelial cells are negative for IMP3.

GLUT1: Glucose transporter 1 (GLUT1) is a member of the Glut transporter family and a membrane-associated erythrocyte glucose transport protein maintaining the basal glucose transport in most cell types. In diagnostic histopathology, GLUT1 is a potential marker for malignant transformation and is overexpressed in many types of malignant epithelial and non-epithelial tumors. It is helpful marker to discriminate between benign and malignant pancreatic glands and between reactive proliferation of mesothelial cells and malignant mesothelioma.

BAP-1: BAP-1 is a nuclear ubiquitin hydrolase functioning as transcriptional regulator and tumor suppressor listed in the mesothelioma chapter. The genomic region is found to be deleted in different fractions of several human malignancies, including mesotheliomas, uveal and cutaneous melanomas, clear cell and renal cell carcinomas, pulmonary adenocarcinomas, and meningiomas. In routine immunohistochemistry, BAP-1 is a helpful marker to discriminate reactive mesothelial proliferation or benign melanocytic lesions positive for BAP-1 and malignant mesothelioma and malignant melanoma that lack the nuclear expression of BAP-1.

Carcinoembryonic Antigen (CEA): CEA is an oncofetal glycoprotein normally expressed by colonic mucosa of fetal colon and to a lesser degree in adult colonic mucosa. CEA is highly expressed in different adenocarcinoma types of various origins. The overexpression of CEA in adenomas or premalignant lesions correlates with

the grade of dysplasia and can be an indicator for malignant transformation.

CD24: CD24 is a glycoprotein and cell adhesion molecule expressed on the surface of most B-lymphocytes and mature granulocytes, squamous epithelium, renal tubules, and differentiating neuroblasts in addition to regenerating tissue.

In neoplastic lesions, CD24 plays an important role as a mediator for proliferation and invasion. Generally, the overexpression of CD24 in tumors is associated with an aggressive behavior and poor prognosis. The overexpression of CD24 was reported in different tumor types including colorectal carcinoma, cholangiocarcinoma, breast carcinoma, prostatic carcinoma, and uterine cervix carcinomas. The overexpression of CD24 detected by immunohistochemistry on paraffin sections is a putative marker for dysplasia in oral and cervical mucosa.

P16: The p16 protein is a cyclin-dependent kinase inhibitor A2 encoded by the CDKN2A gene. The p16 protein plays an important role in preventing the cell cycle to progress from G1 to S phase acting as a tumor-suppressor gene. The expression of p16 is regulated by the activity of the retinoblastoma gene (Rb), which in turn is effected by the E7 oncogene which is one of the HPV genes. p16 is overexpressed in HPV-associated intraepithelial dysplasia and squamous cell carcinomas of different origin including vulvar, vaginal, and cervical squamous cell carcinoma in addition to oropharynx carcinoma.

The CDKN2A gene is also the subject of different mutations or deletions seen in many other epithelial and mesenchymal tumors.

p16 is a very useful marker to distinguish between benign lipoma negative for p16 and well-differentiated liposarcoma positive for p16 (see markers of adipocytic tumors).

Recommendations for the Utility of Immunohistochemistry in Tumor Diagnosis

32

Immunohistochemistry is a powerful and sensitive diagnostic tool for tumor diagnosis that requires high level of practical and theoretical knowledge. The precise tumor diagnosis begins with the adequate processing of tissue samples and includes the standardized stain technique, the optimal choice of diagnostic antibody panels, and ends with critical interpretation of stain results. In order to utilize all the benefits of immunohistochemistry and to minimize the possibilities of errors in tumor diagnosis, we recommend to consider the following points:

1. Initially it is important to remember that the careful histopathologic examination and clinical correlation remain the cornerstone of morphologic diagnosis. The immunoprofiling is to support or rule out one or more of possible differential diagnoses.
2. The laboratory of immunohistochemistry must be under the supervision of well-trained pathologist, highly skilled in methods and techniques of immunohistochemistry and has the necessary morphologic knowledge to do good and critical interpretation of immunohistochemical staining results.
3. The single marker immunohistochemistry is one of the most frequent sources of errors in tumor diagnosis. No single marker can be relied on exclusively. The use of adequate panel of antibodies helps to avoid misinterpretation; it is always advisable to confirm or exclude the diagnosis by two or more additional immunohistochemical markers.
4. Knowledge of the nature of targeted antigens is an important factor in the interpretation of the results. The following details are always to consider:
   - The expression pattern of the antigens (nuclear, cytoplasmic, membranous, or extracellular).
   - Stability of antigens during tissue processing. Optimal and standardized tissue fixation and processing and as a rule, bad H&E sections mean bad immunohistochemistry results.
   - Histopathologists deal with neoplasia with heterogeneous cell populations with high potential of genotypic and phenotypic variations. The reason for the atypical antigen expression can be in the biology of the tumor or the nature of the antibodies used.
5. Features of any new antibody must be carefully studied, and the following parameters are to consider:
   - Type of the antibody: polyclonal or monoclonal in addition to the clone type of the monoclonal antibody.
   - Sensitivity and specificity of the antibody in addition to the recommended dilution of concentrated antibodies.
   - Care must be exercised when using newly developed antibodies. New antibodies are

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often introduced as being highly specific, but after prolonged use or testing on tissue microarrays, many of them prove to be less specific.
   - The specificity and sensitivity of used detection system.
6. The standardization of the immunohistochemical staining method is one of the essential factors for correct interpretation of stain results. Positive and negative controls are valuable for good interpretation.
7. The interpretation and documentation of immunohistochemical results must be standardized. It is not enough to interpret the staining result as positive or negative. Quality and intensity of stain and staining pattern must be also considered and documented, and any conflicting results must be analyzed. Standardized reporting is very helpful in organizing the information to reach an accurate diagnosis.
8. Despite the high sensitivity of immunohistochemistry and the large number of available antibodies, immunohistochemistry—as any method—has its own limits. We should never force the diagnosis based on unclear or unspecific results. Some cases must be clarified or confirmed by additional methods. The detection of specific translocations or other genetic abnormalities associated with various types of neoplasia by molecular methods is an example where we need other methods to obtain a precise tumor diagnosis.

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