Immunohistochemistry in Tumor Diagnostics

Muin S.A. Tuffaha
Hans Guski
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Second Edition

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This book is dedicated to the memory of my parents, Sami and Haya, and to my wife, Ayah.

Muin S. A. Tuffaha

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

Hans Guski

I dedicate this book to my lovely children, Charlotte, Clara, and Karl.
Glen Kristiansen

Preface

In recent years, there has been a significant surge in the development of methods in diagnostic tumor histopathology, particularly in the areas of molecular techniques and immunohistochemistry.

In diagnostic immunohistochemistry, this book provides an extensive overview of the antibodies employed in diagnostic tumor histopathology and presents concise summaries of the immunoprofiles of most tumors. Additionally, it offers practical diagnostic algorithms and invaluable tips to facilitate the interpretation of results.

In the second edition, all chapters have undergone careful review, ensuring that the immunohistochemical profiles of various tumors are updated considering the newest available antibodies and the most recent fifth edition of the WHO classification of tumors. Furthermore, a chapter on biomarkers for theranostic applications has been added to enhance the book's utility.

Designed as a practical and user-friendly bench reference for diagnostic tumor histopathology, this book is recommended to histopathologists of all levels, from aspiring residents to seasoned specialists seeking a reliable and comprehensive resource for tumor diagnostics. Moreover, it is highly recommended for oncologists, hematologists, and researchers involved in these fields.

Cottbus, Germany
June 2023

Muin S. A. Tufaha

Introduction

During the last years, classical histopathology was rapidly developed and has now beside conventional microscopy and electron microscopy a number of additional highly sensitive diagnostic tools, including immunohistochemistry, cytogenetics, and molecular pathology. These methods provide further objective and reproducible criteria for the 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. In the last 30 years, immunohistochemistry has dramatically developed into a highly specialized molecular technique combining the principles of immunology, biochemistry, and histology and has become a potent tool in daily diagnostic histopathology. Nowadays, we have several thousands of monoclonal and polyclonal antibodies specific to cellular and extracellular structures and biomarkers. To merge proteomics or epitomics into a morphological context is an invaluable asset to the discerning, knowledgeable pathologist and immunohistochemistry is now an essential tool 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 essential for tumor staging and planning of therapeutic strategies.

Immunohistochemistry is also helpful in determining the sensitivity of different tumors to several types of specific therapeutic agents such as steroid-receptor-antagonists, humanized monoclonal antibodies, and drug-conjugated antibodies in addition to different enzyme antagonists, including tyrosine-kinase inhibitors. Furthermore, immunohistochemistry offers several significant prognostics and etiopathological markers interesting for tumor follow-up and research. However, it must also be said that quantitative immunohistochemistry is still evolving, and it is doubtful that cut-off-based prognostic immunohistochemistry, as practiced today in many research papers, will largely contribute to future precision medicine.

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Immunohistochemistry in Tumor Diagnosis

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1.1 Expression Pattern and Diagnostic Pitfalls

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

1. Nuclear expression pattern: characteristic for antigens expressed in cellular nuclei or on the nuclear membrane. This pattern is characteristic for transcription factors, intranuclear enzymes, and steroid hormone receptors.
2. Cytoplasmic expression pattern: characteristic for antigens located in the cytoplasm. Typical examples are the cytoskeleton proteins, including microfilaments, microtubules, and intermediate filaments such as Vimentin, Actin, Desmin, and Cytokeratins. Some antigens display a further restricted cytoplasmic staining pattern and stain-specific organelles, such as mitochondria (leading to granular cytoplasmic staining) or the Golgi apparatus (unilateral perinuclear pattern).
3. Membranous expression pattern: characteristic for antigens located within the cell membrane; typical examples are the majority of cluster of differentiation (CD) antigens and adhesion molecules.
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 carcinoembryonic antigen (CEA).

The intensity of the stain and the quantity of positive cells are additional parameters to consider in the interpretation of stained sections. It is noteworthy to mention that some antigens have

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different expression patterns depending on the phase of the cell cycle or the differentiation stage of examined cells, such as the immunoglobulin expression in lymphoid tissue and lymphoid neoplasm. Other antigens have a unique expression pattern characteristic for some specific tumors.

Finally, it is essential to remember that the interpretation of immunohistochemical results is not the description of positive or negative stains. The conventional hematoxylin and eosin (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 of Primary Tumors and 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 an initial diagnostic panel, the histomorphology of the examined tumor, the tumor location and clinical data, as well as the specificity and sensitivity of the available antibodies must be considered.

For the initial classification of tumors with ambiguous morphology or tumors with undetermined histogenic differentiation, we found that the most informative, time and money saving is a primary panel that includes broad spectrum antibodies reacting with epithelial, mesenchymal, neuroendocrine, hematopoietic, and germ cell lines (Algorithm 1.1) [1–4].

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

1. Pan-Cytokeratin (Cytokeratin cocktail)
2. LCA (leukocyte common antigen)
3. Chromogranin/S100 or INSM-1
4. Sox-10 (or another melanoma marker)
5. Oct4/SALL-4

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

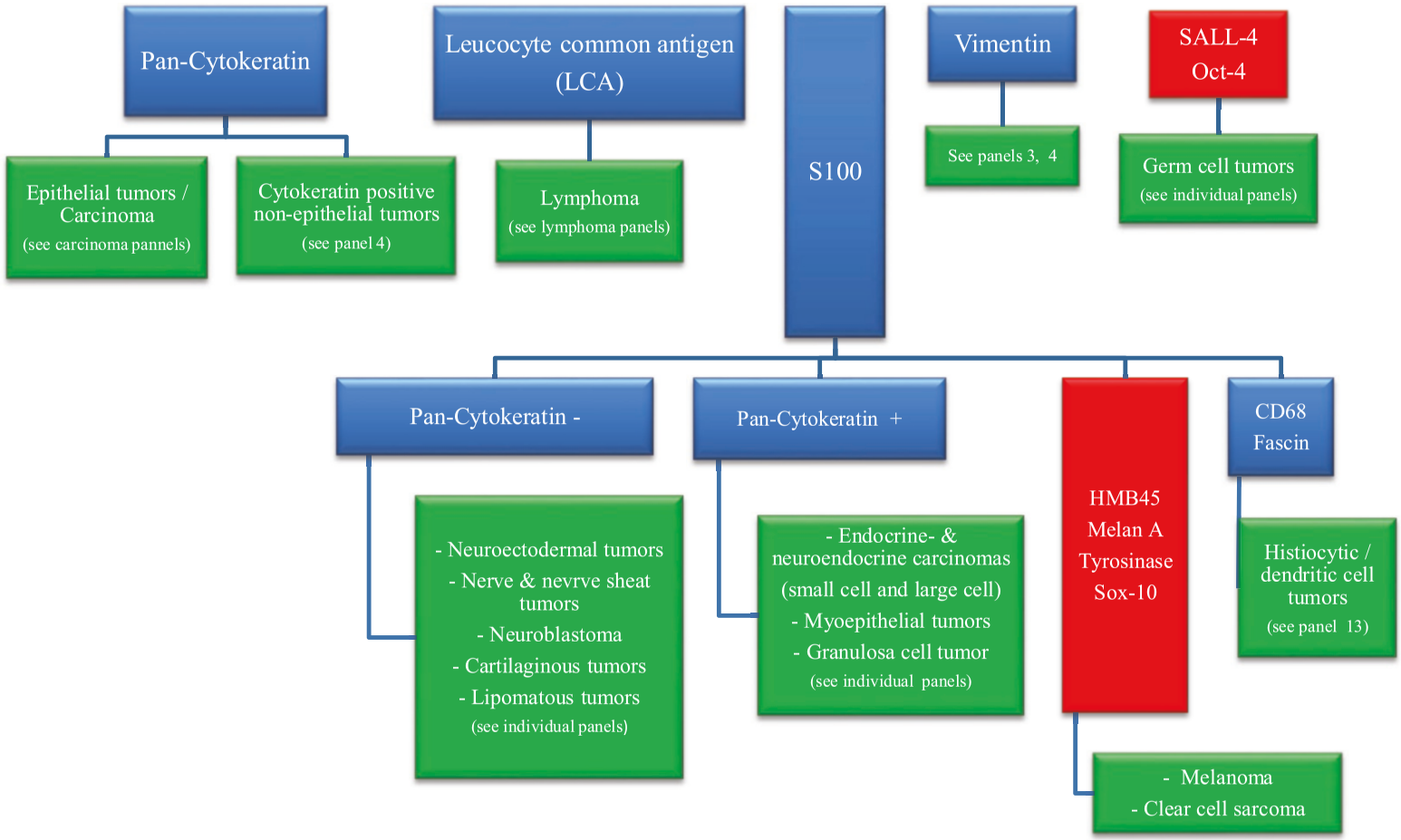
If the tumors reveal the typical small round blue cell morphology, another screening antibody panel is necessary and can include the following antibodies (Algorithm 2):

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

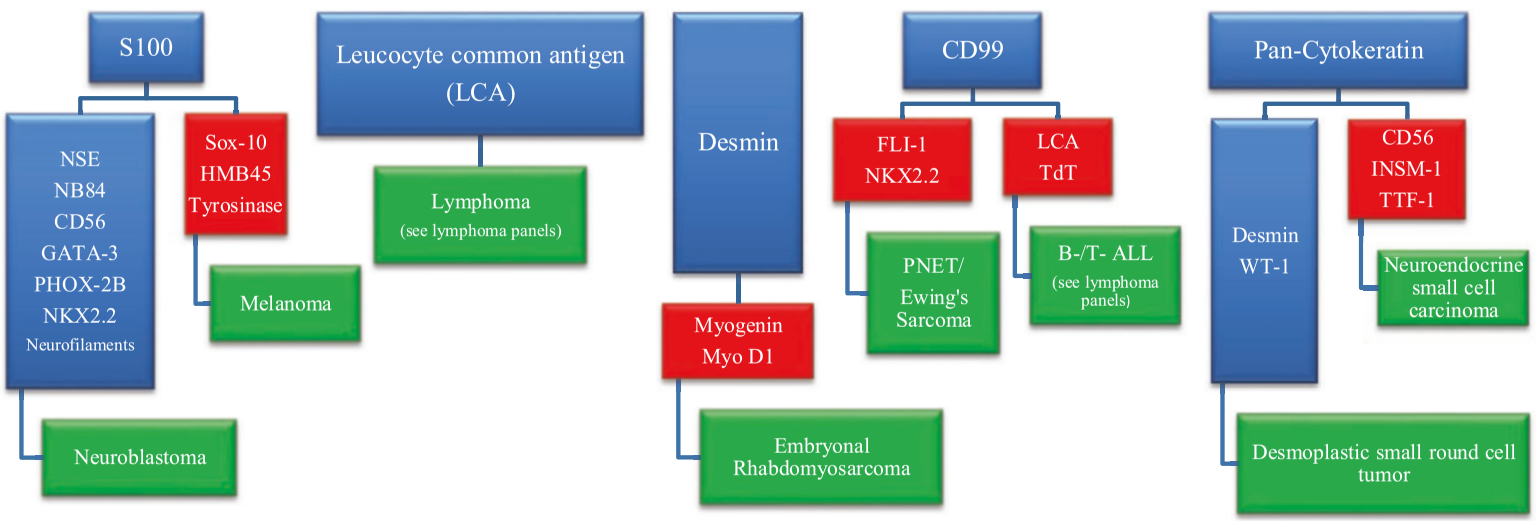
This panel can be modified according to the patient's age, tumor location, and clinical history. Adding one or more tissue—or organ-specific markers to the initial diagnostic panel can give additional valuable diagnostic information.

For orientation, we suggest a group of diagnostic algorithms to ease solving the most common diagnostic problems (Algorithms 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 1.10, 1.11, 1.12, and 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. It is always important to remember that there is no antibody exclusively specific for a certain tissue type or particular tumor entity.

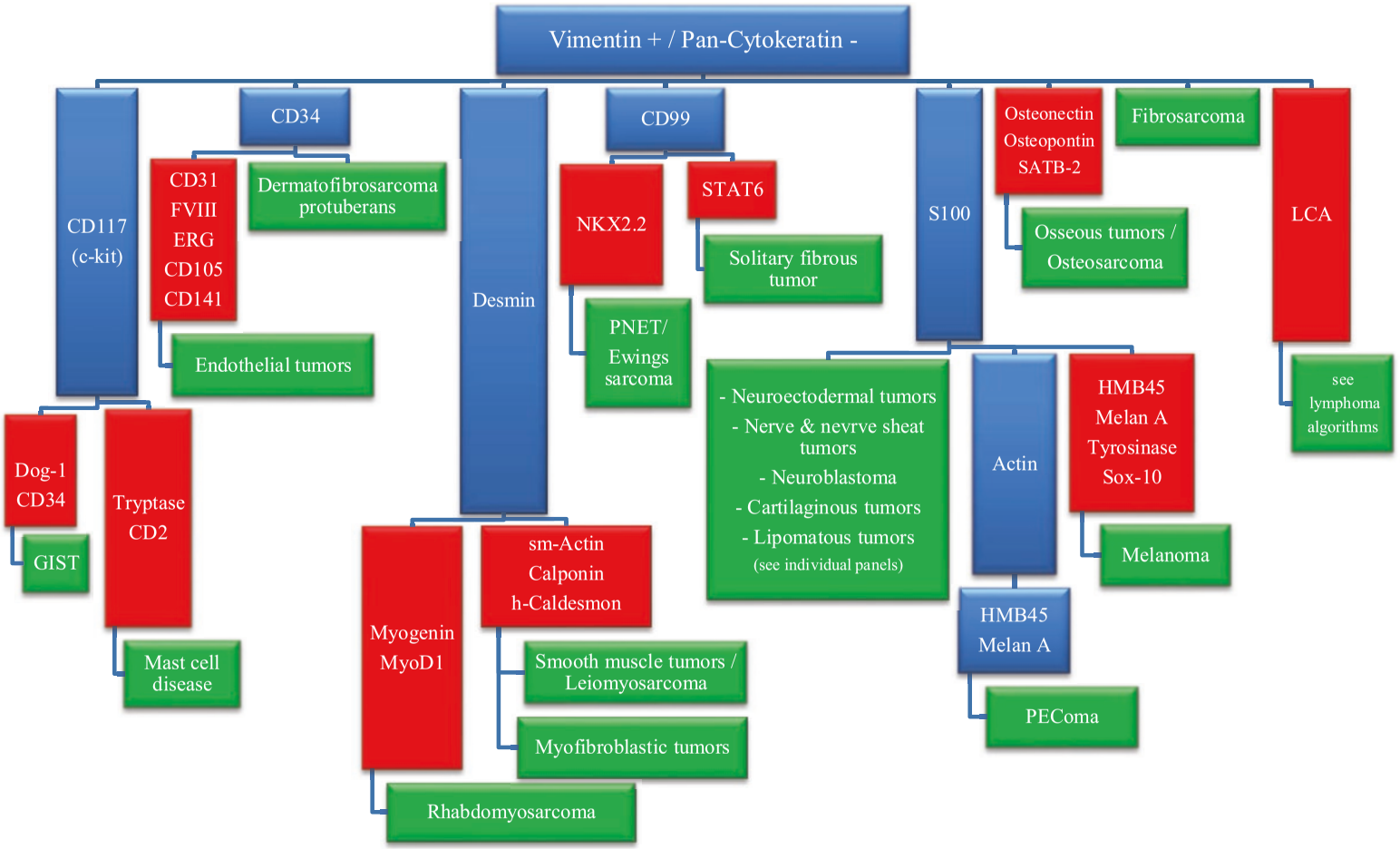
In the following 13 algorithms, general screening antibodies are placed in blue boxes, more specific antibodies in red boxes, and the most probable diagnosis in green ones. It is important to remember that the immunoprofile of tumors may be a subject of exceptions or an aberrant expression of different antigens, which may cause misdiagnosis. Finally, all immunohistochemical markers have to be interpreted in the appropriate morphological context.



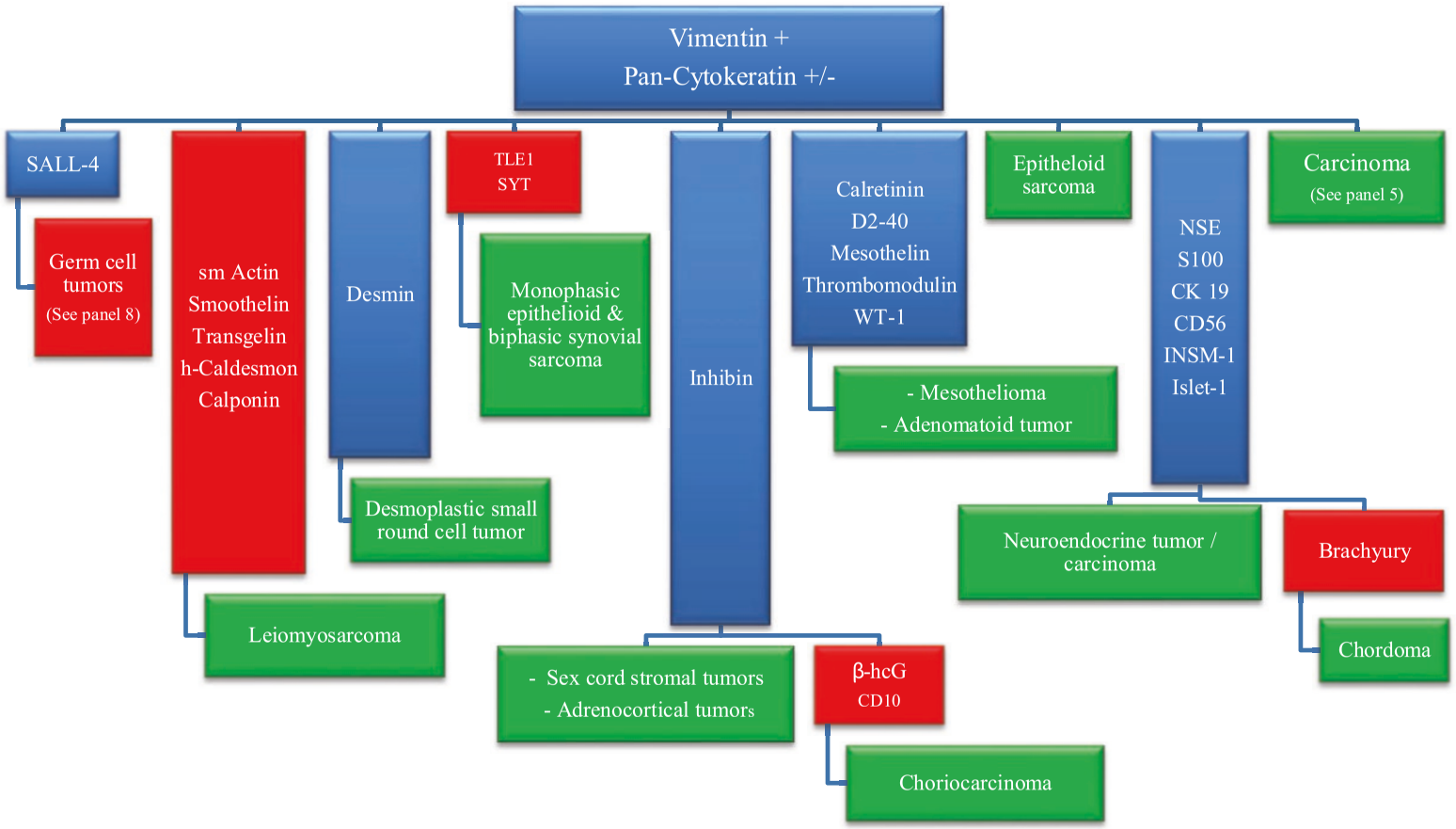
Algorithm 1.1 Primary screening antibody panel for undifferentiated tumors



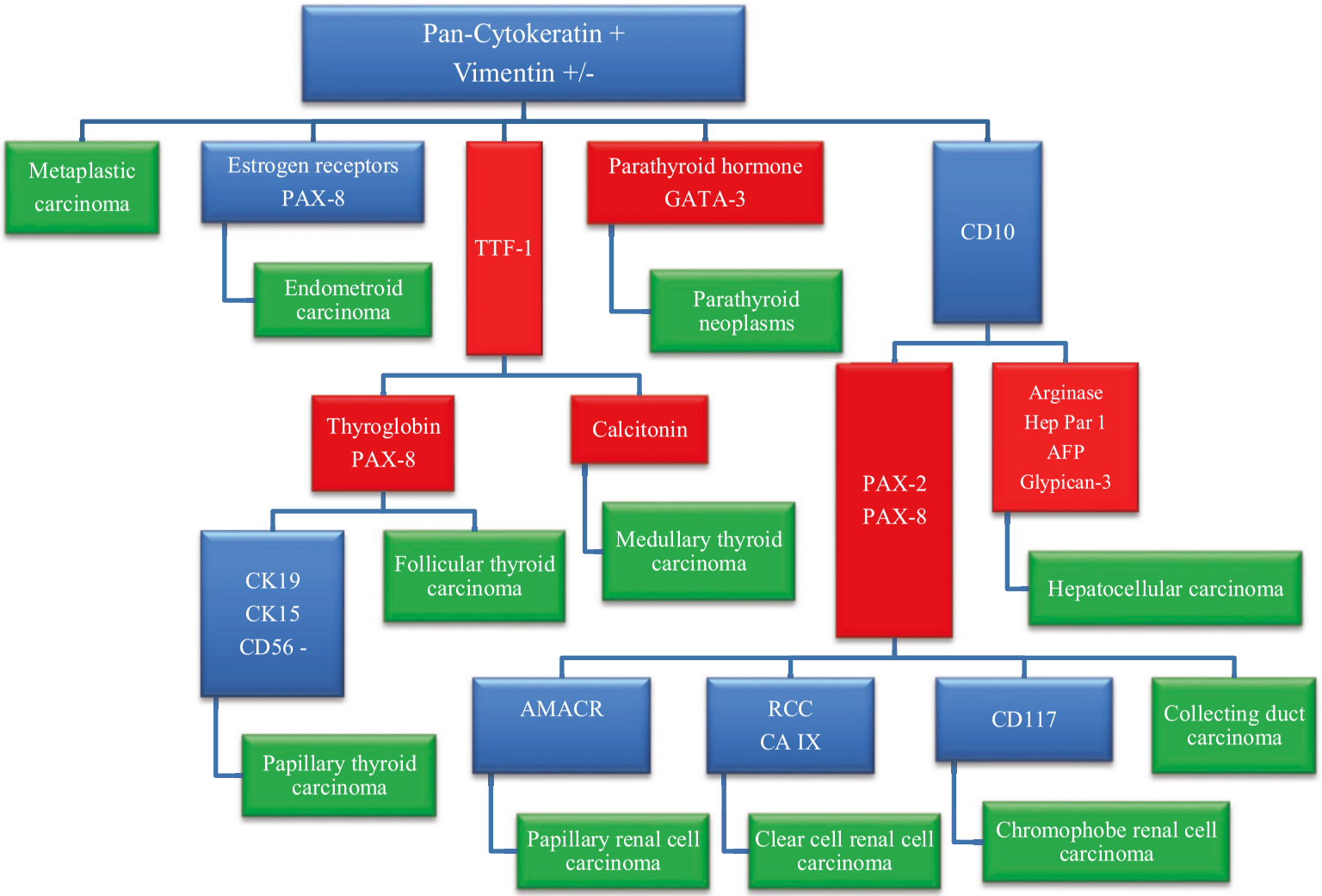
Algorithm 1.2 Screening antibody panel for tumors with small round blue cell morphology



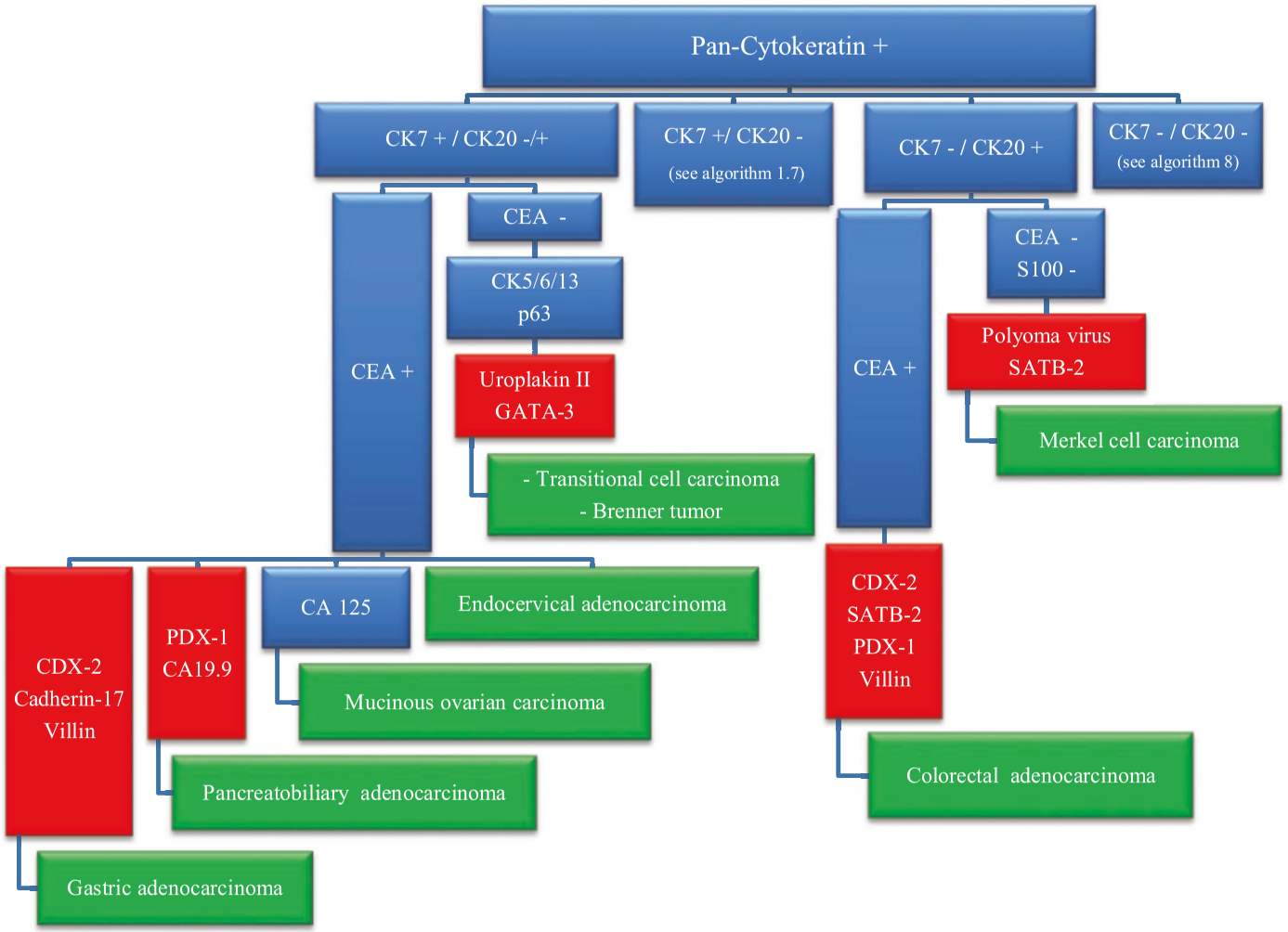
Algorithm 1.3 Cytokeratin-negative tumors/soft tissue tumors



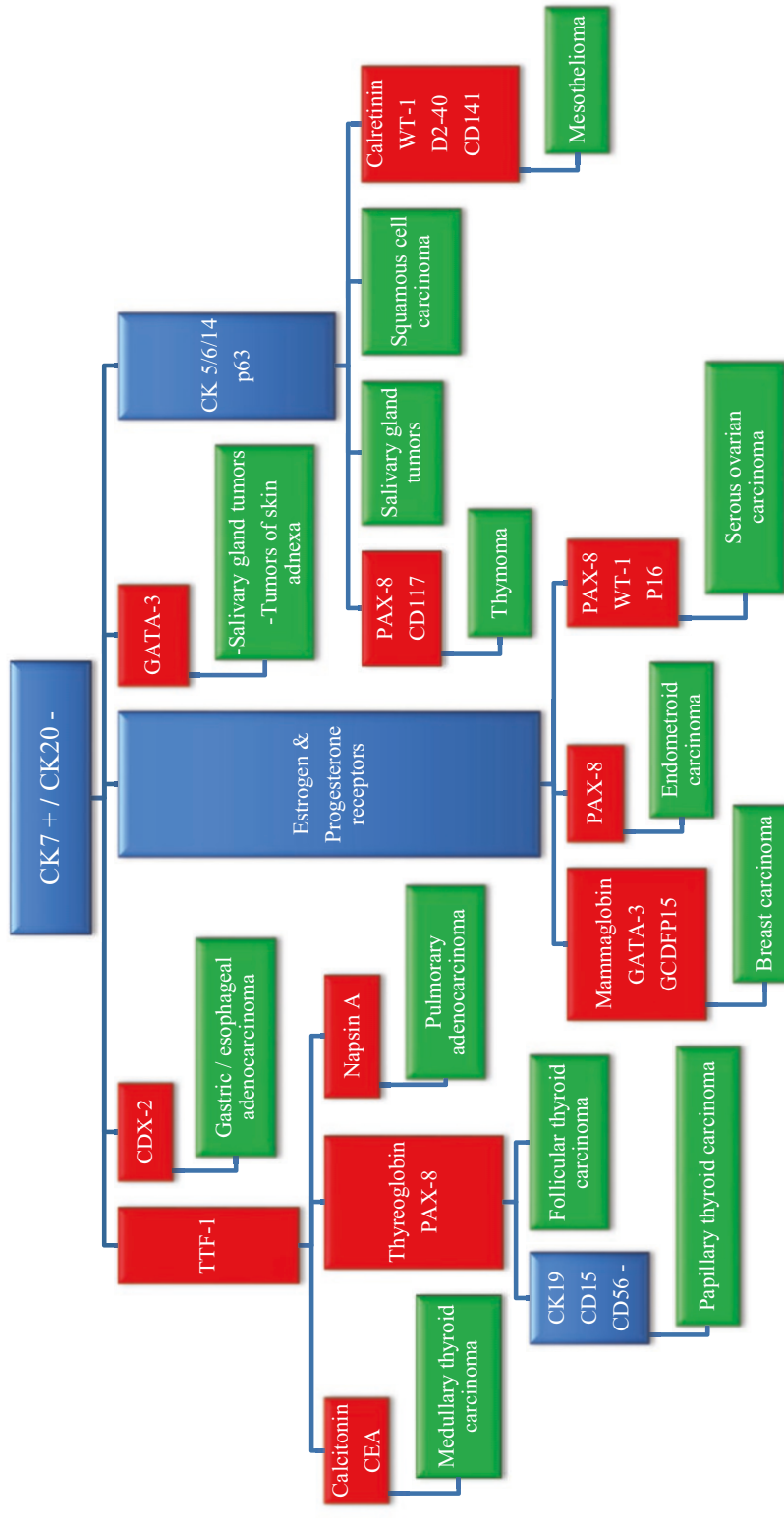
Algorithm 1.4 Tumors with Cytokeratin/Vimentin co-expression



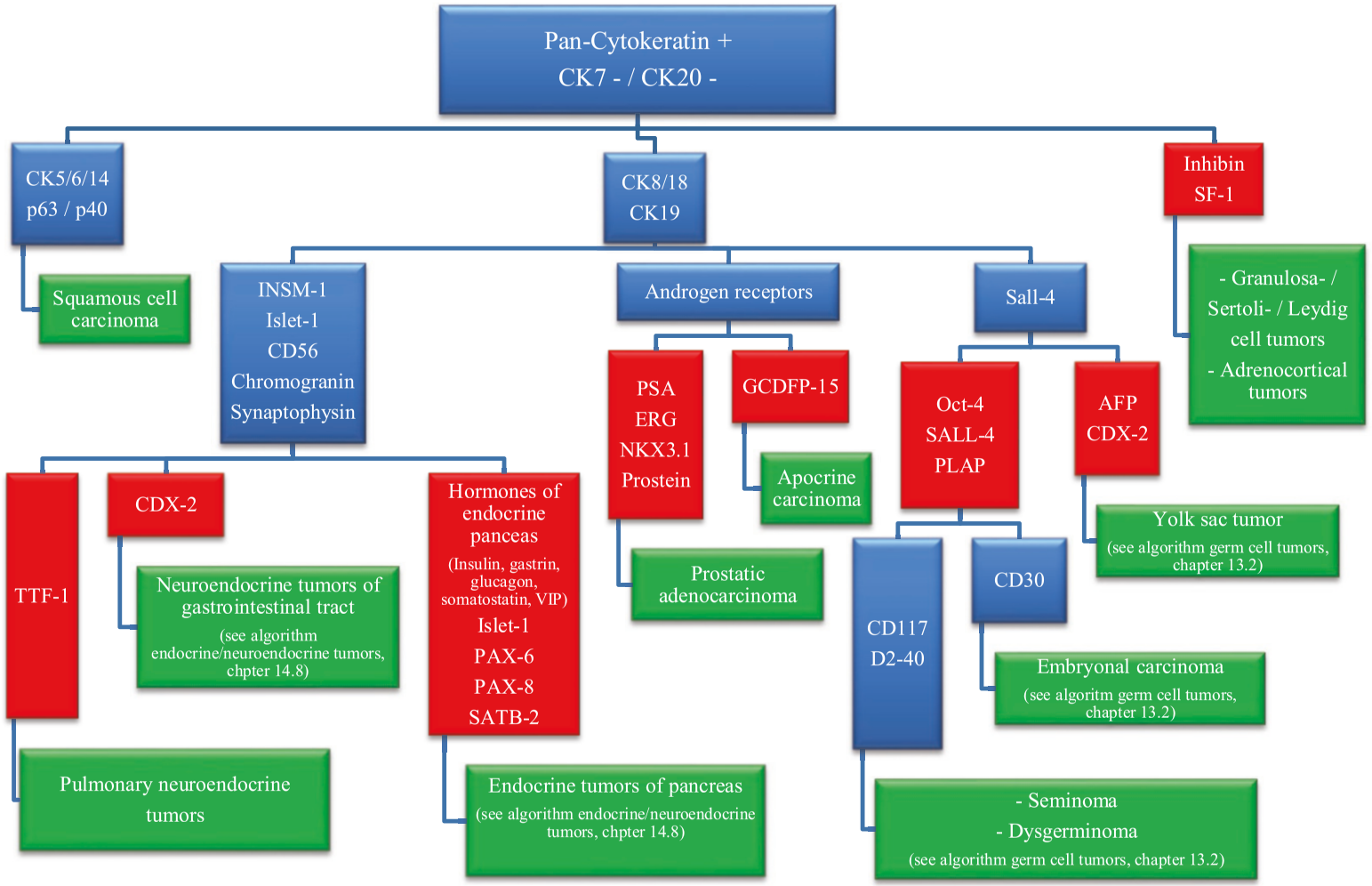
Algorithm 1.5 Carcinomas with Cytokeratin/Vimentin co-expression



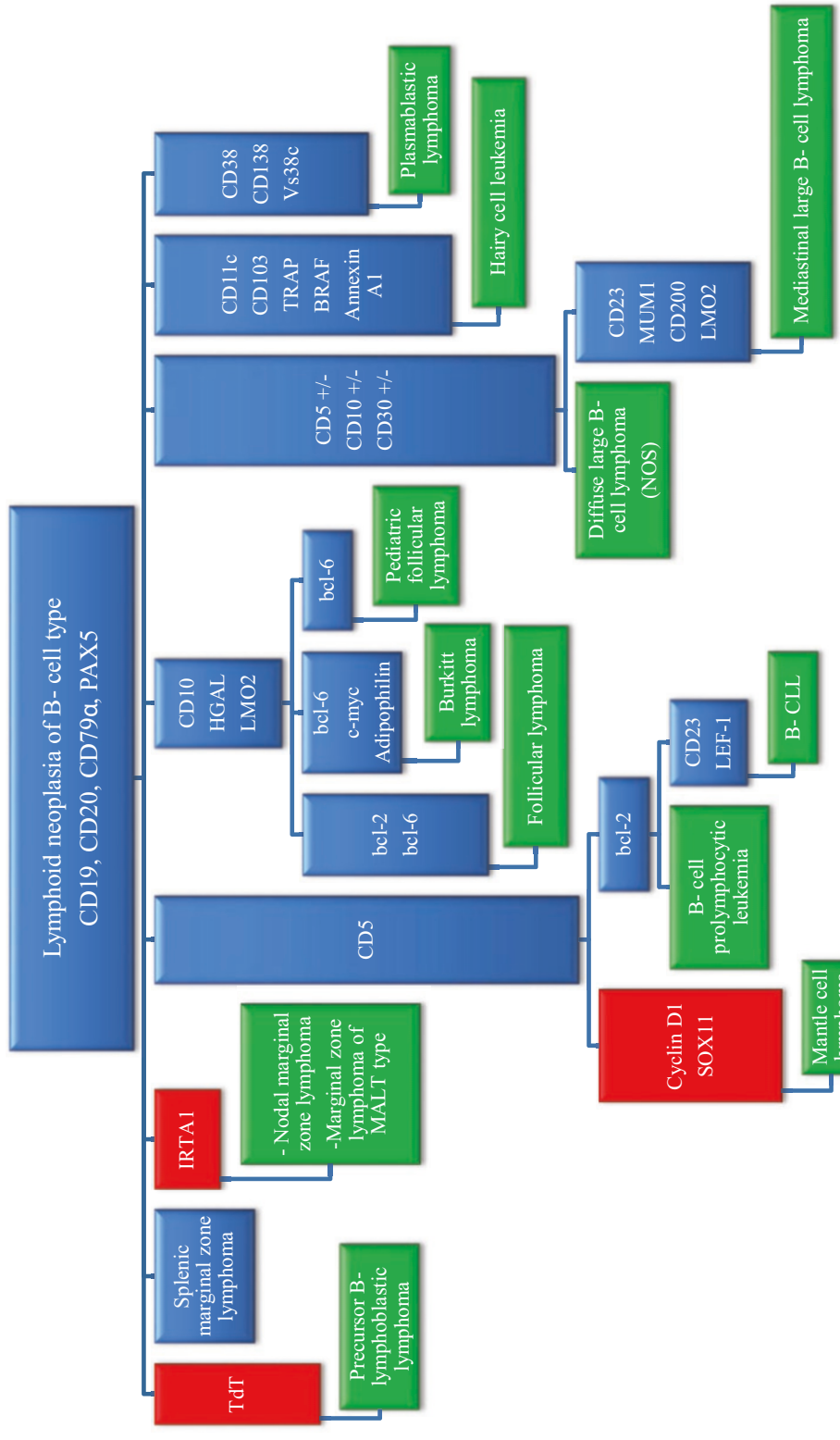
Algorithm 1.6 CK7/CK20 expression pattern in carcinomas



Algorithm 1.7 Cytokeratin CK7 +/CK20—carcinoma

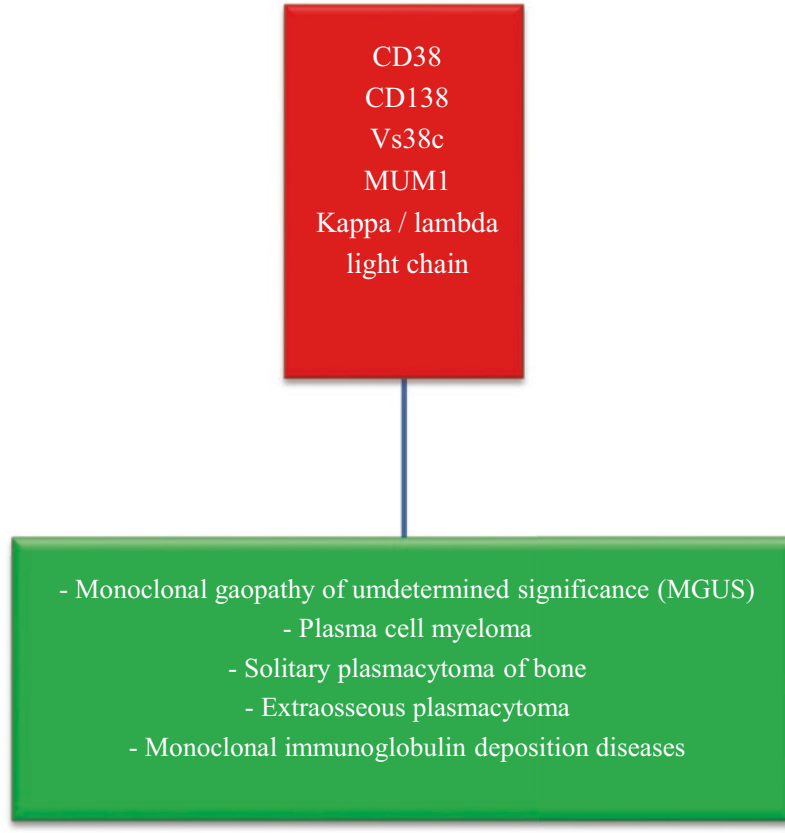


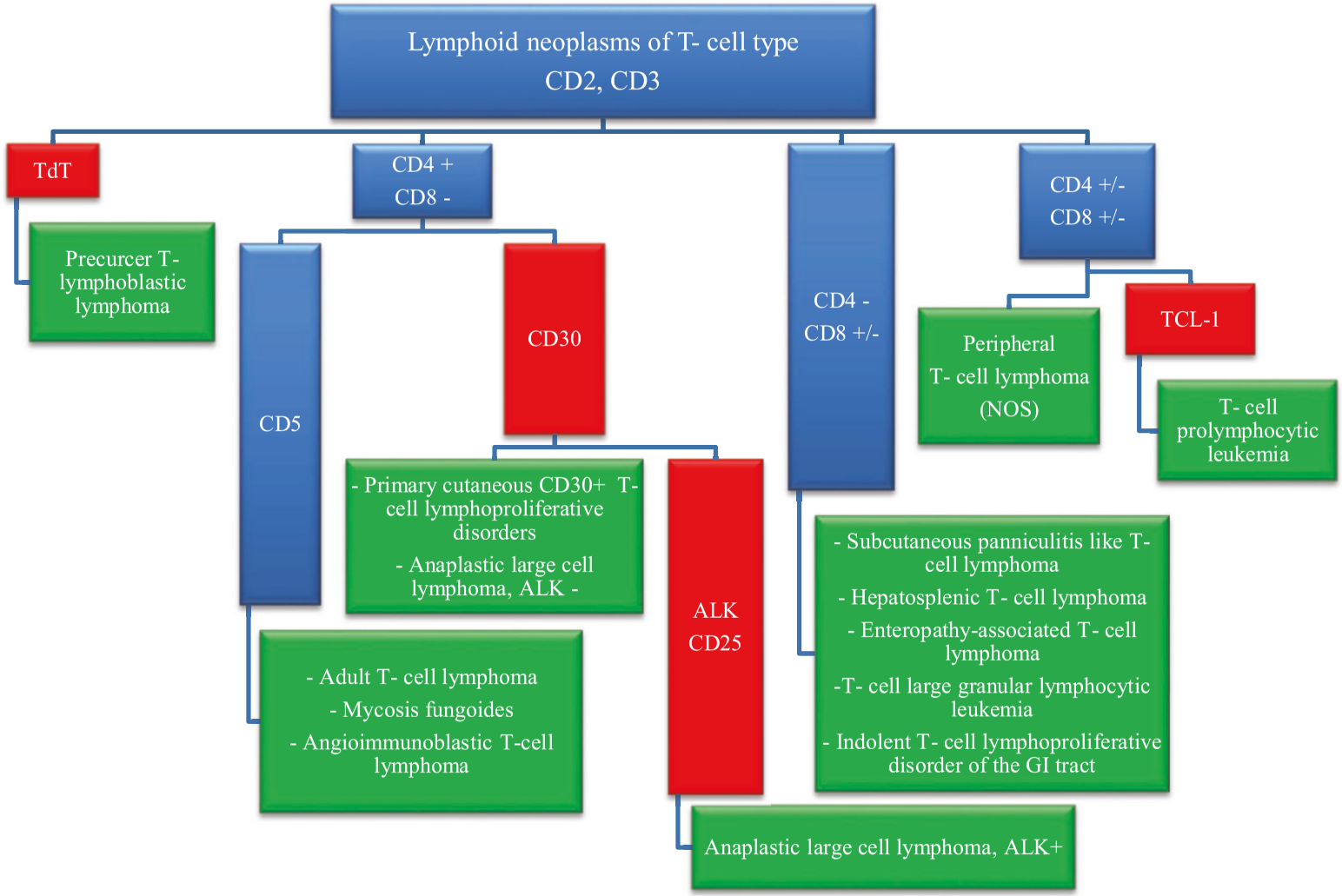
Algorithm 1.8 Cytokeratin CK7-/CK20—carcinoma



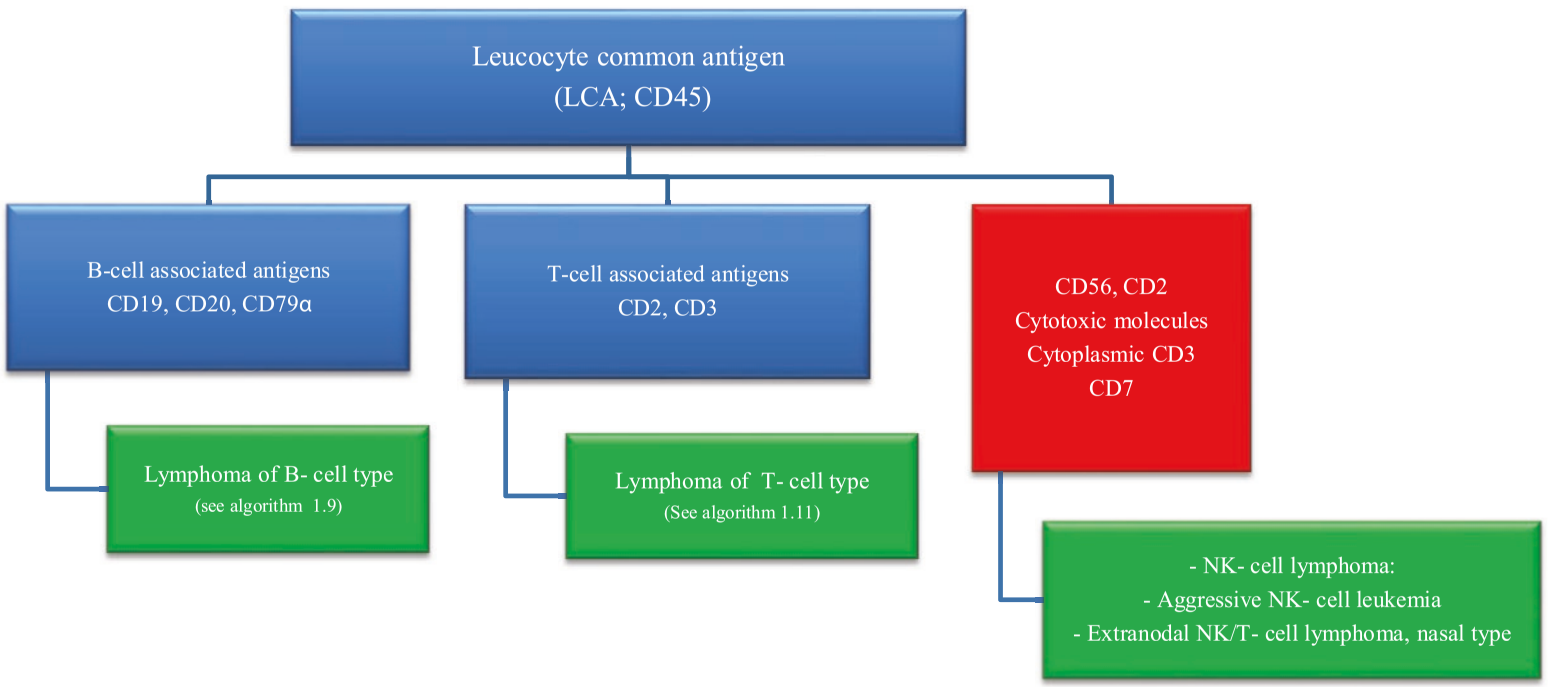
Algorithm 1.9 B-cell neoplasm

Algorithm 1.10 Plasma cell neoplasms

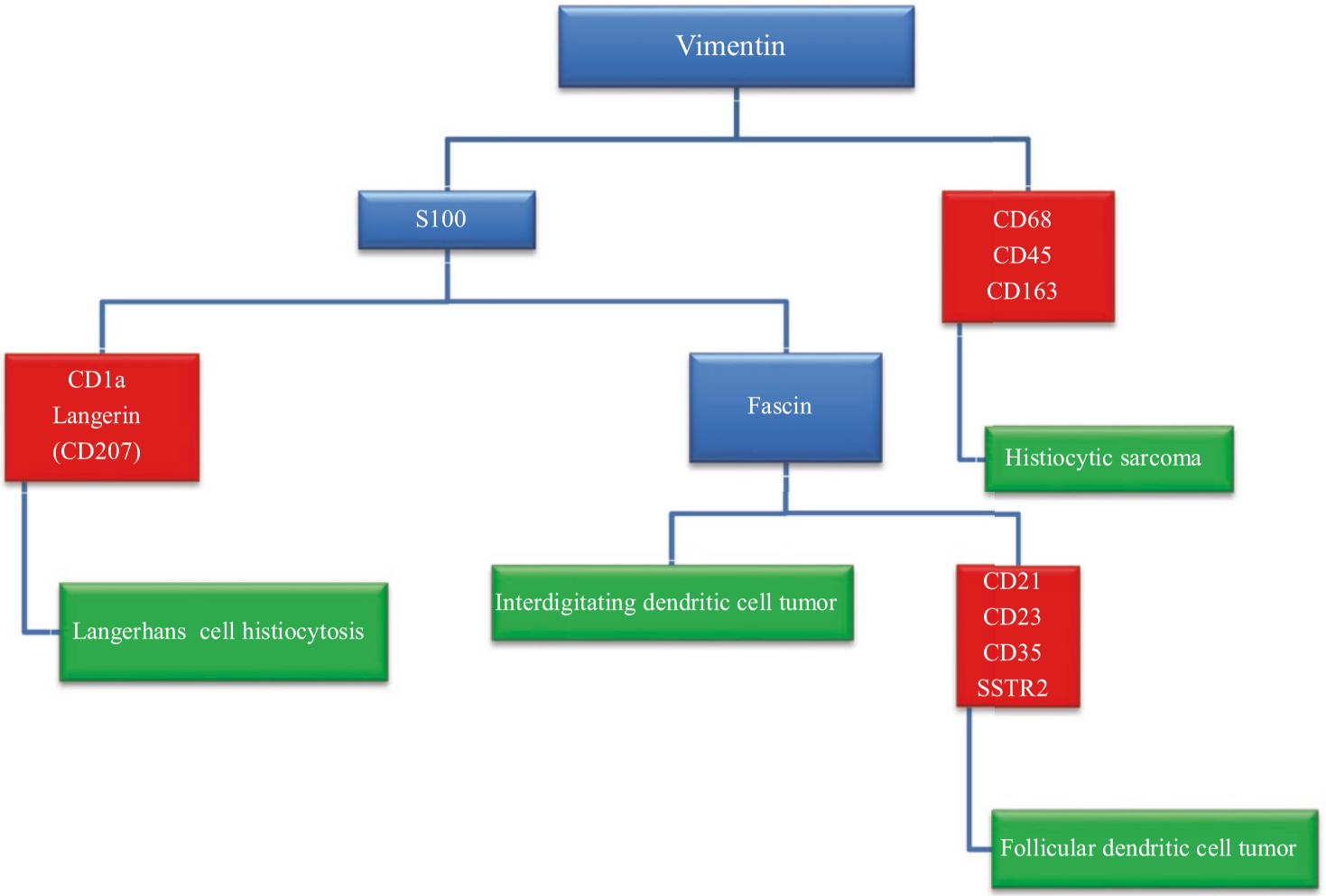




Algorithm 1.11 T-cell neoplasms



Algorithm 1.12 T-/NK-cell neoplasms



Algorithm 1.13 Histiocytic, dendritic cell tumors and stroma-derived neoplasm of lymphoid tissue

1.3 Common Immunohistochemical Markers, Diagnostic Approach, Pitfalls and Immunoprofiles of Most Common Tumors

Modern immunohistochemistry is a highly dynamic diagnostic tool and 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 now available. Many 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 also have a broad expression spectrum. CD15, CD10, CD30, CD34, Desmin, PAX-8, GATA-3, Sox-10, and S100 are typical antigens with multilineage expression patterns. On the other hand, many tumors exhibit a bilineage or atypical expression of different antigens. This phenomenon can be found 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. Examples or such tumors 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.[5]

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

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2 Immunohistochemical Markers for the Diagnosis of Epithelial Tumors

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2.1 Cytokeratins

Cytokeratins are the most important markers used for the diagnosis and classification of epithelial neoplasms. Cytokeratins are intermediate filament proteins in all mammalian epithelial cells building an intracytoplasmic network connecting the nuclear membrane with the cell membrane and desmosomes. Cytokeratins are a complex family comprising more than 20 isotypes and divided into two types [1–3].

- Type I (acidic group) including the Cytokeratins 9–20.
- Type II (basic group) including the 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 that usually remain constant after neoplastic transformation.

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 typically reveal a diffuse cytoplasmic expression pattern; nevertheless, abnormal staining patterns such as perinuclear and dot-like expression patterns are characteristic for different tumors, mainly for neuroendocrine tumors [4]. The following examples demonstrate this phenomenon, which is also of diagnostic value:

1. Merkel cell carcinoma with perinuclear Cytokeratin deposits (typically 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. Pituitary somatotroph adenoma.
6. Medullary thyroid carcinoma.
7. Seminoma (with low molecular weight Cytokeratins).
8. Granulosa cell tumor.
9. Rhabdoid tumor.
10. 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 as well as other frequently used epithelial markers, including epithelial membrane antigen (EMA), epithelial-specific antigen, carcinoembryonic antigen (CEA), p63, p40, Claudins, Cadherins, and different mucins.

2.1.1 Pan-Cytokeratin and Cytokeratin Cocktails

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 Pan-Cytokeratin markers are broad-spectrum anti-Cytokeratin antibodies or antibody cocktails that bind to different type I (acidic) and II (basic) Cytokeratins and help to recognize the epithelial differentiation in tumors. In interpreting a pan-Cytokeratin stain, it is always important to remember that no pan-Cytokeratin reacts absolutely with all Cytokeratins; nevertheless, cytokeratin cocktails are very effective in screening for epithelial differentiation or epithelial neoplasms [5]. Different Pan-Cytokeratin markers are now available and the following Cytokeratin-cocktails and antibody clones are the most commonly used 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 including the Cytokeratins 1/2/3/4/5/6/7/8/10/14/15/16/17/19. AE1/AE3 is widely used as a 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, and neuroendocrine tumors. Cross-reactivity of this cocktail with glial fibrillary acidic protein (GFAP) is reported and can be a source of interpretation errors [6].
- *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. Similar to the AE1/AE3 cocktail, KL1 also shows cross-reactivity with GFAP.
- *MNF116* is a Cytokeratin clone that reacts with the Cytokeratins 5/6/8/17/19 (Fig. 2.1).
- *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 8/14/15/16/18/19.
- *Oscar* Cytokeratin is a broad-spectrum Cytokeratin that reacts with the majority of epithelial cell types and carcinomas derived from these cells. Cytokeratin Oscar reacts with Cytokeratins 7, 8, 18, and 19. Cytokeratin Oscar does not show cross-reactivity with GFAP but reacts with follicular dendritic cells in lymphatic tissue.

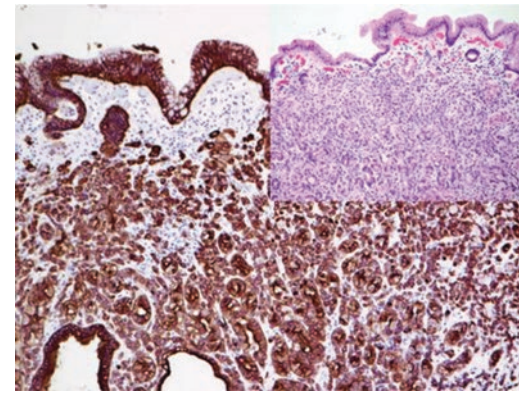

Fig. 2.1 Pan-Cytokeratin (CK MNF116) highlighting the neoplastic cells in diffuse gastric adenocarcinoma

Diagnostic Pitfalls Different Cytokeratins and Cytokeratin cocktails may be expressed in various non-epithelial tissue types and neoplasms or in tumors with features of epithelial differentiation which is essential to consider in the differential diagnosis. The following list includes such tumors that can mimic carcinomas:

- Mesothelial cells and mesothelioma
- Smooth muscle and smooth muscle tumors [7]
- Meningioma
- Chordoma
- Epithelioid sarcoma
- Synovial sarcoma
- Desmoplastic small round cell tumor
- Angiosarcoma
- Dedifferentiated chondrosarcoma
- A small subset of alveolar rhabdomyosarcoma
- Clear cell sarcoma
- Solitary fibrous tumor
- 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
- Dendritic cell sarcoma

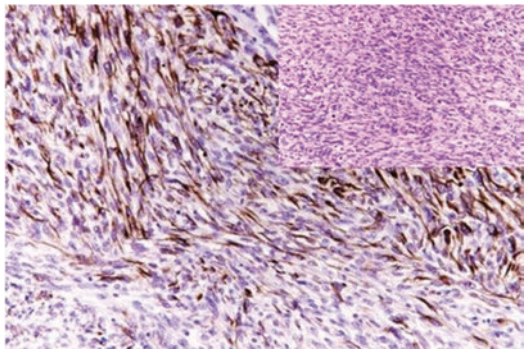

Fig. 2.2 Pan-Cytokeratin expression in malignant peripheral nerve sheath tumor cells

The aberrant expression of Cytokeratin in mesenchymal tumors is usually patchy or may show a dot-like expression pattern (Fig. 2.2). 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 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.

2.1.2 Cytokeratin 5

Cytokeratin 5		
Expression pattern: Cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Squamous cell carcinoma – Myoepithelial tumors – Mesothelioma	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 encoded on chromosome 12q12–13. It is the main component of the cytoskeleton of basal cells of stratified epithelium. Cytokeratin 5, 6, and 14 are related Cytokeratins expressed in stratified squamous epithelium, myoepithelial and mesothelial cells. This expression spectrum makes these Cytokeratins valuable markers for diagnosing squamous cell carcinoma. These Cytokeratins also clearly label normal myoepithelial cells, myoepithelial cell components in some tumors such as salivary gland 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. 2.3). This group of Cytokeratins is usually absent in gastrointestinal adenocarcinomas, germ cell tumors, prostatic carcinoma, thyroid tumors, and hepatocellular and renal cell carcinomas.

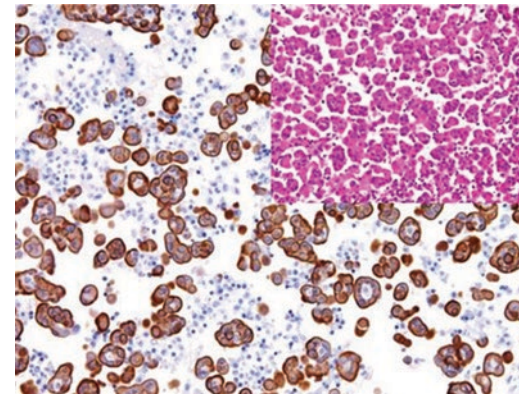

Fig. 2.3 Mesothelioma cells labeled by Cytokeratin 5 in pleural effusion

Recently, CK5/14 has been frequently replaced by p63 and p40, which 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.

2.1.3 Cytokeratin 6

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

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

2.1.4 Cytokeratin 7

Cytokeratin 7		
Expression pattern: Cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
Adenocarcinomas of different origin: 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 upper gastrointestinal tract, salivary glands, biliary tract, pancreas, lung, female genital tract, renal collecting ducts, transitional epithelium, mesothelial cells, thyroid follicle cells, endothelial cells
Positive control: Appendix		

Diagnostic Approach Cytokeratin 7 (sarcolectin) is a type II Cytokeratin expressed in most ductal and glandular epithelium in addition to the transitional epithelium of the urinary tract. Cytokeratin 7 is one of the main markers expressed in different adenocarcinomas; hence it cannot be used alone to determine the origin of the adenocarcinoma or to differentiate between primary and metastatic adenocarcinoma. An important diagnostic criterion is the co-expression of Cytokeratin 7 and Cytokeratin 20 (see diagnostic algorithms 6, 7, and 8) [2]. Cytokeratin 7 is strongly expressed by mesothelial cells and is unsuitable for discriminating between adenocarcinoma and mesothelioma.

Diagnostic Pitfalls In the differential diagnosis between adenocarcinoma and squamous cell carcinoma, it is essential to keep in mind that a minor component of Cytokeratin 7 positive cells can be found in squamous cell carcinoma of different locations, including carcinomas of the head and neck, lung, esophagus, and uterine cervix, mainly in poorly differentiated carcinomas. Cytokeratin 7 is usually absent or weakly expressed in colorectal adenocarcinomas. 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, prostatic carcinoma, epidermal squamous cell carcinoma, tumors of the adrenal cortex, and pituitary tumors. A weak CK7 expression is commonly seen in endothelia, which may serve as an internal positive control.

2.1.5 Cytokeratin 8

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 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, endothelia
Positive control: Appendix		

Diagnostic Approach Cytokeratin 8 is a type 2 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 the adult simple epithelium. Cytokeratin 8 is usually positive in non-squamous carcinomas and, accordingly, cannot be used to discriminate between adenocarcinoma types.

Diagnostic Pitfalls Cytokeratin 8 reacts with several non-epithelial tissue types and tumors, such as smooth muscle cells, leiomyosarcoma, and malignant rhabdoid tumor.

In contrast to normal squamous epithelium, squamous cell carcinomas of different origins are also positive for Cytokeratins 8/18.

2.1.6 Cytokeratin 10

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 1 Cytokeratin encoded on chromosome 17q21. This intermediate filament is usually associated with Cytokeratin 1. Cytokeratin 10 is expressed in keratinizing and non-keratinizing squamous epithelium. In routine immunohistochemistry, Cytokeratin 10 is used in a cocktail with Cytokeratins 13 and/or 14 as a marker for squamous cell carcinoma.

2.1.7 Cytokeratin 13

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

Diagnostic Approach Cytokeratin 13 is a type I Cytokeratin paired with Cytokeratin 4 and expressed in suprabasal and intermediate layers of stratified epithelium. Cytokeratin 13 is usually used in cocktails with Cytokeratin 10 or Cytokeratin 14 as a marker for squamous cell carcinoma.

2.1.8 Cytokeratin 14

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 non-keratinizing 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 heterotetramer with two Cytokeratin 5 molecules and expressed in the basal cell layer of stratified squamous epithelium. Cytokeratin 14 is a good marker for diagnosing squamous cell carcinoma (see Cytokeratin 5). In combination with Cytokeratin 5, it is an excellent marker to stain the myoepithelial/basal cells in breast and prostatic biopsies. The frequently used Cytokeratin 34βE12 to stain myoepithelial/basal cells reacts with Cytokeratins 1, 5, 10, and 14.

2.1.9 Cytokeratin 17

Cytokeratin 17		
Expression pattern: Cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Basal cell carcinoma – Immature metaplasia of the uterine cervix – Pancreatobiliary adenocarcinoma	Basal type triple-negative breast carcinoma, papillary thyroid carcinoma, cholangiocarcinoma, skin adnexal tumors, urothelial carcinoma, epithelioid sarcoma	Myoepithelial cells, cervical reserve cells, skin appendages (hair follicles, nail bed, sebaceous glands)
Positive control: Skin		

Diagnostic Approach Cytokeratin 17 is a type I, a 46 kDa intermediate filament encoded by the KRT17 gene on 17q21.2. Cytokeratin 17 is a basal type cytokeratin and is considered an epithelial stem cell marker, normally expressed in the basal cells of complex epithelia but not in stratified or simple epithelia. Cytokeratin 17 is also a myoepithelial marker helpful in distinguishing myoepithelial cells from luminal glandular epithelium of various origins.

In routine histopathology, Cytokeratin 17 is a helpful marker to discriminate between immature cervical metaplasia, usually positive for Cytokeratin 17 but negative for p16 and HPV-associated high-grade cervical intraepithelial neoplasia (CIN II-III) and cervical squamous carcinoma negative for Cytokeratin 17 but positive for p16. Atypical metaplastic lesions expressing both Cytokeratin 17 and p16 are considered to be high-grade cervical intraepithelial neoplasia.

Furthermore, Cytokeratin 17 is a helpful marker to distinguish pancreatobiliary adenocarcinoma typically positive for Cytokeratin 17, PDX-1, and MUC-1 from extra-pancreatobiliary non-mucinous gastrointestinal adenocarcinoma positive for CDX-2, PDX-1, and MUC-2, negative for CK17 [9, 10].

2.1.10 Cytokeratin 18

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, neuroendocrine carcinoma	Hepatocellular carcinoma, renal cell carcinoma, leiomyosarcoma, chordoma	Epithelium of 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 type I Cytokeratin, an intermediate filament that forms a heteropolymer with Cytokeratin 8. Cytokeratin 18 is expressed in single-layer 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 also positive for Cytokeratin 18, which can also be a component of different Cytokeratin cocktails and might mimic the intravascular tumor spread. Cytokeratin 18 is also expressed in smooth muscle cells and smooth muscle tumors.

2.1.11 Cytokeratin 19

Cytokeratin 19		
Expression pattern: Cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Neuroendocrine tumors – Papillary thyroid carcinoma	Adenocarcinoma of lung, gastrointestinal and pancreatobiliary adenocarcinoma, breast and endometrium carcinoma, transitional cell carcinoma, mesothelioma	Epithelium of gastrointestinal tract, salivary glands, biliary tract, pancreas, lung, female genital tract, transitional epithelium, mesothelial cells, thyroid follicle cells, pituitary gland, 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 limited use in differentiating between carcinoma types. Cytokeratin 19 strongly labels papillary thyroid carcinoma and can be combined with other markers such as CD56 and p63 to differentiate between papillary and follicular thyroid carcinomas, as the latter is usually negative or very weakly positive for this Cytokeratin (see Sect. 14.3) [11].

2.1.12 Cytokeratin 20

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 and enteric type pulmonary adenocarcinoma, hepatocellular carcinoma, transitional cell carcinoma, ductal adenocarcinoma of the prostate	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. Cytokeratin 20 is almost constantly expressed by colorectal adenocarcinomas, mucinous ovarian carcinoma, and less frequently in transitional cell carcinoma (Fig. 2.4). Also, characteristic is the dot-like perinuclear staining pattern in Merkel cell carcinoma (Fig. 2.5). Cytokeratin 20 is a helpful marker to discriminate between reactive atypia and dysplasia of transitional epithelium of urinary tract (Fig. 2.6). In normal and reactive transitional epithelium, the expression of Cytokeratin 20 is restricted to the umbrella cells. In contrast, carcinoma in situ shows a transepithelial expression of Cytokeratin 20. Focal Cytokeratin 20 expression can also be noted in prostatic ductal adenocarcinoma. Cytokeratin 20 is consistently negative in squamous cell carcinoma; thyroid carcinoma; and breast, acinic prostatic, and endometrial adenocarcinomas in addition to mesothelioma.

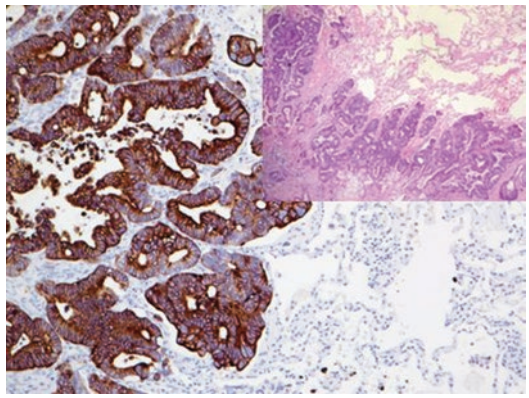

Fig. 2.4 Metastatic colorectal adenocarcinoma with strong Cytokeratin 20 expression

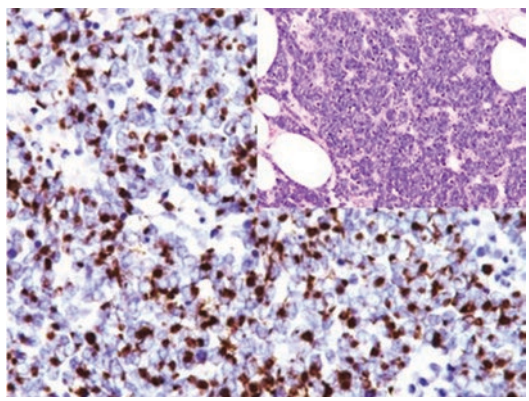

Fig. 2.5 Characteristic dot-like perinuclear expression of Cytokeratin 20 in neoplastic cells of Merkel cell carcinoma

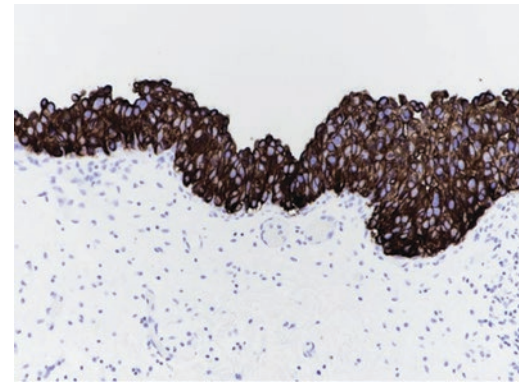

Fig. 2.6 Urothelial carcinoma in situ with transepithelial CK20 expression (see Sect. 12.2)

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, 7, and 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 and able to form gel-like substances [12]. 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 membrane-bound mucins such as MUC-1, MUC-3A, MUC-3B, MUC-4, MUC-12, MUC-13, and MUC-17.

Combining PAS and alcian blue stains in routine histopathology is a very helpful pan-mucin stain. The expression pattern of mucins is characteristic for some tumors and tissue types and can be helpful in the classification of tumors derived from these cell types, and many specific antibodies are now available for the characterization of mucins. This chapter lists the most important mucins used in routine immunohistochemistry.

2.2.1 Epithelial Membrane Antigen

Epithelial membrane antigen (EMA, 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 – Nodular lymphocyte predominant Hodgkin 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 cell types; nevertheless, very low EMA 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 lymphoma, making the EMA positivity a helpful criterion for the diagnosis since L&H cells in this Hodgkin lymphoma type are negative for CD30, CD15, and Fascin. EMA is constantly negative in basal cell carcinoma, adrenocortical tumors, melanoma, hepatocellular carcinoma, and germ cell tumors, that is, seminoma, embryonal carcinoma, and yolk sac tumor.

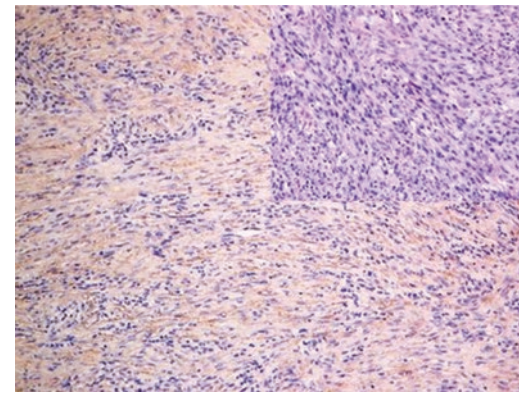

Fig. 2.7 EMA expression in the cells of atypical meningioma

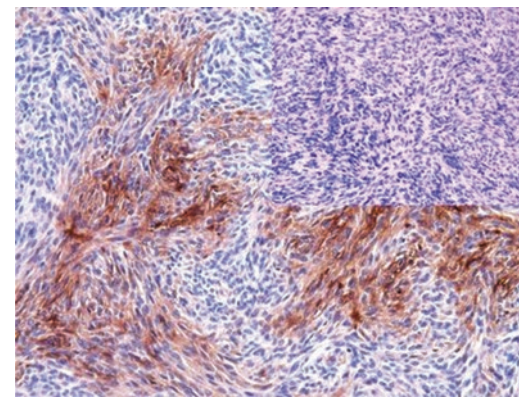

Fig. 2.8 Focal EMA expression in the cells of malignant peripheral nerve sheath tumor

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; [13] plasma cell neoplasms; meningioma; epithelioid mesothelioma; perineurioma; angiosarcoma; leiomyosarcoma; and synovial, epithelioid, and neurogenic sarcomas (Figs. 2.7 and 2.8). Since EMA is highly glycosylated and some antibodies detect

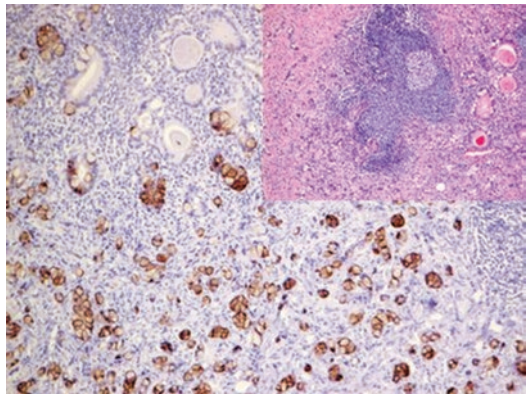

Fig. 2.9 MUC-2 highlighting tumor cells of appendicular mucinous carcinoma

carbohydrate domains, the stain results may show marked differences using different antibodies. Overexpression of EMA in carcinomas has been associated with a worse prognosis.

2.2.2 Mucin-2

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 colonic, gastric, pancreatic, breast, and ovarian mucinous adenocarcinomas as well as enteric-type pulmonary adenocarcinoma (Fig. 2.9). Pancreatic ductal adenocarcinoma and cholangiocarcinoma are usually negative for MUC-2.

2.2.3 Mucin-3

Two closely related subtypes of this mucoprotein have been identified in humans, type 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.

2.2.4 Mucin-4

Mucin-4 (MUC-4) is a transmembrane mucoprotein composed of alpha and beta chains and found on the apical surface of many types of epithelial cells. MUC-4 is involved in the regulation of cellular adhesion and cell surface signaling. MUC-4 is normally expressed in tracheal and bronchial epithelium, epithelium of gastrointestinal mucosa, and prostatic glands. MUC-4 is highly expressed in pulmonary, gastric, and pancreatic adenocarcinomas besides pancreatic intraepithelial neoplasia (PanIN). MUC-4 is also a sensitive and specific marker for low-grade fibromyxoid sarcoma, sclerosing epithelioid fibrosarcoma, and secretory carcinoma of the salivary glands. MUC-4 is also expressed in the glandular cells of biphasic synovial sarcomas.

2.2.5 Mucin-5AC

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 the respiratory tract. MUC-5 AC is a marker for many carcinoma types such as esophageal, gastric, colonic, pancreatic, large duct type cholangiocellular, endometrial, endocervical adenocarcinomas, and mucinous ovarian carcinoma. MUC-5 AC is also expressed in preinvasive pancreatic neoplasia, including all types of intraductal papillary neoplasm (IPMN), intraductal oncocytic papillary neoplasm (IOPN), and intraductal tubulopapillary neoplasm (ITPN).

2.2.6 Mucin-5B

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

2.2.7 Mucin-6

Mucin-6 (MUC-6) is a gel-forming mucoprotein and one of the major mucins protecting gastric mucosa. MUC-6 is synthesized by gastric and pyloric glands, mucosa of the gall bladder, and bile and pancreatic ducts, in addition to colonic and endocervical mucosa. MUC-6 is a marker for invasive ductal carcinoma of the breast and gastric adenocarcinomas. Similar to MUC-5 AC, MUC-6 is also strongly expressed in cells of pre-invasive pancreatic neoplasia IPMN, IOPN, and ITPN in addition to large duct-type cholangiocellular carcinoma.

2.2.8 Mucin-16

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 and mesothelioma and may also occur in breast carcinomas. This marker is listed in detail in a later chapter (see markers for ovarian epithelial tumors, Sect. 11.6).

2.3 Claudins

Claudins are a family of integral transmembrane proteins that includes 23 members. These integral transmembrane tight junction–associated proteins are found in all types of tight junction–bearing cells, including epithelial and endothelial cells. Claudins form a paracellular barrier and pores and regulate the transport of molecules through the intercellular space. Different Claudin types are expressed in different tissue types.

2.3.1 Claudin-1

Claudin-1 is mainly expressed in epithelial, endothelial, and perineural cells. Claudin-1 is a marker for gastric adenocarcinomas, meningioma, and perineurioma (Fig. 2.10).

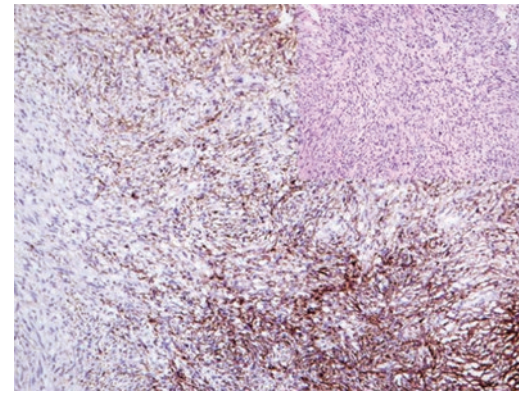

Fig. 2.10 Claudin-1 staining tumor cells of neurofibroma

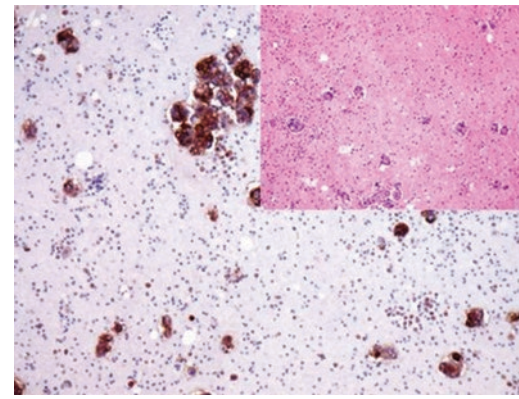

Fig. 2.11 Claudin-4 highlighting tumor cells of ovarian carcinoma in ascitic fluid

2.3.2 Claudin-4

Claudin-4 is used in routine immunohistochemistry as a marker to discriminate between reactive mesothelial cells and carcinoma cells in pleural and peritoneal effusion (Fig. 2.11). Claudin-4 is normally expressed in most types of epithelial cells and related carcinomas, including colorectal adenocarcinoma, cholangiocarcinoma, ovarian carcinoma, and breast and prostatic carcinomas but constantly negative in mesothelial cells, mesothelioma, and cholangiocarcinoma [14, 15]. The expression of Claudin-4 is also found in endothelial cells and cells of the submucosal and myenteric plexus.

2.3.3 Claudin-5

Caludin-5 is typically expressed in endothelial cells and glomerular podocytes. It is also found in the gastrointestinal mucosa, luminal epithelium of the breast ducts and sweat glands, as well as prostatic gland and thyroid follicular cells. Claudin 5 labels 95–100% of angiosarcomas and different adenocarcinoma types.

2.3.4 Claudin-7

In diagnostic immunohistochemistry, Claudin-7 may help distinguish between renal oncocytoma (negative) and chromophobe renal cell carcinoma (positive).

2.3.5 Claudin-18

This Claudin has two splice variants, 18.1 and 18.2.

Claudin-18.1 is the lung-specific isoform. Claudin-18.2 is selective for gastric mucosal epithelial cells but not expressed in undifferentiated gastric stem cells. Claudin-18.2 is expressed in ~80% of gastric adenocarcinomas but can also be expressed in other adenocarcinoma types, including pancreatic, esophageal, breast, ovarian, and pulmonary adenocarcinomas. Claudin-18.2 is also expressed in the majority of well-differentiated gastric neuroendocrine tumors (NET G1).

Claudin-18.2 is the molecular target for specific therapeutic antibodies. The expression of Claudin-18 can be estimated by immunohistochemistry.

2.4 Cadherins

Cadherins are a family of calcium-dependent transmembrane adhesion molecules linked to the cytoskeleton by the catenin molecules forming the cadherin-catenin complex involved in the formation of different types of intercellular junctions and stabilization of cell contacts. Cadherins are divided into different subclasses, including classical, desmosomal, protocadherins, and unconventional, and include more than 100 different cadherin types according to the tissue or cell type carrying these molecules. The most interesting cadherin members targeted in routine histopathology are E-cadherin (CDH-1), N-cadherin (CDH2), N-cadherin-2 (CDH12), P- cadherin (CDH-3), Ksp-cadherin (cadherin-16), and LI-cadherin (CDH-17).

2.4.1 Epithelial Cadherin

E-cadherin is a member of the cadherin superfamily and the major calcium-dependent cell adhesion molecule of epithelial cells clustered as CD324 and encoded on 16q22.1. The E-cadherin molecule is composed of extracellular, transmembrane, and intracellular domains. The expression of E-cadherin is associated with epithelial stratification and polarization in addition to gland formation [16]. E-cadherin is expressed in various types of epithelial and myoepithelial cells, and carcinomas originate from these cells. In routine histopathology, E-cadherin is a helpful marker to discriminate between ductal and lobular breast carcinoma (see Chap. 10).

In effusion cytology, E-cadherin is an important marker to differentiate between benign reactive mesothelial proliferation, typically negative for E-cadherin and carcinoma cells, mostly strong positive for E-cadherin taking into consideration the loss of E-cadherin expression in some tumor types. The loss of E-cadherin expression usually appears due to mutations in the E-cadherin gene, which cause the loss of cohesiveness of tumor cells, which is characteristic for lobular breast carcinoma and poorly cohesive and signet cell carcinoma, in addition to a subset of undifferentiated carcinomas. Also, it is essential to consider that cells of malignant mesothelioma are usually positive for E-cadherin (Fig. 2.12). In conclusion, strong E-cadherin-positive cells in effusion cytology are most likely to be malignant (Fig. 2.13). It is also important to consider that a subset of malignant melanomas shows at least a focal E-cadherin expression.

Interestingly, in diagnostic histopathology the aberrant nuclear expression of E-cadherin is

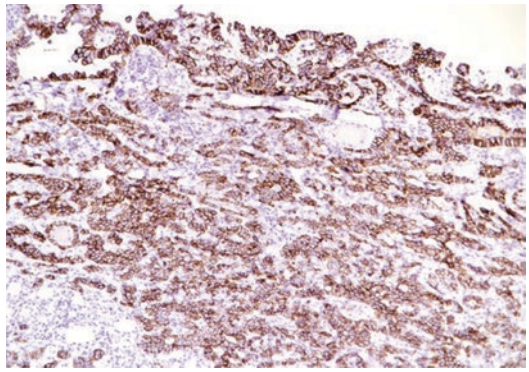

Fig. 2.12 Membranous E-cadherin expression in malignant mesothelioma

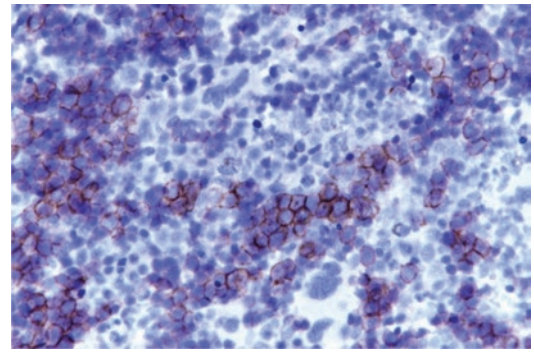

Fig. 2.14 Bone marrow trephine biopsy with E-cadherin staining the cells of erythropoiesis

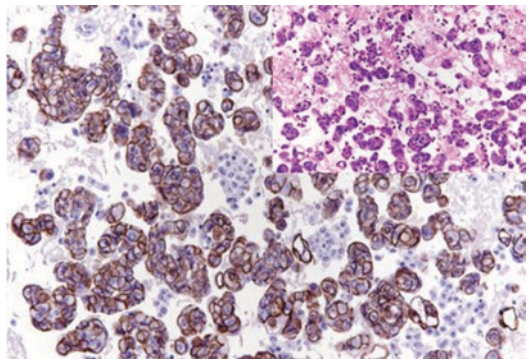

Fig. 2.13 Metastatic carcinoma cells in pleural effusion labeled by E-cadherin

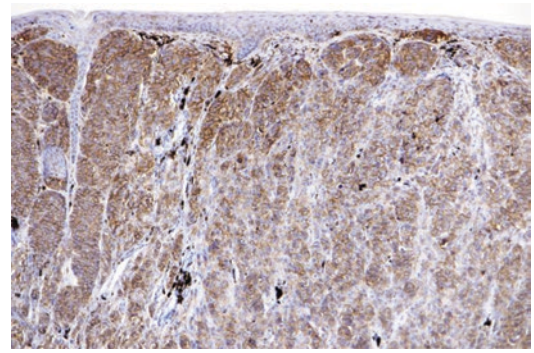

Fig. 2.15 Strong E-cadherin expression on melanoma cells of nodular melanoma

described in several tumors, including gastrointestinal and pancreatic adenocarcinomas, neuroendocrine tumors, and Merkel cell carcinoma, in addition to renal cell carcinoma. In pancreatic epithelial tumors, the nuclear expression of E-cadherin is diagnostic for solid pseudopapillary neoplasm of the pancreas [17–19].

The co-expression of Podoplanin, Somatostatin receptor 2 (SSTR2), and E-cadherin is a characteristic profile for different meningioma types.

E-cadherin is also a marker for erythroid precursors (erythroblasts and normoblasts) and takes part in the regulation of erythroid differentiation, while mature erythrocytes lack E-cadherin (Fig. 2.14). E-cadherin is also a marker for most biphasic and a significant subset of monophasic synovial sarcoma. A moderate E-cadherin expression is also noted in malignant melanoma (Fig. 2.15) [20].

2.4.2 Neural Cadherin

N-cadherin is also a calcium-dependent cell adhesion molecule clustered as CD325 expressed in neural tissue playing a role in neuron-neuron interaction. N-cadherin is also found in intercalated discs of cardiac muscles. In effusion cytology, N-cadherin is expressed in normal, reactive, and malignant mesothelial cells.

2.4.3 Cadherin-16

Cadherin-16 is a member of the cadherin superfamily encoded on chromosome 16q22.1, expressed exclusively in the kidney and known as kidney-specific cadherin (Ksp-cadherin). In normal renal tissue, Cadherin-16 is expressed in the basolateral membrane of renal tubular and col-

lecting duct epithelium, whereas glomerular and interstitial cells lack the expression of cadherin-16 (Fig. 2.16). The expression of cadherin-16 in different renal tumors is listed with the markers of renal tumors.

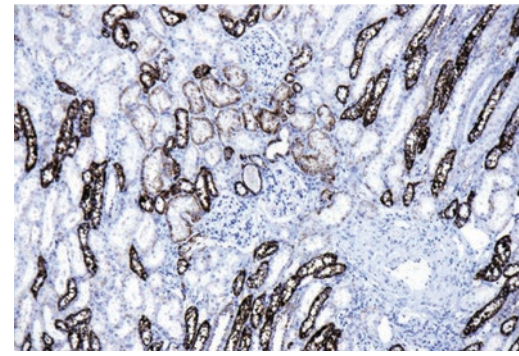

Fig. 2.16 Cadherin-16 (Ksp-cadherin) highlighting tubular and collecting duct epithelium; glomerular and interstitial cells lack the cadherin-16 expression

2.4.4 Cadherin 17

Cadherin 17, also known as liver-intestine cadherin (LI-cadherin), is a further member of the cadherin superfamily acting as an intestinal peptide transporter regulated by CDX-2. Cadherin 17 is strongly expressed in the intestinal mucosa and can be used as a helpful marker for gastrointestinal carcinomas. Cadherin 17 is listed in detail with the markers of gastrointestinal carcinomas.

2.5 Miscellaneous Epithelial Markers

2.5.1 Epithelial-Specific Antigen

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 and 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 (clustered as CD326), also known as human epithelial antigen or epithelial cell adhesion molecule (EPCAM), is a transmembrane glycoprotein mediating calcium-independent cell-cell adhesion and is involved in cell signaling, migration, proliferation, and differentiation [16].

In routine immunohistochemistry, Ber-EP4 is the most commonly used antibody clone. EPCAM is expressed on most normal epithelial cells except 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 benign and malignant mesothelial cells; accordingly, it can be used as a diagnostic marker to distinguish between carcinoma cells and mesothelial cells in pleural and peritoneal effusions and between pulmonary adenocarcinoma and malignant mesothelioma (Fig. 2.17). EPCAM is also a helpful marker to discriminate between basal cell carcinoma (EPCAM & bcl-2 positive, EMA negative) and squamous cell carcinoma (EPCAM & bcl-2 negative, EMA positive) (Fig. 2.18). Furthermore, it is a valuable marker to differentiate between various types of hepatoid carcinomas positive for EPCAM and hepatocellular carcinoma, usually lacking the EPCAM expression.

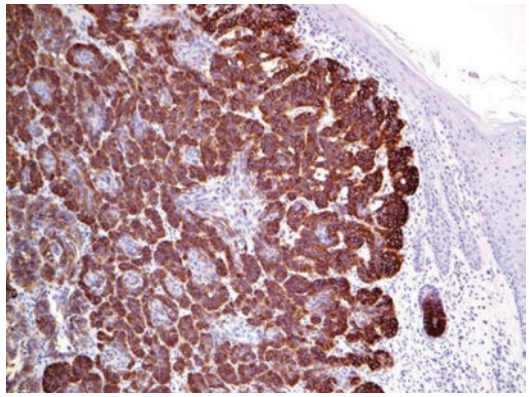

Fig. 2.17 Basal cell carcinoma with strong EPCAM (clone Ber-EP4) expression

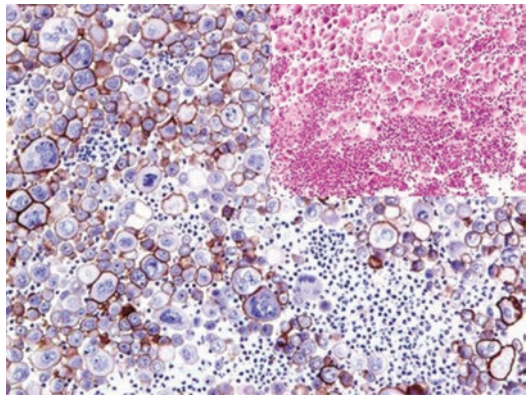

Fig. 2.18 Malignant pleural effusion; metastatic carcinoma cells labeled by EPCAM (clone Ber-EP4)

Diagnostic Pitfalls Up to 20% of reactive mesothelial cells and malignant mesotheliomas may express the EPCAM antigen (usually as a focal weak stain), which must be considered in the differential diagnosis in pleural and peritoneal effusions. The use of other tissue-specific transcriptional markers, such as TTF-1 and CDX-2, in addition to other epithelial markers, such as E-cadherin, is important to establish the diagnosis. It is also important to consider that a small subset of plasma cells (up to 7%) may be positive for EPCAM [21].

The loss of EPCAM expression can be noticed in a subset (up to 3%) of adenocarcinomas with microsatellite instability with MSH2/MSH6 deficiency. The loss of MSH2, in this case, occurs as a result of bi-allelic mutations of the EPCAM gene located on chromosome 2 upstream of the MSH2 gene, causing the inactivation of MSH2 expression (see Chap. 35).

2.5.2 Epithelial-Related Antigen

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 similar features to the above-mentioned EPCAM antigen. It is usually used to label the cells of epithelial tumors of different origins and to discriminate between metastatic carcinoma and atypical mesothelial proliferation in effusion cytology. MOC31 stains chromophobe renal cell carcinoma, whereas clear cell renal cell carcinoma lacks the expression of MOC31.

2.5.3 p63/p40

p63/p40		
Expression pattern: Nuclear		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Squamous cell carcinoma – Myoepithelial tumors – Urothelial carcinoma – Marker for basal/myoepithelial cells in prostatic and mammary glands	Thymoma, transitional cell carcinomas, Brenner tumor, papillary thyroid carcinoma, neuroendocrine carcinoma, choriocarcinoma, placental site nodule and epithelioid trophoblastic tumor, diffuse large B-cell lymphoma, primary cutaneous diffuse large B-cell lymphoma, leg type, primary mediastinal large B-cell lymphoma	Stratified epithelium, transitional epithelium, myoepithelial basal cells, a subset of lymphocytes, skeletal muscle (cytoplasmic stain)
Positive control: Prostate		

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 located on chromosome 3q27–29 encodes two groups of three isoforms with different N-termini, including the TA and ΔN isoforms. The TA isoforms contain the N-terminal domain and are involved in the regulation of apoptosis. The ΔN isoforms (known as p40) lack the N-terminal domain and are highly expressed in squamous and basal cells. Both isoforms can be labeled by specific antibodies, such as clone 4A4 to p63 or p40 directed to the ΔNp63-a isoform, whereas the latter seems to be more specific for squamous and basal cells [22, 23]. Both antibodies are excellent markers for squamous cell carcinoma of different origins, basal myoepithelial cells and myoepithelial tumors, in addition to transitional cell carcinoma of the urinary tract and choriocarcinoma.

The strong expression of p63 and p40 in the myoepithelial and basal cells makes them very helpful markers for discriminating between benign and malignant prostatic and breast lesions (See Sect. 13.1) (Fig. 2.19). p63 is also a helpful marker to discriminate between the follicular variant of papillary thyroid carcinoma and other benign follicular lesions, as follicular structures in non-papillary carcinoma usually lack the p63 expression [11].

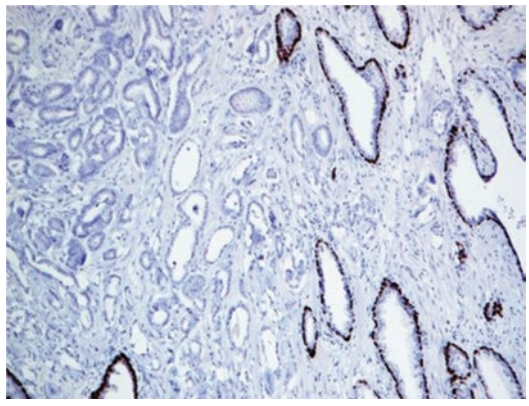

Fig. 2.19 Acinar adenocarcinoma of the prostate with p63 highlighting the basal cells in normal prostatic glands, whereas neoplastic glands lack the p63 positive basal cell layer

p63 and p40 are also markers for placental site nodule and epithelioid trophoblastic tumors in addition to a subset of choriocarcinoma, whereas exaggerated placental site and placental site trophoblastic tumors lack the expression of p63/p40.

Diagnostic Pitfalls p63 has been detected in up to 30% of pulmonary adenocarcinoma, specifically poorly differentiated types, 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 with p40 to classify pulmonary non-small cell carcinomas. Remarkably, p63 but not p40 expression was found in a subset of soft tissue tumors, including Ewing sarcoma/PNET, neurothekeoma, perineurioma, giant cell tumor, synovial sarcoma, rhabdomyosarcoma, MPNST, extraskeletal myxoid chondrosarcoma, and salivary gland tumors [24]. The expression of p63 in different soft tissue types is to be considered in interpreting tumors with epithelioid appearance. p63 is not a suitable marker to differentiate between poorly differentiated squamous cell carcinoma or transitional carcinoma and lymphoma as p63 exhibits nuclear expression in different types of non-Hodgkin lymphoma, including high-grade diffuse large B-cell lymphoma (up to 50% of cases), primary mediastinal large B-cell lymphoma, and follicular lymphoma (Fig. 2.20) [25]. A correlation between the p63 expression and a high proliferation index (Ki-67) is described.

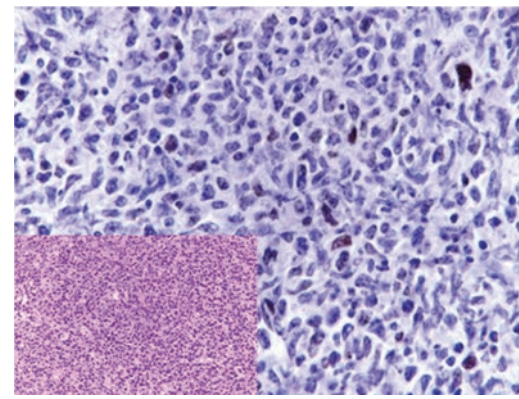

Fig. 2.20 Nuclear p63 expression in the cells of diffuse large B-cell lymphoma

2.5.4 Carcinoembryonic Antigen

Carcinoembryonic antigen (CEA; CD66e)		
Expression pattern: Cytoplasmic/extracellular		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Gastrointestinal and pancreatic adenocarcinoma – Cholangiocarcinoma and hepatocellular carcinoma – Pulmonary adenocarcinoma – Cervical adenocarcinoma	Breast carcinoma, non-keratinizing lung squamous cell carcinoma, 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 the fetal colon and to a lesser degree in adult colonic mucosa. CEA is highly expressed in different carcinoma types of various origins. CEA-negative malignant tumors are of importance in the differential diagnosis. Prostatic carcinoma, endometrioid carcinoma, renal cell carcinoma, serous ovarian 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.

2.5.5 Epidermal Growth Factor Receptor-1

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 – Choriocarcinoma – Triple-negative breast carcinoma (basal-like)	Glioblastoma, endometrial stromal sarcoma, 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 the type 1 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 origins, mostly carcinomas, including head and neck, renal, colonic, pancreatic, ovarian, and bladder carcinomas, in addition to basal-like triple-negative breast carcinoma. 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, head, and neck squamous cell carcinomas and colorectal adenocarcinoma. Colorectal adenocarcinomas sensitive to spe-

cific immunotherapy must have a wild RAS gene. Semi-quantitative evaluation of the EGFR expression on tumor cells might be required to estimate the response to specific immunotherapy, and the 3-point scoring system used for HER-2 can be used. Additionally, pulmonary carcinomas associated with driver mutations within the EGFR gene show an excellent therapeutic response to different EGFR tyrosine kinase inhibitors, whereas the EGFR protein expression itself is not a predictive marker.

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3 Markers and Immunoprofile of Pulmonary Tumors and Tumors of the Upper Respiratory Tract and Middle and Inner Ear

Contents

3.1 Diagnostic Antibody Panel for Tumors of the Upper Respiratory Tract and Middle and Inner Ear

Cytokeratin profile, p63/p40, p16, CD56, Synaptophysin, Chromogranin, INSM-1, EBV, and NUT.

3.2 Diagnostic Antibody Panel for Non-small Cell Pulmonary Tumors

Cytokeratin profile, TTF-1, Napsin A, p40, p63, Surfactant proteins, CDX-2, PDX-1, and NUT [1, 19].

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https://doi.org/10.1007/978-3-031-45024-2_3

3.3 Diagnostic Antibody Panel for Neuroendocrine Pulmonary Tumors

Synaptophysin, Chromogranin, CD56, S100, INSM-1, Islet-1, SSTR, orthopedia homeobox protein (OTP), and Ki-67 (see also Sect. 14.1, Neuroendocrine Markers).

3.4 Diagnostic Antibody Panel for Pulmonary Mesenchymal Tumors

CD1a, Langerin (CD207), HMB45, Melan A, STAT6, CD31, CD34, and CD99.

3.5 Therapy-Related Biomarkers

PD-L1, ALK, NTRK, ROS-1, c-Met, and HER-2.

3.5.1 Thyroid Transcription Factor-1

Thyroid transcription factor-1 (TTF-1)		
Expression pattern: Nuclear		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
• Pulmonary adenocarcinoma: – Non-mucinous – Mucinous – Adenosquamous carcinoma • Pulmonary small cell carcinoma • Pulmonary blastoma – Carcinomas of the thyroid gland: Papillary, follicular, and medullary carcinoma – Large duct type and extrahepatic cholangiocarcinoma	Non-pulmonary small cell carcinoma of different locations, nasopharyngeal papillary adenocarcinoma, mesonephric and mesonephric-like adenocarcinoma, glial-ependymal and choroid plexus tumors, pituicytoma, granular cell tumor and spindle cell oncocytoma of the sellar region, meningeal tumors	Type II pneumocytes and Clara cells of the lung, thyroid follicular and parafollicular C cells, pituitary gland (neurohypophysis), glial cells of basal hypothalamus, 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 also plays an active role in the regulation of development and transcriptional activity of the lung and central nervous system (diencephalon). In the adult thyroid gland, TTF-1 is expressed in both follicular and parafollicular cells and controls the synthesis of different thyroid hormones and the thyrotropin receptor. In normal lung, TTF-1 is strongly expressed in type II alveolar cells, Clara bronchiolar cells, and to 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 [13].

In routine immunohistochemistry, TTF-1 is widely used as a specific and sensitive marker for the majority of pulmonary adenocarcinomas, including non-mucinous and mucinous adenocarcinomas and to a lesser degree in colloid,

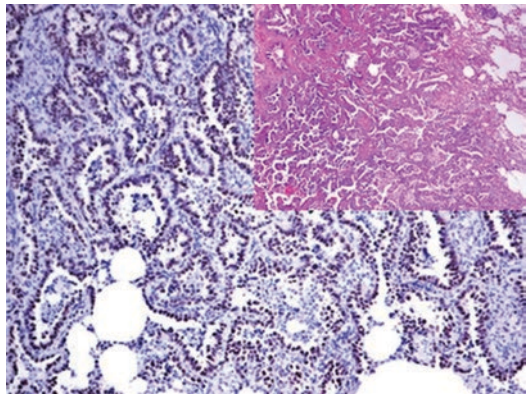

Fig. 3.1 Strong nuclear TTF-1 expression in pulmonary adenocarcinoma

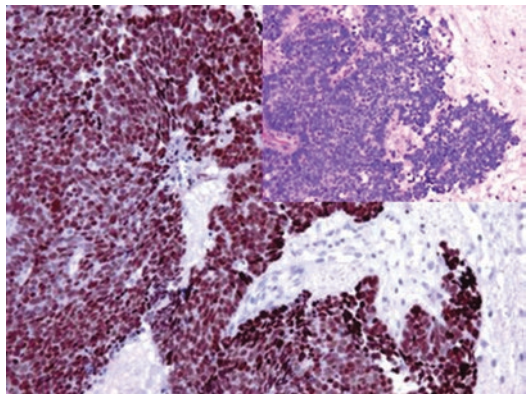

Fig. 3.2 Strong nuclear TTF-1 expression in pulmonary small cell neuroendocrine carcinoma

fetal, and enteric type adenocarcinomas, in addition to pulmonary small cell carcinoma and large cell neuroendocrine carcinoma. At the same time, a weak expression may also be found in pulmonary carcinoids (NET G1–2). Large cell carcinoma of the lung is constantly negative for TTF-1 and p40 (null immunophenotype). Furthermore, TTF-1—also in combination with Pax-8—is an important diagnostic marker for follicular, papillary, and medullary thyroid carcinomas (Figs. 3.1 and 3.2) and undifferentiated thyroid carcinoma (See Sect. 14.3) [2, 3]. Pulmonary squamous cell carcinoma 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, clone-independent TTF-1 expression is reported in different epithelial and non-epithelial extrapulmonary tumors. The TTF-1 expression is found in small cell neuroendocrine carcinomas of different origins, such as urinary bladder, ovarian and prostatic small cell carcinomas, in addition to Merkel cell carcinoma and in rare cases of breast, uterine and ovarian carcinomas beside mixed Müllerian Tumors [16, 23].

The aberrant expression of TTF-1 is also reported in about one-half of extrahepatic cholangiocarcinomas, including gallbladder adenocarcinoma, while non-neoplastic biliary epithelium lacks the TTF-1 expression (Fig. 9.10) [4].

The TTF-1 expression in various types of CNS tumors—especially those in the third ventricle region—is also to be considered when searching for primary brain metastases [12]. The nuclear TTF-1 expression in tumors of the neurohypophysis is remarkable, including pituicytoma and granular cell tumors of the sellar region [11]. Remarkable is also the aberrant nuclear expression of TTF-1 in more than 50% of Schwannomas and very rare non-Hodgkin lymphomas [15, 17].

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 (Fig. 9.9) [5].

Despite the abovementioned unexpected phenomena, TTF-1 remains a valuable, specific, and sensitive immunohistochemical marker for diagnosing pulmonary and thyroid tumors.

3.5.2 Napsin A

Napsin A		
Expression pattern: Cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Pulmonary adenocarcinoma – Papillary renal cell carcinoma – Ovarian and endometrial clear cell carcinoma	Sclerosing pneumocytoma, a subset of clear renal cell carcinoma, a 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		

Diagnostic Approach Napsin A is a 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 normally found in Type 2 pneumocytes, respiratory epithelium, alveolar macrophages, and in the cells of renal tubules in addition to plasma cells and a subset of lymphocytes. Napsin A is expressed in the majority of pulmonary adenocarcinomas (more than 80% of conventional adenocarcinomas and in about 30% of large cell carcinoma); consequently, Napsin A is used as a specific marker for pulmonary adenocarcinoma whereas primary pulmonary squamous cell carcinoma and small cell carcinoma 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.

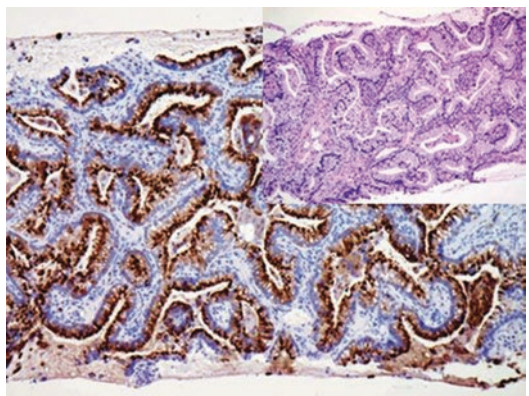

Fig. 3.3 Metastatic pulmonary adenocarcinoma with strong cytoplasmic Napsin A expression

Diagnostic Pitfalls The expression of Napsin A may be found in other non-pulmonary tumors. A low expression level of Napsin A is observed in up to 80% of papillary renal cell carcinoma and a small subset of clear renal cell carcinoma, but those constantly lack the expression of TTF-1 [18].

The expression of Napsin A is also reported in up to 90% of endometrial and ovarian clear cell carcinomas and about one-third of extrahepatic cholangiocarcinoma, and later may also be positive for TTF-1 [4, 6, 7]. Weak Napsin A expression is also reported in a small subset of colorectal and esophageal and pancreatic adenocarcinomas. Noteworthy is the expression of Napsin A in different primary and metastatic thyroid carcinomas, which are usually markedly positive for TTF-1. Micropapillary pattern of thyroid carcinoma commonly shows a strong Napsin A expression, whereas a small subset of anaplastic and poorly differentiated thyroid carcinomas is Napsin A positive [14]. 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.

3.5.3 Surfactant Proteins

Surfactant proteins		
Expression pattern: Cytoplasmic/membranous		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Pulmonary adenocarcinoma	Surface cells of sclerosing hemangioma of the lung	Type 2 pneumocytes, bronchiolar cells
Positive control: Lung tissue		

Diagnostic Approach Surfactant proteins, including surfactant proteins A, B, C, and D, in addition to surfactant precursors, are lipoproteins synthesized by type II pneumocytes and Clara bronchiolar epithelial cells essential to maintain the surface tension of the alveoli while type I pneumocytes lack the expression of surfactant proteins. Antibodies to surfactant proteins are good markers for pulmonary adenocarcinoma. Pulmonary squamous cell carcinoma, large cell carcinoma, and non-pulmonary adenocarcinomas, besides 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 also be positive for surfactant. The diagnosis of primary or metastatic pulmonary adenocarcinoma must be based on clinical data, microscopic appearance, cytokeratin profile, TTF-1, and Napsin A expression. The expression of surfactant and the absence of CDX-2, GATA-3, and steroid receptors help support the diagnosis of primary pulmonary carcinoma.

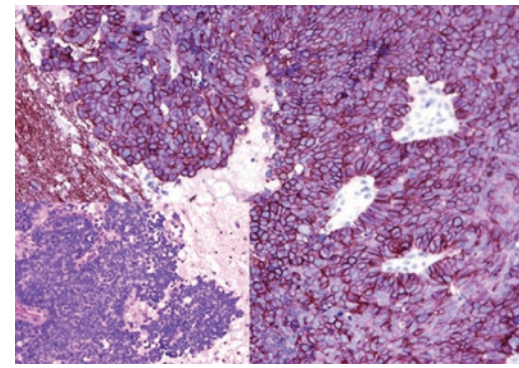

Fig. 3.4 Small cell carcinoma with the typical membranous CD56 expression pattern

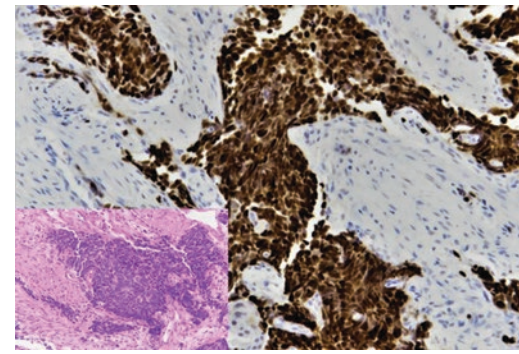

Fig. 3.5 Small cell carcinoma with strong nuclear Islet-1 expression

3.5.4 Neuroendocrine Markers

CD56 is the marker of choice to label the cells of small cell carcinomas with the typical membranous stain pattern. However, CD56 is an adhesion molecule and not a specific neuroendocrine marker that can be focally expressed in a subset of non-endocrine tumors which makes it challenging to interpret the expression in small lung biopsies (Fig. 3.4). To confirm the neuroendocrine origin, other more specific neuroendocrine markers can be used, such as INSM-1 and Islet-1. In our series, Islet-1 labels about 60% of small cell carcinomas, 70% of which with very strong nuclear stain (Fig. 3.5). Chromogranin and synaptophysin are not ideal to label small cell carcinomas as the neurosecretory granules are not accumulated in the scanty cytoplasm of the small tumor cells, especially chromogranin may appear negative, while synaptophysin is usually superior. Chromogranin and synaptophysin are appropriate markers for carcinoid tumors. Orthopedia homeobox protein (OTP) is an additional interesting marker for primary pulmonary neuroendocrine tumors (see below).

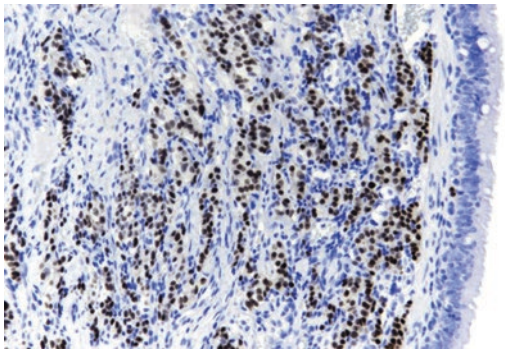

Fig. 3.6 Bronchial wall infiltrated by the cells of typical carcinoid. Carcinoid cells with strong nuclear OTP stain

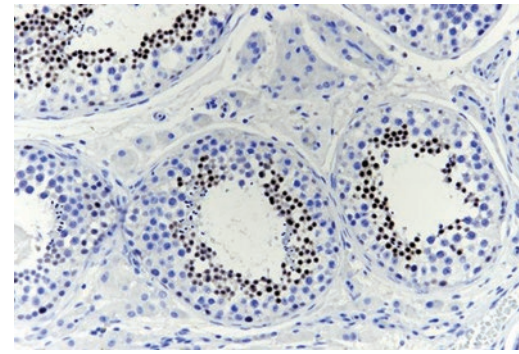

Fig. 3.7 Testicular biopsy with NUT highlighting spermatogonia and spermatids

3.5.5 Orthopedia Homeobox Protein

Orthopedia homeobox protein is a member of the homeodomain family and a transcription factor involved in neuroendocrine and neuroepithelial differentiation. OTP has been recently described as a marker for pulmonary well-differentiated neuroendocrine tumors. OTP is expressed in the majority of typical (~90%) and less common in atypical (~50%) carcinoids but negative in pulmonary small cell carcinoma and large cell neuroendocrine carcinoma and in other non-small cell carcinomas of the lung (Fig. 3.6) [21]. Neuroendocrine tumors of the digestive system usually lack the expression of OTP. Low OTP expression levels are reported in a subset of genitourinary endocrine tumors [22].

3.5.6 Nuclear Protein in Testis

The nuclear protein in testis (NUT) protein is the product of the NUT (NUTM1) gene located on chromosome 15q14 and normally expressed in testicular spermatogenic cells (spermatogonia and spermatids) in addition to oocytes of the ovary (Fig. 3.7). Midline carcinoma is a rare highly malignant carcinoma that accrues in the thorax, head, and neck region and is characterized by the t(15;19) translocation caused by the fusion of the NUT gene to the BRD3 gene located on chromosome 19p13.1, resulting in the overexpression of the NUT protein. This translocation is found in up to 70% of the midline carcinoma cases, whereas additional translocation partners other than the BRD3 gene cause the other 30% of the cases [20].

Antibodies to NUT are specific markers for midline carcinoma [8–10]. The expression of NUT is also found in the majority of spermatocytic seminomas and in a subset of primary and metastatic seminomas in addition to embryonal carcinoma. Additionally, NUT is also a marker for various NUTM1 rearranged neoplasms of different origins, including the cytokeratin, CD117 and DOG-1 negative NUTM1 rearranged gastrointestinal sarcoma, and a subset of B-ALL.

3.5.7 Anaplastic Lymphoma Kinase

Anaplastic lymphoma kinase (ALK) is a membrane-associated kinase encoded on chromosome 2p23 and clustered as CD246, listed in Sect. 16.4 with other lymphoma markers.

Multiple activating genetic alterations are found in association with non-small-cell lung carcinomas, whereas the EML4-ALK rearrangement is the most common ALK anomaly found in ~4% of non-small-cell lung carcinomas, specifically adenocarcinomas. It is formed by a small inversion within the short arm of chromosome 2 (2p) involving the ALK gene and the echinoderm microtubule-associated protein-like 4 (EML4), generating a constitutively active ALK tyrosine kinase. Other ALK fusion partners have also been described in pulmonary carcinomas. The EML4-

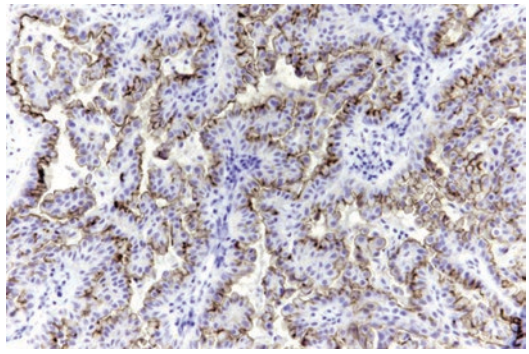

Fig. 3.8 ALK-positive pulmonary adenocarcinoma with strong membranous ALK expression

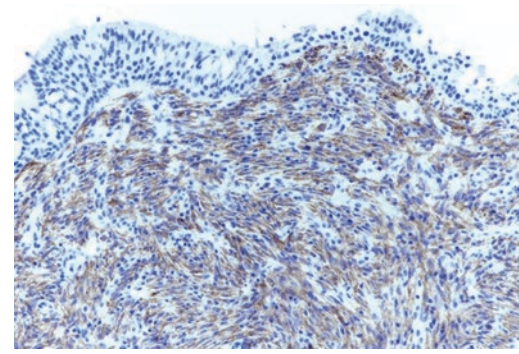

Fig. 3.9 Inflammatory myofibroblastic tumor of the lung with strong cytoplasmic ALK expression

ALK rearrangement is found mainly in adenocarcinomas arising in young, non-smoking females.

The ALK molecule is the target for specific tyrosine kinase inhibitors used for the therapy of different ALK-positive tumors including pulmonary adenocarcinoma. The immunohistochemical detection of ALK in tumor cells using specific antibodies is a sensitive surrogate for a possible ALK gene rearrangement. Immunohistochemistry is an approved assay to predict the response for the specific ALK tyrosine-kinase inhibitor (ALK-TKI) therapy, nevertheless, the positive result obtained by immunohistochemistry can be later confirmed by one of the molecular methods, including Fluorescence in situ hybridization (FISH) (See Sect. 16.4) (Fig. 3.8) [24].

In mesenchymal lung tumors, ALK is a diagnostic marker and therapeutic target for the inflammatory myofibroblastic tumor. ALK translocations are associated with ~70% of the cases and 30% with other genes, including ROS-1, NTRK, and RET (Fig. 3.9).

3.5.8 ROS-Associated Oncogene 1

The ROS-associated oncogene 1 (ROS-1) gene is located on chromosome 6q22 and encodes a receptor tyrosine kinase, a member of the insulin receptor family. Gene amplification or different rearrangements involving the ROS-1 gene with other gene fusion partners, such as the CD74, EZR, or SLC34A2, are described as potential driver mutations in 1–2% of non-small-cell lung carcinomas, mainly adenocarcinomas causing the overexpression of the ROS-1 protein. These adenocarcinomas typically show a solid or cribriform morphology with signet-ring cells or extracellular mucin and occur mainly in young, non-smoking females.

Immunohistochemistry is an acceptable screening test for the detection of ROS-1 overexpression in related adenocarcinomas, but the gene rearrangement must be confirmed by FISH or one of the molecular methods to predict the response to the specific ROS1-TKI therapy. Because of intracellular mucin, the immunohistochemical staining of the ROS-1 frequently shows a false positive background, whereas the specific immunohistochemical expression pattern depends on the ROS-1 gene fusion partner, mostly cytoplasmic but may also be membranous [25].

3.5.9 C-Mesenchymal-Epithelial Transition Factor

The C-mesenchymal-epithelial transition factor (c-Met) gene is located on chromosome 7 q21–3 and encodes a transmembrane tyrosine kinase receptor activated by its ligand, the hepatocyte growth factor (HGF). The c-Met HGF pathway promotes several cellular processes, including cellular proliferation, regeneration, differentiation, and angiogenesis. Due to different c-Met genomic alterations, including point mutations, amplification, fusion, translocation, or dysregulation of transcription that cause the overexpres-

sion of the c-Met protein, the expression of the c-Met is found in 20–80% of lung tumors with different intensities. Therapy-related genomic alterations are reported only in ~2% of pulmonary non-small-cell carcinomas, mainly alterations in exon 14 of the Met gene in addition to gene amplification [26].

Immunohistochemistry is only a screening test to detect therapy-related c-Met genetic alterations. Further confirmation and characterization of these genetic alterations by FISH and/or molecular methods (NSG) are necessary to predict the response to specific c-Met inhibitor therapy [27].

3.5.10 Programmed Death-Ligand 1

Programmed death-ligand 1 (PD-L1) (clustered as CD274) is a cell surface ligand transmembrane protein and the therapeutic target for selective checkpoint inhibitors in non-small cell carcinoma. PD-L1 is listed in detail in Chap. 31.

Immunoprofile of lung and respiratory tract tumors and tumors of the middle and inner ear				
Tumor type	+ in > 90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in < 10% (−)
A. Tumors of the upper respiratory tract (nasal cavity, paranasal sinuses, nasopharyngeal, and skull base)				
Squamous cell carcinoma:	**CK5/6**, **p63**, **p40**, CK8, CK13, CK19, EMA			CK4, CK7, CK10, CK14, **p16**, bcl-2, EBV
Adenocarcinoma, intestinal-type:	CK20, PDX-1, CDX-2		CK7	CK5/14, p63, GATA3
Adenocarcinoma, non-intestinal-type:	CK7		CK20	CK5/14, p63, CDX-2
Sinonasal undifferentiated carcinoma:	CK-OSCAR, CK8, **p16**	CK7, CD117	CK19, EMA	CK4, CK5, CK6, CK10, CK13, CK14, CK20, NUT
Nasopharyngeal (undifferentiated) carcinoma:	**CK5/6**, CK8, CK13, CK19, EMA	Bcl-2, **EBV**, HLA-DR		CK4, CK7, CK10, CK14, p16, NUT
Low-grade nasopharyngeal papillary adenocarcinoma:	Pan-CK, CK7, CK19, **TTF-1**		CK5/6/14,	Thyroglobulin, PAX-8, CK20, p63
Midline carcinoma (NUT carcinoma):	Pan-CK, **NUT**[a]	CK5/14, CD56, p16, p63, CD34	CK7	EBV, CD99, chromogranin, Synaptophysin
Neuroendocrine carcinoma (sinonasal, laryngeal):	Pan-CK, **INSM-1**, Synaptophysin, chromogranin, CD56	Calcitonin, CEA	TTF-1	CK5/6/10/14
Olfactory neuroblastoma (esthesioneuroblastoma):	**CD56**, CD57, NSE, INSM-1, Islet-1, PGP9.5, Fli-1, **SSTR2A**, Neurofilaments *Sustentacular cells*: Sox-10, S100, GFAP	Synaptophysin, chromogranin, SATB-[2b], Calretinin	Pan-CK	GATA-3, EMA, WT-1, CD99, NUT, Sox-10[c]
Sinonasal glomangiopericytoma:	Actin, **LEF-1**[d], β-catenin, vimentin	FXIIIa, cyclin D1	S100	Pan-CK, CD31, CD34, ERG, Sox-10, CD117, CD99, STAT-6
Biphenotypic sinonasal sarcoma[e]:		S100, actin	Desmin, Myogenin	Sox-10, STAT6, pan-CK
Nasopharyngeal angiofibroma (stromal cells):	B-catenin (nuclear), androgen receptors		Actin, CD117	CD34, ER, PgR

B. Tumors of the middle and inner ear				
Middle ear adenoma:	*Luminal cells:* CK7 *Basal cells:* CK5/14, p63, chromogranin, Synaptophysin, INSM-1, Islet-1			GFAP, TTF-1, CDX-2, PAX-8, CK20
Endolymphatic sac tumor:	Pan-CK, CK7, PAX-8, Sox-10			TTF-1, S100
Aggressive papillary tumor:	Pan-CK, EMA			
Vestibular schwannoma:	See tumors of peripheral nerves			
Meningioma:	See meningeal tumors			
C. Lung epithelial tumors				
Alveolar adenoma:	CK7, TTF-1, Napsin A, surfactant			
Papillary adenoma:	CK7, TTF-1, surfactant			
Squamous cell carcinoma:	**CK5/6/10/13/14**, CK8/18, CK19, **p40**	**p63**	CK7[f], Calretinin	TTF-1, CK4, CK20, p16
Pulmonary adenocarcinoma non-mucinous type: – Lepidic adenocarcinoma – Acinar adenocarcinoma – Papillary adenocarcinoma – Micropapillary adenocarcinoma – Solid adenocarcinoma	**CK7**, CK8, CK18, CK19, **TTF-1**[g], CEA	**Napsin A**, surfactant proteins	p63, CK5/6/14[h], Mesothelin, villin	CD141 Calretinin, p40, CK20, CDX-2
Pulmonary adenocarcinoma mucinous type:	CK7	**CK20**, PDX-1	TTF-1, CDX-2, 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 (nuclear)	Chromogranin, ER-β	AFP[i], SALL4	CK5/6/14, p40
Pulmonary adenocarcinoma enteric type:	**CK7, CK20**	CDX-2, MUC2, villin		CK5/6/14, p40
Large cell carcinoma:	**CK7**, CK8, CK14, CK18, CK19, EMA		CK5/6/14	**TTF-1**, **Napsin A** , CK20
Neuroendocrine tumors: – NET G1 (typical carcinoid) – NET G2 (atypical carcinoid)	Pan-CK[j], CK8, CK18, **INSM-1**, **chromogranin**, **Synaptophysin**, **OTP**, NSE, PGP 9.5	S100, CD56, E-cadherin, EMA, CEA	CD99, CD117, TTF-1	CK5/6/14, p40, CK20
	Proliferation: NET G1 (carcinoid): Proliferation index (Ki-67): <3%; mitotic rate: <2/2mm^2 NET G2 (atypical carcinoid): Proliferation index (Ki-67): 3–20%; mitotic rate: 2–20/2mm^2			
Small cell carcinoma (neuroendocrine carcinoma small cell type; NET G3):	Pan-CK[j], CK8, CK18, CK19, **CD56**, **INSM-1**, S100, NSE Proliferation index (Ki-67): > 80%	Neurofilaments, **TTF-1**, **Islet-1**, CD99, PAX-5, Synaptophysin, chromogranin, CD117, CK7, vimentin	TdT	CK5/6/14, CK20

Neuroendocrine carcinoma large cell type (NET G3):	CK7, CK8, CK18, CK19 **INSM-1**, **CD56**, **chromogranin** Proliferation index (Ki-67): >20%; mitotic rate:>20/2mm^2	TTF-1, **Islet-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: – Adenoid cystic carcinoma – Mucoepidermoid carcinoma – Epithelial-myoepithelial carcinoma – Pulmonary hyalinizing clear cell carcinoma	See salivary gland tumors			
Pulmonary blastoma	*Epithelial component:* CK7, EMA, TTF-1, CEA	Chromogranin	Synaptophysin	
Sclerosing pneumocytoma (sclerosing hemangioma, inverting alveolar pneumocytoma hemangioma):	*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)[k] Vimentin	CK5/6, CK20, CD31, CD34, surfactant, Calretinin CK5/6, CK20 Calretinin, ER, and PgR
D. Lung mesenchymal tumors				
Clear cell tumor (sugar tumor):	**HMB45**[l], HMB50, Cathepsin K, CD68		CD57 (leu7), Synaptophysin, NSE, CD34	Pan-CK, EMA, S100, Chromogranin, CD56
Epithelioid hemangioendothelioma (intravascular bronchoalveolar tumor):	**CD31**, CD34, Vimentin			Pan-CK, Calretinin
Inflammatory myofibroblastic tumor (inflammatory pseudotumor):	Actin (in spindle cells), Vimentin	Desmin, Cyclin D1, **ALK (p80)**	bcl-2, NTRK^m, ROS-1^m, RET^m	Pan-CK, EMA, CD56
Pulmonary lymphangiomyomatosis:	Smooth muscle component: Actin, Caldesmon, **HMB45**	Estrogen and progesterone receptors		S100
Solitary fibrous tumor of the pleura:	CD34, **STAT6**, Vimentin	CD99, **Glutamate receptor-2**, bcl-2	Actin, TLE1, CD10, β- Catenin	Desmin, S100, Pan-CK, EMA, CD56, CD68, CD117
Intimal sarcoma:	**Sox-10**, S100, Vimentin	CD56, Actin, MDM2		Pan-CK, MelanA, HMB45, ERG, CD31, CD34, F VIII
Primary pulmonary myxoid sarcoma with EWSR1-CREB1 fusion:	Vimentin	EMA		Pan-CK,TTF-1, CD34, Chromogranin, Synaptophysin, CD56

Pulmonary Langerhans cell histiocytosis:	**CD1a**, [c] **CD207 (Langerin)**, [n] S100, HLA-DR	CD11c, CD68 CD31		Pan-CK

[a] Consider molecular detection of the t(15;19)(q13;p13.1) specific translocation
[b] See Fig. 3.10
[c] Positive in sustentacular cells
[d] See Fig. 3.11
[e] See Fig. 3.12
[f] CK7 found in up to 30% of pulmonary squamous cell carcinoma
[g] TTF-1 can be absent in poorly differentiated pulmonary adenocarcinomas
[h] Frequently positive in poorly differentiated pulmonary adenocarcinoma
[i] Can also be positive in poorly differentiated carcinoma
[j] Often dot-like expression pattern
[k] Atypical membranous and cytoplasmic stain pattern is noted when the MIB-1 clone is used
[l] See Fig. 3.13
[m] Expression may be found only in ALK-negative tumors
[n] See Fig. 3.14

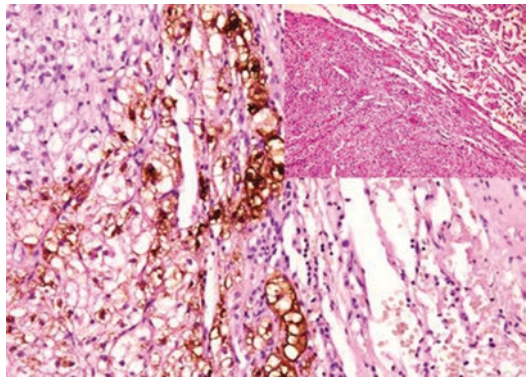

Fig. 3.10 Olfactory neuroblastoma; tumor cells with nuclear SATB-2 expression

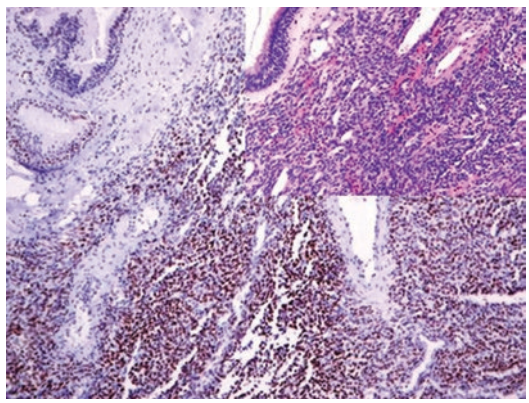

Fig. 3.11 Sinonasal glomangiopericytoma, tumor cells labeled by LEF-1 with nuclear stain

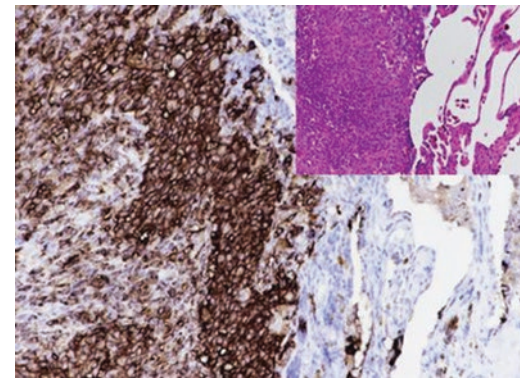

Fig. 3.12 HMB45 expressed in cells of pulmonary clear cell tumor (sugar tumor)

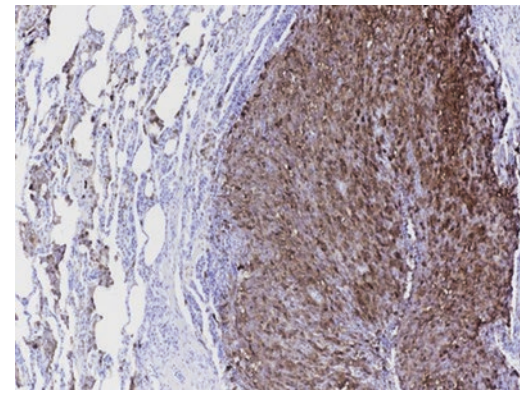

Fig. 3.13 Pulmonary Langerhans cell histiocytosis with strong CD1a expression

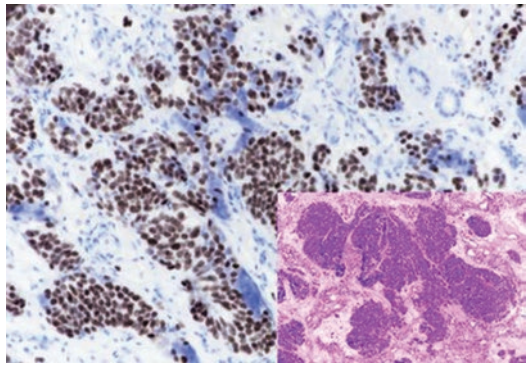

Fig. 3.14 Pulmonary Langerhans cell histiocytosis with strong Langerin expression

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Markers and Immunoprofile of Thymic Epithelial Tumors

4

Contents

4.1 Markers for Thymic Epithelium

Cytokeratin profile, p40, p63, PAX-8, CD117, CD5, CD20, FONX-1, and CD205.

4.2 Markers for Thymic Lymphoid Stroma

CD1a, CD3, and TdT [1, 2].

4.3 Markers for Thymic Neuroendocrine Tumors

Chromogranin, Synaptophysin, INSM-1, Islet-1, and CD56.

4.4 Therapy-Related Biomarkers

PDL-1.

Thymomas are a heterogeneous group of tumors composed of a mixture of neoplastic epithelial cells and lymphoid stroma in different proportions. Both components have distinctive morphology and immunoprofile characteristic for each thymoma type (A, AB, B1, B2, B3, and thymic carcinoma), and each thymoma type has its characteristic genetic abnormalities. Thymic neuroendocrine tumors are classified similarly to pulmonary endocrine tumors.

4.4.1 PAX-8 and CD117

PAX-8 and CD117 are two markers helpful in discriminating between normal and neoplastic

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https://doi.org/10.1007/978-3-031-45024-2_4

thymic epithelium as the latter is usually positive for both markers, whereas CD117 is more characteristic for atypical thymoma (B3) and thymic carcinoma (Figs. 4.1, 4.2, and 4.3) and usually negative in type A. Both markers are detailed in later chapters (see Sects. 12.1 and 7.2).

4.4.2 FOXN-1

The FOXN-1 gene encodes a transcription factor functioning as a regulator for the differentiation and involution of the thymic epithelial cells. FOXN-1 is also involved in the organogenesis of the parathyroid gland. FOXN-1 is a marker for thymic epithelial cells and tumors derived from these cells, including different thymoma types and thymic carcinoma [3–5].

4.4.3 CD205

CD205 (also called Lymphocyte antigen 75) is a membrane glycoprotein expressed on cortical thymic epithelial cells and dendritic cells in addition to monocytes involved in antigen uptake, trafficking, and presentation.

Both FOXN-1 and CD205 are sensitive and specific markers for thymomas expressed in up to 90% of these tumors.

4.4.4 Cytokeratin Profile and Lymphoid Stroma

The Cytokeratins 5/6/14 and p63 stain both benign and neoplastic thymic epithelial cells, including all thymoma types (Fig. 4.4).

CD5 is a marker for malignant thymic epithelium (thymic carcinoma), usually negative in benign thymic epithelium and thymomas type A, AB, and B1–3 (Fig. 4.5).

The lymphoid stroma associated with thymomas mainly comprises immature T-lymphocytes positive for CD3, CD1a, CD99, and TdT (Fig. 4.6).

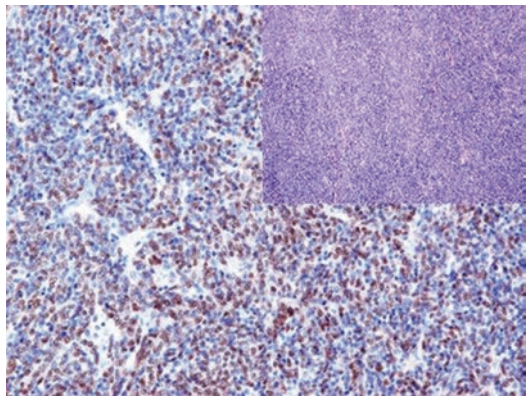

Fig. 4.1 Nuclear PAX-8 expression in neoplastic epithelial cells of thymoma type AB

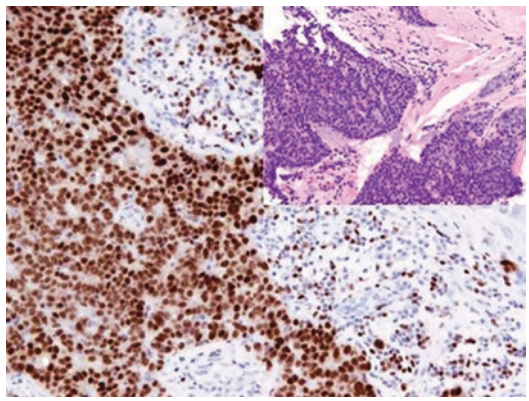

Fig. 4.2 Nuclear PAX-8 expression in malignant epithelial cells of thymic carcinoma

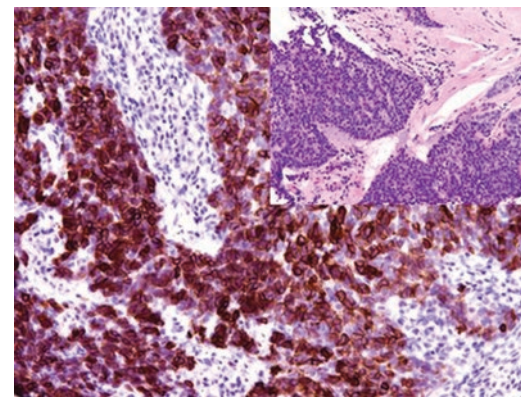

Fig. 4.3 CD117 staining malignant epithelial cells of thymic carcinoma

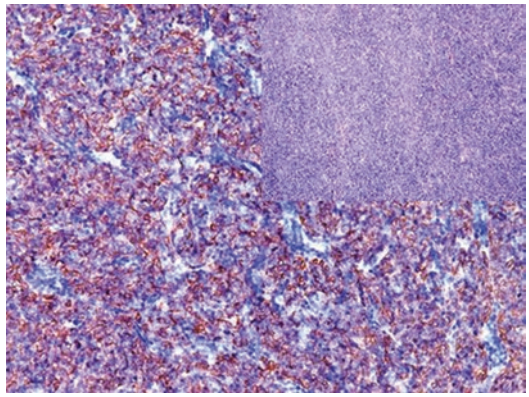

Fig. 4.4 Cytokeratin 5/14 expression in neoplastic epithelial cells of thymoma AB type

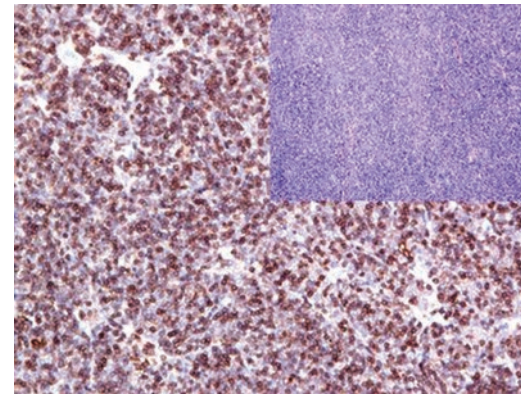

Fig. 4.6 CD1a labeling the tumor-associated T-lymphocytes in thymoma AB type

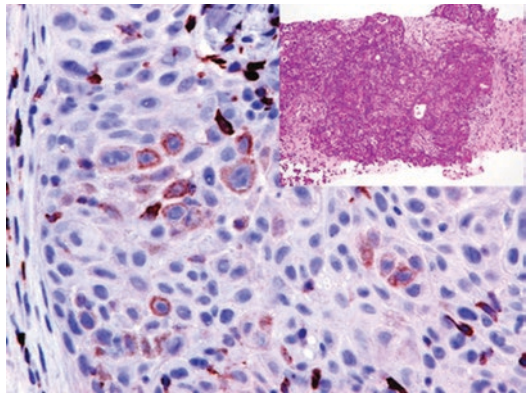

Fig. 4.5 Thymic carcinoma with CD 5 highlighting the neoplastic epithelial cells

Immunoprofile of thymic epithelial tumors				
Tumor type	+ in > 90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in < 10% (−)
Thymoma (type A, AB, and B1, 2 & 3): – 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:* CK5/6/14, CK7, CK8, CK18, CK19, **FOXN-1**, PAX-8, p63 *Tumor-associated lymphocytes*: Predominantly immature T-lymphocytes positive for TdT, CD1a[a], CD3, CD99	CD15, CD57 (leu7), **CD205**, D2–40[b]	CD20[c]	EMA, CD5, bcl-2, CD117, HER-2

Thymic carcinoma (squamous cell carcinoma/basaloid carcinoma):	*Neoplastic epithelial cells:* CK5/6/14, CD5, p63, p40, CD70, bcl-2, EMA *Tumor-associated lymphocytes:* Predominantly mature T- and B-lymphocytes negative for TdT	CD15, CK7, CK8, CK18, CK19, PAX-8, CD117, **FOXN1**, **CD205**	Synaptophysin, chromogranin, HER-2, Mesothelin	TTF-1, NUT
Clear cell carcinoma:	CK5/6/14, p63, p40		CD5, CD117	TTF-1
Low-grade papillary adenocarcinoma of the thymus:	CK7, CK8, EMA		CD5, CD15, CD117, CEA, Calretinin	TTF-1, CK5/14, p63, p40, CK20, PAX-8
Enteric type adenocarcinoma of the thymus:	CK20, PDX-1, CDX-2		CK7, CD5	TTF-1, CD117
Adenocarcinoma (NOS) of the thymus:	CK8	CK7	CEA	CK20, CDX-2
Salivary gland-type tumors: – Adenoid cystic carcinoma – Mucoepidermoid carcinoma	See salivary gland tumors			
Thymic neuroendocrine tumors: – Carcinoid and atypical carcinoid (NET G1 & G2) – Small cell carcinoma (NET G3/NEC) – Large cell neuroendocrine carcinoma (NEC)	See neuroendocrine tumors and neuroendocrine carcinomas of the lung			
Undifferentiated carcinoma of the thymus	Pan-cytokeratin	PAX-8, CD117		CK5/14, p63, p40, Oct-4

[a] See Fig. 4.7

[b] D2–40 is mainly expressed in thymomas type B2-B3

[c] CD20 is focally expressed on the epithelial cells of thymomas type A and AB (~ 50%) (Fig. 4.7) but negative in normal thymic epithelium and thymomas type B and C

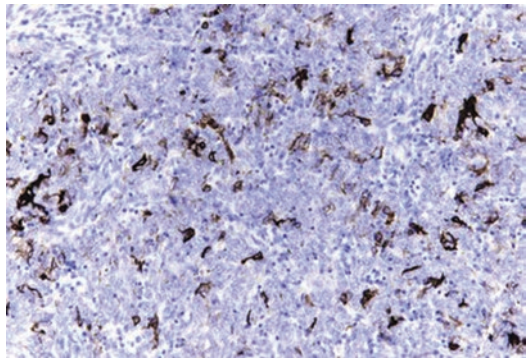

Fig. 4.7 CD20 expression on the epithelial cells of type A thymoma

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Markers and Immunoprofile of Heart and Pericardial Tumors

5

Contents

5.1 Diagnostic Antibody Panel for Heart Tumors

The primary tumors of the heart are heterogeneous with different histogenesis and constellation. The diagnostic immunohistochemical panel depends on the histogenesis and morphology of the tumor.

M. S. A. Tuffaha et al., *Immunohistochemistry in Tumor Diagnostics*,
https://doi.org/10.1007/978-3-031-45024-2_5

Immunoprofile of heart and pericardial tumors				
Tumor type	+ in > 90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in < 10% (−)
Rhabdomyoma/ rhabdomyosarcoma:	Desmin, sr-actin, myosin, myoglobin	Myo-D1	HMB45[a]	Pan-CK, sm-actin, S100
Cardiac myxoma:	Calretinin[(a)], PGP9.5, CD31, CD34, actin,	Desmin, Synaptophysin		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, glutamate receptor-2, bcl-2	Actin, TLE1, CD10, β- catenin	Desmin, S100, pan-CK, EMA, CD56, CD68, CD117
Mesothelioma:	See the chapter on mesothelioma (Chap. 15)			

[a] Positive only in a subset of rhabdomyoma
[b] See Fig. 5.1
[c] See Fig. 5.2

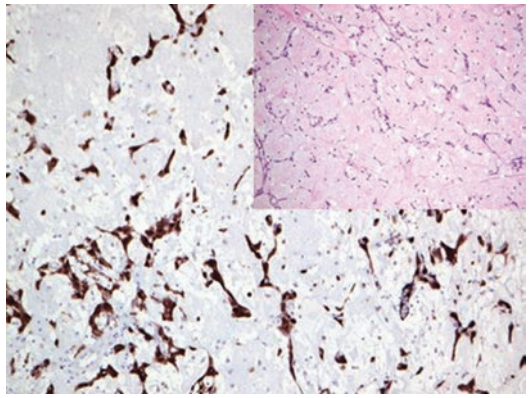

Fig. 5.1 Cardiac myxoma; tumor cells showing strong Calretinin expression

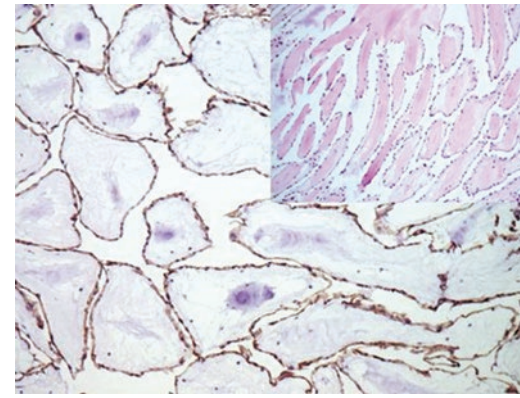

Fig. 5.2 Papillary fibroelastoma lined by CD31 positive endothelial cells

6 Markers and Immunoprofile of Tumors of the Oral Cavity, Oropharynx and Salivary Glands

Contents

6.1 Odontogenic Tumors and Tumors of the Oral Cavity and Oropharynx

6.1.1 Diagnostic Antibody Panel for Odontogenic Tumors and Tumors of the Oral Cavity

Cytokeratin profile, p63, p40, p16, NUT, EBV, and Sox-10.

M. S. A. Tuffaha et al., *Immunohistochemistry in Tumor Diagnostics*,
https://doi.org/10.1007/978-3-031-45024-2_6

Immunoprofile of odontogenic tumors and tumors of the oral cavity and oropharynx				
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, HPV-independent:	**CK5/6/14**, CK8/18, CK19, **p63**, **p40**			CK7, CK20
Squamous cell carcinoma, HPV-associated:	**CK5/6/14**, CK8/18, CK19, **p63**, **p40**, **p16**[a]			CK7, CK20
Sebaceous carcinoma:	**Adipophilin**, EMA, androgen receptors, CEA			
Clear cell odontogenic carcinoma:	CK8, CK 13, CK14, CK18, CK19, EMA			Vimentin, Desmin, actin, S100, HMB45
Ectomesenchymal chondromyxoid tumor of the tongue:	GFAP, vimentin	S100, CD56	Pan-CK, CD56	CK5/14, CK7, p63, actin, Calponin, Desmin
Ameloblastoma/ameloblastic carcinoma:	Pan-CK, CK5/14, p63, p40, CD56[b], CD138, E-cadherin	Calretinin, BRAF-$_{V600E}$		CK7
Melanotic neuroectodermal tumor of infancy:	*Epithelioid cells:* Pan-CK, HMB45 n*euroblast-like cells:* Synaptophysin			Chromogranin
Granular cell tumor:	S100, **Sox-10**, CD56, nestin, vimentin	Inhibin, Calretinin, CD68	TFE-3	Pan-CK, actin, HMB-45, Desmin
Rhabdomyosarcoma with TFCP2 rearrangement:	Pan-CK, Desmin, Myo D1	Myogenin, ALK	P63, Satb-2, CD30	
Mucosal melanoma:	See chap. 22 (melanocytic tumors)			

[a] See Fig. 6.1
[b] Positive only in the peripheral cells of tumor nests (see Fig. 6.2)

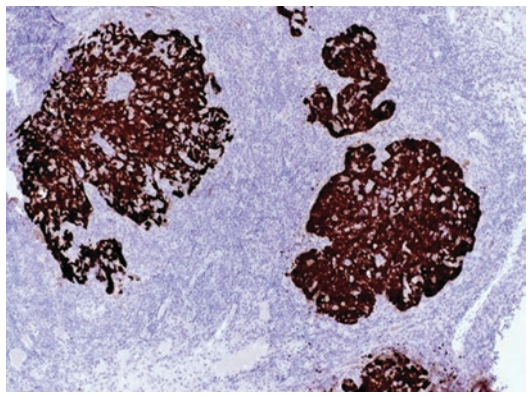

Fig. 6.1 Mucosa-associated lymphoid tissue from the base of the tongue infiltrated by HPV-associated squamous cell carcinoma with strong 16 expression

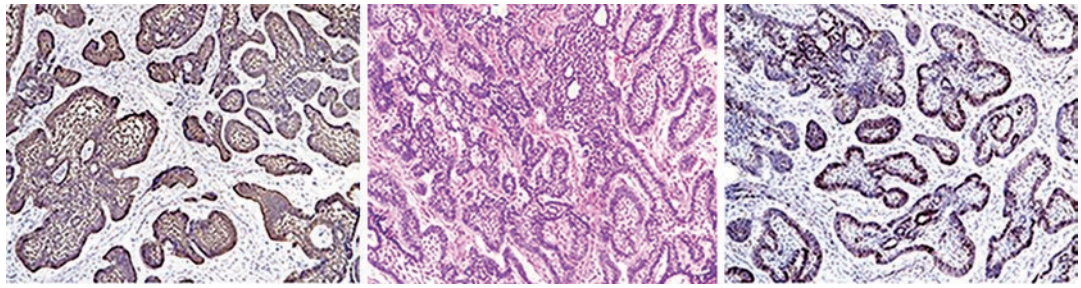

Fig. 6.2 Ameloblastoma with CK5/14 positive cells (***left***) and CD56 expression in the peripheral cells (***right***)

The immunoprofile of miscellaneous soft tissue tumors arising in the oral cavity is listed in related chapters.

6.2 Salivary Gland Tumors

6.2.1 Diagnostic Antibody Panel for Salivary Gland Tumors

6.2.1.1 Markers for Acinar Cells and Acinic Tumors

α-Amylase, DOG-1, CK8/18, and NR4A3.

6.2.1.2 Markers for Ductal/Luminal Cells

CK7

6.2.1.3 Markers for Myoepithelial Cells

CK5/6/14, p63/p40, Sox-10, GFAP, sm-Actin, h-Caldesmon, Calponin, and S100 [1–3].

6.2.1.4 Markers Specific for Salivary Gland Tumors and Genetic Mutations Associated with Salivary Gland Tumors

GATA-3, CD117, Myb, LEF-1, PLAG-1, HMGA-2, NTRK, and $NRAS_{Q61R}$.

6.2.1.5 Therapy-Related Markers

HER-2, NTRK, and PD-L1.

Salivary glands, including the three major glands and minor glands, are composed of three main epithelial components and each of them can be the origin of primary salivary gland neoplasia, which is essential to consider in the interpretation of salivary gland tumors as the immunoprofile of the salivary gland tumor depends on the origin of neoplastic cells:

- Serous acini.
- Mucous acini.
- Ducts: include the intercalated, striated, and excretory ducts composed of ductal/luminal cells and myoepithelial/basal cells.

Lacrimal gland tumors and tumors arising from bronchial mucous glands are similar to salivary gland tumors and show identical histology and immunophenotype.

6.3 Cytokeratin Profile

Generally, the cytokeratin profile is an important tool to highlight the different cell types forming the salivary gland units or tumors derived from these cell types. Cytokeratins 8/18 label the acinar cells, whereas Cytokeratin 7 is a marker for ductal/luminal cells. High molecular weight Cytokeratins (CK5/10/14) label the myoepithelial and basal cells (See Sect. 2.1). p63, actin, and myosin are also additional markers that label these cells. Recently, Sox-10 was also established as a marker for myoepithelial cells and tumors deriving from these cells (see Chap. 22) (Fig. 6.3).

The atypical distribution of different cell types is clearly seen in biphasic tumors composed of two cell types (Fig. 6.4).

Interesting is the p63 positive/p40 negative immunophenotype characteristic for canalicular adenoma, polymorphous adenocarcinoma, and

microsecretory adenocarcinoma. This immunophenotype distinguishes these tumors from other carcinoma types, especially adenoid cystic carcinoma [4].

6.3.1 Anoctamin-1 (DOG-1)

DOG-1 is a transmembrane chloride channel protein highly expressed in the cells of Cajal and 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, lacrimal glands, and the pancreas (Fig. 6.5) [5]. DOG-1 is a diagnostic immunohistochemical marker for acinic cell carcinomas of salivary glands. Weak to moderate expression level of DOG-1 is also found in a small subset of polymorphous low-grade adenocarcinoma (Figs. 6.6 and 6.7), adenoid cystic carcinoma, mucoepidermoid carcinoma, and epithelial-myoepithelial carcinoma.

6.3.2 Alpha-Amylase

Amylases are enzymes that catalyze the cleavage of the glucoside bonds of large sugar molecules into oligosaccharides. Amylases are synthesized by acinic cells of salivary glands and pancreas. Salivary gland and pancreatic amylase are encoded by different genes and have different amino acid sequences with different antigenic proprieties. In diagnostic immunohistochemistry, antibodies to α-amylase are used as specific markers for acinic cell carcinoma of salivary glands. Other salivary gland tumors are usually negative for Amylase. Antibodies specific to salivary gland amylase do not label pancreatic acinic cell carcinoma.

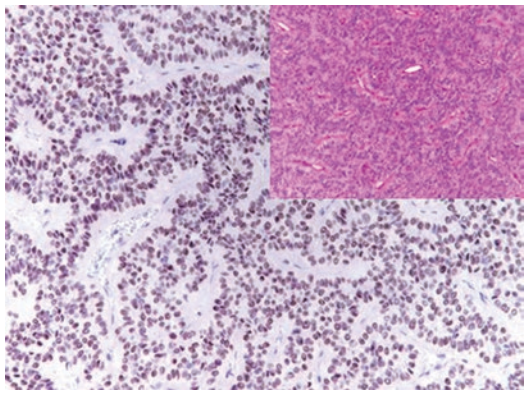

Fig. 6.3 Myoepithelioma, tumor cells with nuclear expression of Sox-10

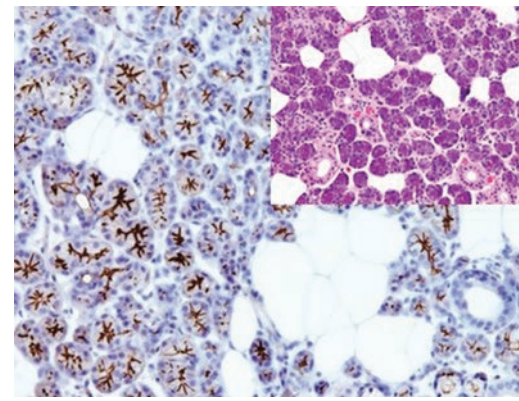

Fig. 6.5 Acinic cell carcinoma of the parotid gland; DOG-1 highlighting the apical surface of the tumor cells

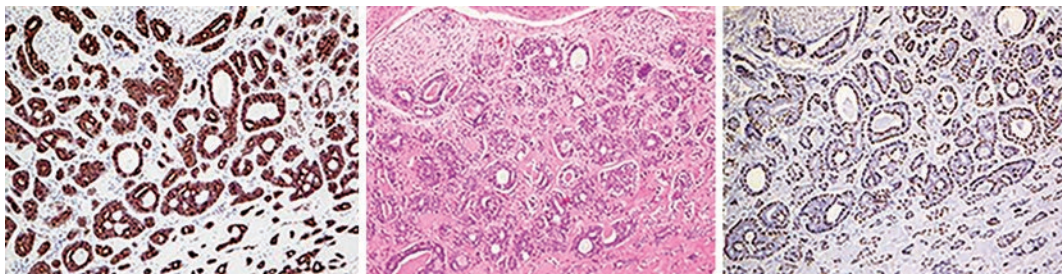

Fig. 6.4 Cytokeratin expression pattern in adenoid cystic carcinoma; CK7, HE, p63. Luminal/ductal cells are positive for CK7 (***left***) and basal cells are labeled by p63 (***right***)

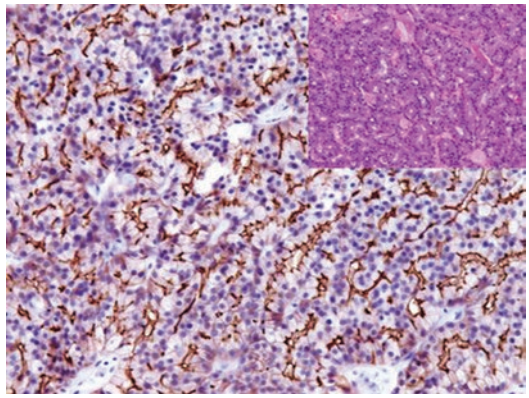

Fig. 6.6 DOG-1 highlighting the apical surface of acinar cells of the parotid gland

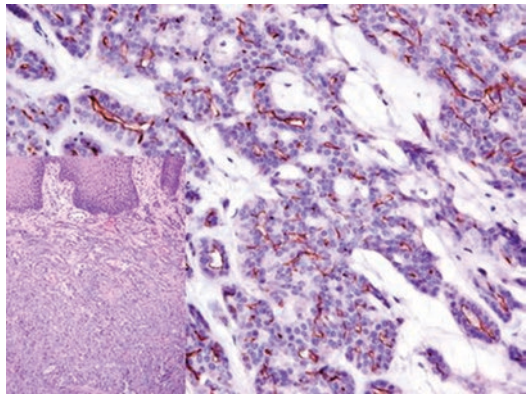

Fig. 6.7 Polymorphous low-grade adenocarcinoma; DOG-1 labels the apical surface of the tumor cells

6.3.3 GATA-3

GATA-3 is a transcription factor frequently used as a marker that strongly labels primary and metastatic breast carcinoma, transitional cell carcinoma of the urinary tract, and skin tumors (see markers of breast tumors, Chap. 10). Less intensive expression level is also found in salivary gland ductal cells, and GATA-3 can be used as a marker for tumors originating from these cells. Strong GATA-3 expression is characteristic for salivary duct carcinoma and mammary analogue secretory carcinoma. Weak to moderate GATA-3 expressed levels in a subset of tumor cells are found in acinic cell carcinoma, adenoid cystic carcinoma, mucoepidermoid carcinoma, polymorphous low-grade adenocarcinoma, and oncocytoma [6]. The expression of GATA-3 in salivary gland tumors is to be considered in the differential diagnosis of tumors of unknown primary [7].

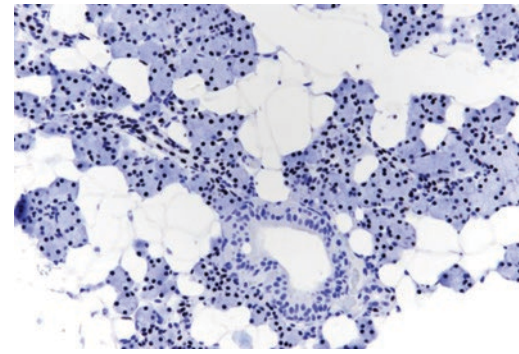

Fig. 6.8 Sox-10 highlighting the nuclei of acinic cells, myoepithelial cells, and cells of intercalated ducts

6.3.4 Sox-10

Sox-10 is a member of the Sox family of transcription factors listed in detail with the melanoma markers (see Chap. 21). Sox-10 is also a very informative marker for diagnosing salivary gland tumors. In normal salivary gland tissue, Sox-10 labels the acinic cells, cells of intercalated ducts, and basaloid and myoepithelial cells (Fig. 6.8) [8, 9]. In salivary gland tumors, Sox-10 labels tumor cells of acinic cell carcinoma, adenoid cystic carcinoma, epithelial-myoepithelial carcinoma, polymorphous low-grade adenocarcinoma, mammary analogue secretory carcinoma, sialoblastoma, basal cell adenoma, and adenocarcinoma, in addition to pleomorphic adenoma (Fig. 6.9) [8]. Salivary duct carcinoma, clear cell carcinoma, adenocarcinoma NOS, and oncocytoma were found to be negative for Sox-10.

6.3.5 MYB

MYB proto-oncogene is a member of the MYB (myeloblastosis) family of transcription factors functioning as a regulator of hematopoietic cells. A balanced translocation between the MYB and NFIB genes in the t (6;9)(q22–23;p23–24) translocation is the most common genetic abnormality

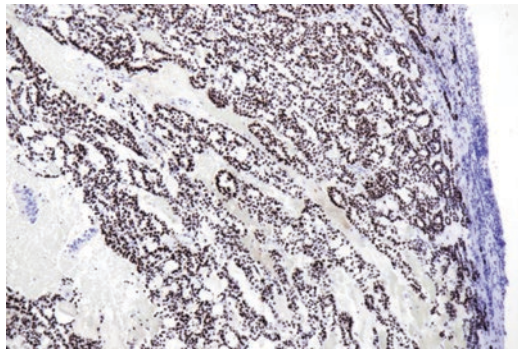

Fig. 6.9 Sox-10 highlighting the nuclei of tumor cells in polymorphous low-grade adenocarcinoma

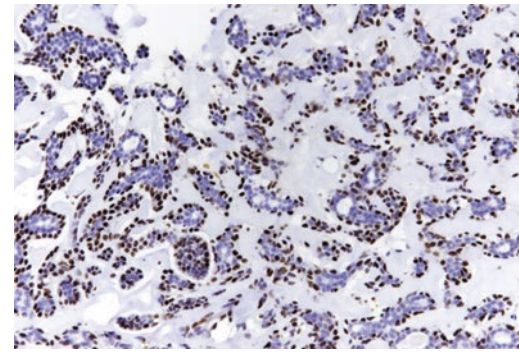

Fig. 6.10 Strong nuclear MYB expression in tumor cells of adenoid cystic carcinoma

associated with adenoid cystic carcinoma, which is found in more than 50% of cases [10–12]. Nevertheless, the same translocation is also reported in association with ~50% of prostatic basal cell carcinoma, and those cases exhibit an adenoid cystic carcinoma-like morphology. Furthermore, the MYB gene is also a partner in other genetic abnormalities associated with different types of neoplasia [13].

In salivary gland tumors and salivary gland–type tumors of other locations, the strong nuclear MYB expression detected by immunohistochemistry is very specific for adenoid cystic carcinomas present in up to 60% of all cases (Fig. 6.10).

Immunoprofile of salivary gland tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Pleomorphic adenoma:	*Luminal epithelial cells:* CK7, CK8/18, CD10, CK11, CK 13, CK14, CK19, EMA *Myoepithelial cells:* Vimentin, S100, Calponin, actin, GFAP, CK5/6/14 Proliferation index (Ki-67)[a]: >2%	PLAG-1, HMGA-2, CEA	GATA-3	CK14, CK20, vimentin, GFAP EMA, CEA
Oncocytoma/oncocytic carcinoma:	CK7, CK8/18, CEA	GATA-3, p63		Actin, Sox-10
Myoepithelioma/myoepithelial carcinoma:	**Sox-10**[b], CK5/6/14, S100, Sox-10, Calponin, 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/basal cell adenocarcinoma:	*Peripheral basaloid/ myoepithelial cells*: S100, actin, Calponin, vimentin, GFAP, CK5/6/14, p63, **Sox-10** *Luminal epithelial cells:* Pan-CK, CK7, CK8/18, EMA, **LEF-1**[c]	CEA		CD43, vimentin
Mucoepidermoid carcinoma:	*Mucous-secreting cells*: CK8/18, CK17, CK19, EMA, CEA *Epidermoid cells*: CK5/6/10/14, CK8, p63, p40	CK7, GATA-3	Mammaglobin	Sox-10
Acinic cell carcinoma:	CK8/18, EMA, **DOG-1**, **Sox-10**, **NR4A3**, transferrin, Lactoferrin	CK19, NSE, **α-amylase**, bone morphogenetic protein 6, Cyclooxygenase-2	CK7, bcl-2, PgR, GATA-3, CEA	CK14, p40, p63, NTRK, mammaglobin
Adenoid cystic carcinoma:	*Myoepithelial and luminal/ductal cells:* CK8/18, CK14, CK17, 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%	**Myb**, CEA, S100, CD117 (c-kit), DOG-1, MUC-1, p63 CEA	GATA-3, GFAP	CK20
Polymorphous adenocarcinoma (polymorphous low-grade adenocarcinoma):	CK7, CK8/18, CK19, EMA, S100, **Sox-10**, **p63**[d], Galactin-3, E-cadherin, vimentin, bcl-2 Proliferation index (Ki-67): 1.5–7%	Mammaglobin, CEA, **DOG-1**, CD117	GFAP, EMA	CK20, **p40**[d], CD43, GATA-3, Calponin, actin,
Salivary duct carcinoma:	CK7, CK8/18, CK14, CK19, EMA Proliferation index (Ki-67): > 25%	Androgen receptors, NKX3.1[e], PSA, GATA-3, GCDFP-15, mammaglobin, p53, HER-2, CEA		S100, CK20, Sox-10
Epithelial-myoepithelial carcinoma:	*Epithelial luminal cells:* CK8/18, EMA *Myoepithelial cells:* S100, actin, CK5/6, CK14, p63, Sox-10, vimentin	$NRAS_{Q61R}$	GATA-3 CEA	

Hyalinizing clear cell carcinoma (clear cell carcinoma)[f]:	CK5/6/14, CK8/18, p40, p63, EMA	CEA, GATA-3	CK7, vimentin	TTF-1, CK20, Sox-10, actin, Calponin, CD10, CD117, PAX-8, GFAP
Mucinous adenocarcinoma:	CK7			CK20, CDX-2, TTF-1, p63, AR
Sclerosing microcystic adenocarcinoma:	*Luminal epithelial cells*: CK7 *Myoepithelial cells*: S100, actin, CK5/6, CK14, p63, Sox-10			CD117
Intraductal carcinoma (low-grade cribriform cystadenocarcinoma):	*Luminal epithelial cells:* CK7 *Myoepithelial cells:* S100, actin, CK5/6, CK14, p63, vimentin			HER-2, GATA-3
Secretory carcinoma (mammary analogue secretory carcinoma)[g]:	CK7, CK8/18, EMA, GCDFP-15, **GATA-3**, **mammaglobin**, **MUC-4**, S100, CD117	**NTRK**, Sox-10		CK5/14, p63, DOG-1, actin, Calponin, ER, PgR
Microsecretory adenocarcinoma:	CK5/14, p63, Sox-10, S100		Actin	**p40**
Sebaceous carcinoma:	**Adipophilin**, CK8/18, CK5/14	EMA, CK19, p63, androgen receptors, Perilipin, CD15	CK7	CK20, CEA, S100
Sialoblastoma:	Sox-10, p63	CK4/14, CK7, CK19, S100, CD117	AFP	

[a] Atypical membranous and cytoplasmic stain patterns may be additionally noted when the MIB-1 clone is used
[b] See Fig. 6.11
[c] Associated with the EWSR1-ATF1 gene fusion
[d] Characteristic immunohistochemical profile for polymorphous low-grade adenocarcinoma positive for p63 but negative for p40 (see Fig. 6.12)
[e] See Fig. 6.13
[f] LEF-1 is positive in basal cell adenoma and a subset of basal cell adenocarcinomas. See Fig. 6.14
[g] Secretory carcinoma is mostly associated with t(12;15)(p13;q25) with ETV6-NTRK gene fusion

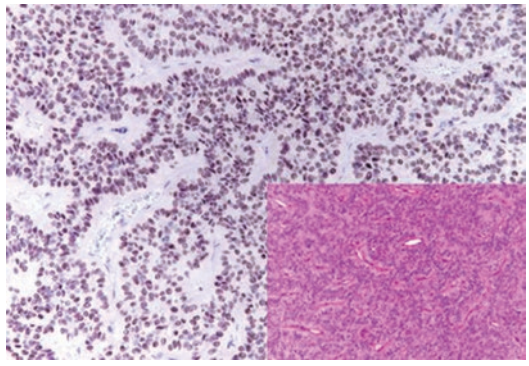

Fig. 6.11 Myoepithelioma with strong nuclear Sox-10 expression

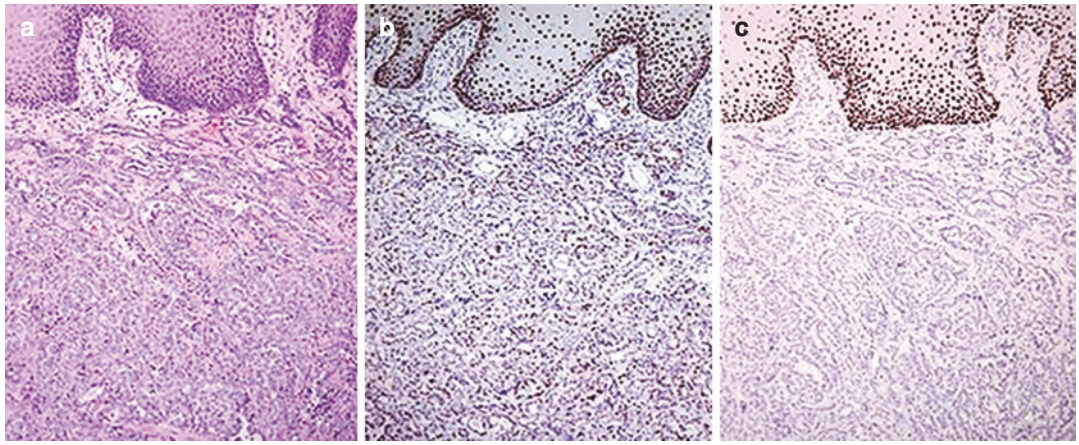

Fig. 6.12 Polymorphous adenocarcinoma with p63+/p40- immune profile: (**a**) H&E, (**b**) p63 expression in tumor cells, and (**c**) p40 negative tumor cells

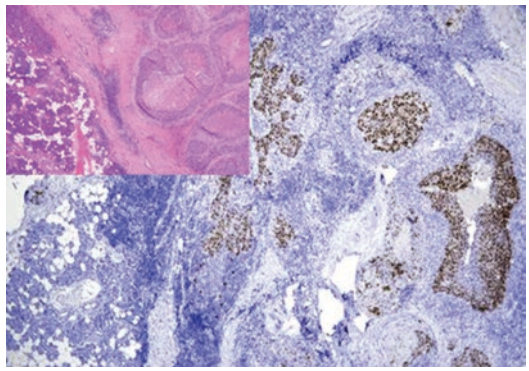

Fig. 6.13 Salivary duct carcinoma. Tumor cells exhibit a strong nuclear NKX3.1 expression

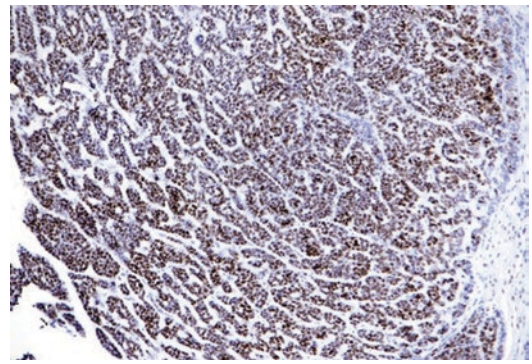

Fig. 6.14 Basal cell adenoma with nuclear LEF-1 expression in tumor cells

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7 Markers and Immunoprofile of Tumors of the Gastrointestinal Tract

Contents

7.1 Gastrointestinal Epithelial Tumors

7.1.1 Diagnostic Antibody Panel for Gastrointestinal Carcinoma

Cytokeratin profile, CDX-2, SATB-2, CDH-17, CEA, Villin, and β-Catenin.

7.1.2 Diagnostic Antibody Panel for Gastrointestinal Neuroendocrine Tumors

Cytokeratin profile, CDX-2, SATB-2, INSM-1, Synaptophysin, Chromogranin, Somatostatin receptor (SSRT-2), and Ki-67.

M. S. A. Tuffaha et al., *Immunohistochemistry in Tumor Diagnostics*,
https://doi.org/10.1007/978-3-031-45024-2_7

7.1.3 Therapy-Related Markers

HER-2, DNA mismatch repair proteins (MLH1, PMS2, MSH2, MSH3, MSH6), BRAF-$_{V600E}$, NRAS-$_{Q61R}$, NTRK, and PD-L1.

7.1.3.1 CDX-2

CDX-2		
Expression pattern: Nuclear		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Colorectal adenocarcinoma – Gastric adenocarcinoma – Carcinoids of the gastrointestinal tract – Yolk sac tumor	Islet pancreas tumors, sinonasal carcinoma, adenocarcinomas of the urinary bladder, ovarian mucinous adenocarcinoma, nests of squamoid differentiation in endometrioid and cervical adenocarcinoma, columnar cell variant papillary thyroid carcinoma, malignant peripheral nerve sheath tumor	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 in the post-gastric mucosa at 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 is usually associated with the expression of Cytokeratin 20.

In routine immunohistochemistry, the expression of CDX-2 protein is characteristic for esophageal, gastrointestinal, and colorectal adenocarcinomas in addition to gastrointestinal neuroendocrine tumors with different expression intensity, whereas the highest frequency and intensity is characteristic for 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 the squamous epithelium into columnar epithelium with goblet cells.

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. A high expression level of CDX-2 is found in bladder adenocarcinoma derived from intestinal urachus, pancreatic adenocarcinoma, biliary adenocarcinoma, and mucinous ovarian carcinoma. The expression of CDX-2 is characteristic for testicular yolk sac tumors and is reported in rare cases of prostatic carcinoma [2]. Pulmonary adenocarcinoma with mucinous differentiation can also be positive for CDX-2; this type of pulmonary adenocarcinoma is also positive for Cytokeratin 20 and commonly lacks the expression of TTF-1 but usually positive for PDX-1 [3, 4]. Some neuroendocrine tumors outside the GIT are also reported to be positive for CDX-2 [5]. The loss of CDX-2 expression has been noted in anaplastic high-grade gastrointestinal adenocarcinomas and medullary adenocarcinomas. Aberrant CDX-2 and nuclear β-catenin expression are found in squamoid morular metaplasia in reactive and neoplastic endometrioid lesions.

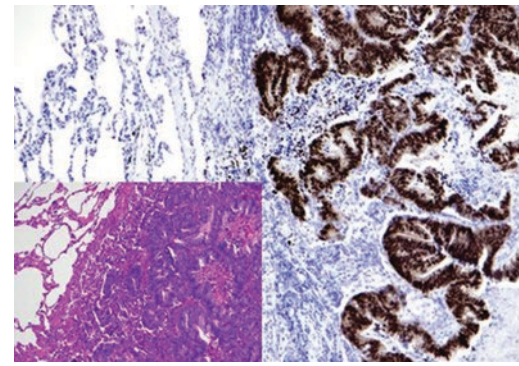


Fig. 7.1 Strong nuclear CDX-2 expression in metastatic tumor cells of primary rectal adenocarcinoma (lung metastases)

7.1.4 SATB-2

SATB-2		
Expression pattern: Nuclear		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Colorectal adenocarcinoma – Medullary colorectal carcinoma – Neuroendocrine tumors of the colon and rectum – Osteoblastic tumors and osteosarcoma	Hepatocellular carcinoma, laryngeal squamous cell carcinoma, olfactory neuroblastoma, clear cell and papillary renal cell carcinoma, nephrogenic adenoma, Merkel cell carcinoma, olfactory neuroblastoma, BCOR rearranged sarcoma, phosphaturic mesenchymal tumor	Colorectal epithelium, a subset of neuronal cells of the central nervous system, hepatocytes, epithelium of proximal nephron, epithelial cells of the epididymis and seminiferous ducts, osteoblasts
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 the central nervous system and osteoblasts. In the gastrointestinal tract, SATB-2 is selectively expressed in the colorectal epithelium, while gastric and small intestinal mucosa, as well as 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 and CDX-2. Only a small proportion of colorectal adenocarcinomas lack the expression of SATB-2 and those are frequently associated with deficiency of the mismatch repair proteins [6]. Adenocarcinomas of the upper gastrointestinal tract and pancreas typically lack the expression of SATB-2. SATB-2 is also strongly expressed in colorectal neuroendocrine tumors, whereas other neuroendocrine tumors are reported to be negative or weakly positive for this marker [7, 8]. In several studies, up to 75% of Merkel cell carcinoma cases were positive for SATB-2 (Fig. 7.3) [9]. SATB-2 is also an important diagnostic marker for tumors and reactive osteogenic lesions. It stains mesenchymal cells with osteoblastic differentiation, including neoplastic osteoblasts of osteosarcoma [10–12].

Diagnostic Pitfalls Low expression level of SATB-2 is reported in a small subset of esophageal, gastric, pancreatic, and pulmonary adenocarcinomas in addition to papillary renal cell carcinoma and to a lesser degree in clear cell carcinoma. A weak expression may also be found in rare cases of ovarian and transitional cell carcinomas (see Algorithm 7.1) [12]. Contrary to CDX-2, mucinous ovarian carcinoma usually lacks the expression of SATB-2. Similar to CDX-2, the expression of SATB-2 can also be noted in squamoid morula associated with reactive and neoplastic endometrioid lesions.

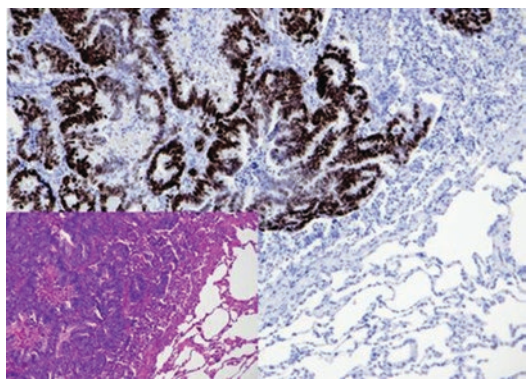


Fig. 7.2 Lung metastasis of rectal adenocarcinoma, metastatic cells with strong SATB-2 expression

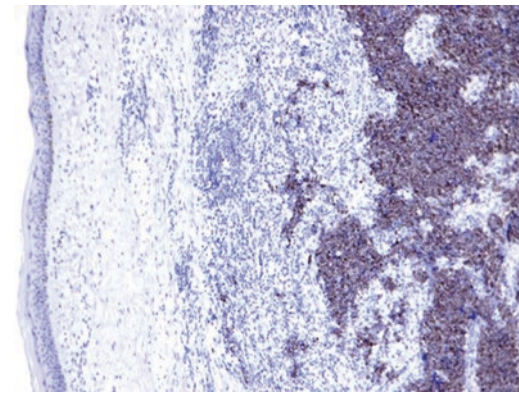
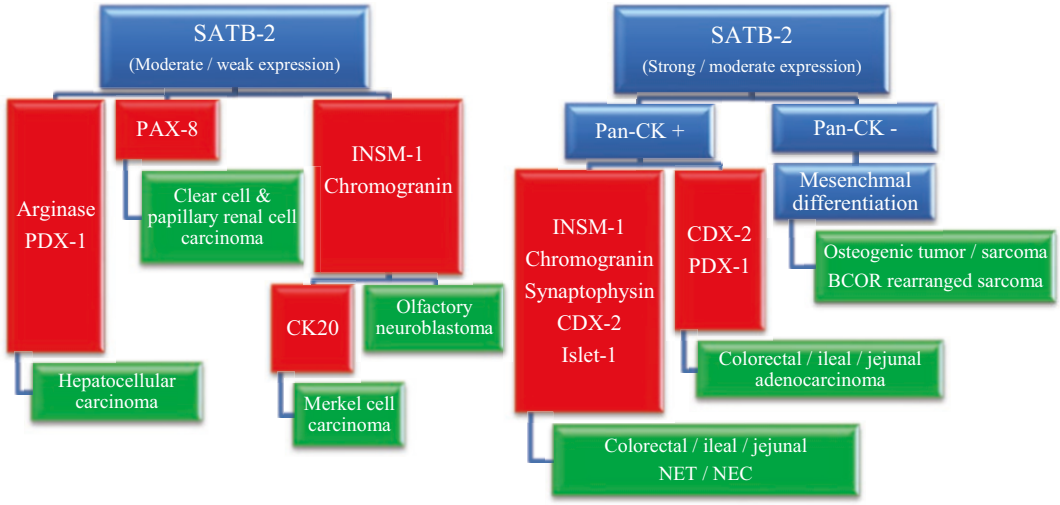


Fig. 7.3 Merkel cell carcinoma with nuclear SATB-2 expression in tumor cells



Algorithm 7.1 Differential diagnosis of SATB-2 positive tumors

7.1.5 Cadherin-17

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, hepatocellular carcinoma, gastrointestinal and pancreatic neuroendocrine tumors, cholangiocellular carcinoma, metanephric adenoma, osteosarcoma	Intestinal epithelium, pancreatic, gall bladder and bile ducts mucosa, fetal liver, adrenal cortex, pituitary gland
Positive control: Appendix		

Diagnostic Approach **C**alcium-dependent **adh**esion molecules **17** (CDH17), also known as liver-intestine cadherin (LI-cadherin), is a member of the cadherin family acting as an intestinal peptide transporter and regulated by CDX-2. In normal mucosa, CDH17 is strongly expressed in the epithelium of intestinal mucosa, while gastric antral and body mucosa usually lack the expression of CDH17. Pancreatic and bile duct mucosa might show focal weak CDH17 expression. In gastrointestinal adenocarcinomas, CDH17 is found in more than 98% of colorectal adenocarcinomas, including medullary carcinoma, in up to 70% of esophageal adenocarcinomas, and one-third of gastric adenocarcinomas (Fig. 7.4) [13, 14]. Less than 20% of pancreatic adenocarcinomas are positive for CDH17.

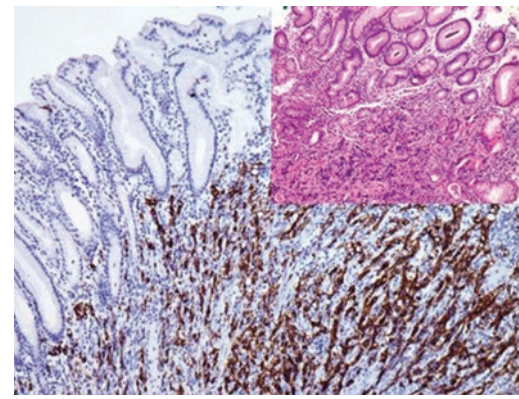


Fig. 7.4 CDH17 expression in cells of gastric adenocarcinoma

CDH17 is negative or focally weak positive in pulmonary adenocarcinoma, breast carcinoma, papillary thyroid carcinoma, transitional cell carcinoma, renal cell carcinoma, hepatocellular carcinoma, and mesothelioma.

7.1.6 Villin

Villin is an Actin-binding cytoskeletal protein that binds Actin filaments into parallel bundles. Villin is a component of the brush border of different epithelial types, including intestinal mucosa cells, ductal cells of the pancreas and biliary tract, mucosa of fallopian tubes and seminiferous ducts, and cells lining proximal renal tubules. Villin is a marker for gastrointestinal adenocarcinomas in addition to pancreatobiliary adenocarcinoma, whereas poorly differentiated diffuse and signet ring cell adenocarcinomas frequently lack the expression of Villin.

Diagnostic Pitfalls Villin is not a specific marker for gastrointestinal carcinomas, as ovarian, endometrioid, and renal cell carcinomas may also be positive for Villin. Villin expression is also found in several neuroendocrine tumors (NETs) of different origins—mainly gastrointestinal NETs—with a characteristic apical membranous expression pattern. Merkel cell carcinoma may also be positive for Villin.

7.1.7 Catenins

Catenins are a family of proteins that include α, β-, γ-, and δ- Catenins and play an important role in cell-to-cell and intracellular adhesion to maintain the function and polarity of the cells besides their function as modulators for the expression of different genes. β-Catenin is the most targeted Catenin in routine immunohistochemistry. β-Catenin binds directly to the cytoplasmic domain of E-cadherin, which in turn binds the actin molecule, but the β-Catenin molecules can also shuttle between the cytoplasm and nucleus. Normal cells show a submembranous staining pattern, whereas the accumulation of cytoplasmic or nuclear β-Catenin indicates mutated β-Catenin. The expression of β-Catenin is encoded by the CTNNB1 gene on chromosome 3p21 and regulated by the adenomatous polyposis coli (APC) gene; consequently, an abnormal nuclear expression is noted in colorectal adenomas and serrated lesions. The nuclear β-Catenin accumulation is also characteristic for different types of fibromatoses, including desmoid and mesenteric fibromatosis, in addition to β-Catenin activated type hepatocellular adenoma. Other carcinoma types, such as carcinoma of the endometrium, can also show nuclear expression depending on the genetic anomalies associated with these tumor types.

7.1.8 Markers of neuroendocrine tumors are listed in Chap. 14

7.1.8.1 HER-2

Human epidermal growth factor receptor-2 (HER-2), also known as ERBB2 (clustered as CD340), is one of the four members of the epidermal growth factor receptor family listed in detail in Chap. 10. The HER-2 molecule is expressed in normal epithelial cells, and overexpression is found in up to 30% of breast cancer cases and in 9–38% of gastric adenocarcinomas and adenocarcinomas of the gastroesophageal

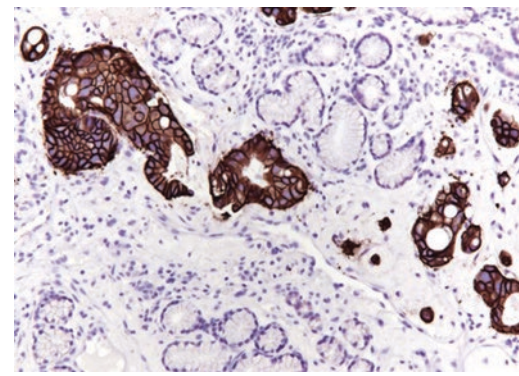


Fig. 7.5 Gastric mucosa infiltrated by neoplastic glands of adenocarcinoma. Tumor cells exhibiting strong membranous HER-2 expression (score 3+)

junction (Fig. 7.5). The expression intensity correlates with the grade of tumor differentiation, and the highest expression intensity is found in well-differentiated adenocarcinomas. In contrast, the expression in gastric carcinomas is more heterogeneous than in breast carcinomas. Similar to breast cancer, the assessment of HER-2 status can be achieved by immunohistochemistry or FISH/CISH assays. The HER-2 expression score can be estimated considering the expression intensity and percentage of immunoreactive tumor cells (see table below).

7.1.9 Scoring of HER-2 Expression in Gastric Cancer

IHC Score	HER-2 overexpression	Staining result
0	Negative No gene amplification	No membranous immunoreactivity in any tumor cell
1+	Negative No gene amplification	Tumor cell cluster[a] with a faint membranous immunoreactivity irrespective of the percentage of positive tumor cells
2+	Equivocal with uncertain gene amplification	Tumor cell cluster[a] with a weak to moderate complete, basolateral, or lateral membranous immunoreactivity irrespective of the percentage of positive tumor cells
3+	Positive High gene amplification	Tumor cell cluster[a] with a strong complete basolateral or lateral membranous immunoreactivity irrespective of the percentage of positive tumor cells

[a] A cluster consists of ≥5 tumor cells

Adenocarcinomas with an IHC score of 2+ need further investigation to clarify the gene amplification status by FISH/CISH or other molecular methods similar to breast carcinoma (see Chap. 10).

7.1.9.1 Mismatch Repair Proteins and Microsatellite Instability

Microsatellite instability (MSI) is detected in ~15% of all colorectal adenocarcinomas, and the mismatch repair protein deficiency is the hallmark of tumors associated with Lynch and related syndromes such as Muir-Torre syndrome, Turcot syndrome, and constitutional mismatch repair deficiency. Colorectal adenocarcinomas with microsatellite instability show distinct morphology with increased intratumoral-activated T-lymphocytes and are commonly localized in the right hemicolon. Poorly differentiated, mucinous, and medullary adenocarcinomas and adenocarcinomas with signet ring cell features are frequently associated with microsatellite instability. These tumors have a better prognosis and exhibit a response to immune checkpoint inhibitors.

The assessment of microsatellite instability and the detection of mismatch repair proteins by immunohistochemistry is discussed in detail in Chap. 31.

Immunoprofile of gastrointestinal epithelial tumors				
Tumor type	+ in > 90% (+)	+ in 50–90% (+/–)	+ in 10–50% (–/+)	+ in < 10% (–)
A. Tumors of the esophagus and esophagogastric junction				
Squamous cell carcinoma of the esophagus:	**CK5/6**, CK8, CK14, CK18, CK19, **p63**, **p40**	β-Catenin, cyclin D1	CK7	CK20
Barrett's esophagus	**CDX-2**	See table below		
Adenocarcinoma of the esophagus:	CK7, CK8, CK18, CK19, CDX-2, E-cadherin	PDX-1, villin, cyclin D1		CK20, CK5/6, p40
Undifferentiated carcinoma:	Pan-CK, vimentin			CK5/14, p40, CD56, INSM-1, Sox-10
Salivary gland-type tumors: – Adenoid cystic carcinoma – Mucoepidermoid carcinoma	See salivary gland tumors			
B. Tumors of the stomach				
Gastric adenocarcinoma: – Tubular adenocarcinoma – Papillary adenocarcinoma – Poorly cohesive carcinoma/signet-ring cell carcinoma – Mucinous adenocarcinoma – Adenocarcinoma with lymphoid stroma	CK8, CK18, CK19, villin, EMA,	CK7, CDX-2, CDH-17, CEA, Muc5AC, glicentin	CK20	CK5/6, CK14, CK17, CA125, **SATB-2**
Undifferentiated carcinoma:	Pan-CK, vimentin			CK5/14, p40, CD56, INSM-1, Sox-10, Dog-1
C. Tumors of the small intestine and ampulla				
Adenocarcinoma of the duodenum and small bowel:	CK8, CK18, CK19, **CDX-2**[a], CDH-17, villin	**CK7**, CK20, **PDX-1**,	SATB-2, AMACR, Hep Par-1	
Adenocarcinoma of the ampullary region:	CK8, CK18, CK19, CK7, **PDX-1**		CK20, CDX-2	
D. Tumors of the appendix, colon, and rectum				
Appendiceal goblet cell adenocarcinoma:	CK20, CDX-2, SATB-2	Synaptophysin[b], Chromogranin[b]		CK7
Colorectal adenocarcinoma:	CK8, CK18, CK19, **CK20**, **CDX-2**, **SATB-2**, CDH-17, CEA, villin, MUC-2	β- catenin [c], CD10, AMACR	CK7	CA125, CK5/6, CK14, GATA-3, Thrombomodulin
Colorectal mucinous adenocarcinoma:	**CK20**, **CDX-2**, **SATB-2**, villin, β- Catenin[c]		CK7, PDX-1	
Medullary colorectal carcinoma:	Pan-CK, **SATB-2**, CDH-17, EMA	Calretinin, CD10, TFF-3	CK20, CDX-2, MSI markers (MLH1, PMS2, MSH2, MSH6)[d]	CK7, villin
E. Tumors of the anal canal and perianal skin				
Rectal squamous cell carcinoma:	CK5/6, CK10, CK17, CK18, CK19, p63/p40	**p16**		CK7, CK20

Basal cell carcinoma (basaloid carcinoma):	**CK5/6**, CK8, CK15, CK17, CK18, CK19, BerEp4	CK10	CK7	CK20
Intestinal adenocarcinoma:	CK20, CDX-2, SATB-2	CK7		
Anal gland adenocarcinoma:	CK7, CK18, CK19,	GATA-3		CK20, CDX-2, SATB-2
Primary anal Paget's disease:	**CK7**,[e] CK8, CK18, EMA, GATA-3	**GCDFP-15**,[e] TRPS-1, CEA		CK20, CDX-2, SATB-2
F. Gastrointestinal neuroendocrine neoplasms (see also Chap. 14: Immunoprofile of tumors of endocrine organs and neuroendocrine tumors)				
Classification and screening markers for gastrointestinal neuroendocrine neoplasms: **Neuroendocrine tumors:** – NET G1 (carcinoid) – NET G2 (atypical carcinoid) – NET G3 **Neuroendocrine carcinoma:** – NEC of small cell type – NEC of large cell type	Neuroendocrine markers: Synaptophysin, chromogranin, INSM-1, Islet-1, CD56, SSTR2, NSE, S100, Epithelial markers: Pan-CK, CK8/18, CK19	Islet-1, CDX-2, PDX-1, SATB-2, villin		CK20
	Proliferation: NET G1 (carcinoid): Proliferation index (Ki-67): < 3%; mitotic rate: <2/2mm^2 NET G2 (atypical carcinoid): Proliferation index (Ki-67): 3–20%; mitotic rate: 2–20/mm^2 NET G3 & NEC: Proliferation index (Ki-67): > 20%; mitotic rate:>20/2mm^2			
Gastric ECL[f] cell NET:	Broad-spectrum neuroendocrine markers		Histamine, gastrin	
Gastric EC cell NET:	Broad-spectrum neuroendocrine markers		Serotonin	
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] Found only in scattered neuroendocrine cells associated with the tumor (Fig. 7.6)
[c] Nuclear stain
[d] Enterochromaffin-like cells
[e] See Figs. 7.7 and 7.8
[f] Microsatellite instability in more than 80% of medullary colorectal carcinomas

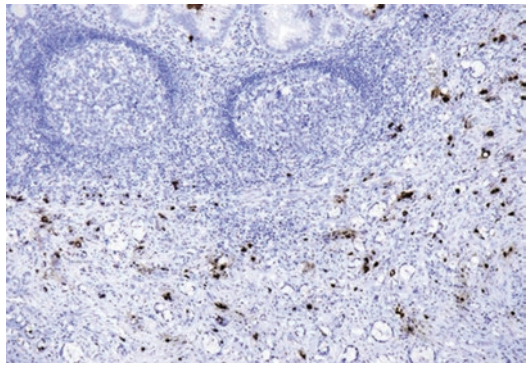


Fig. 7.6 Chromogranin staining the neuroendocrine cells associated with appendiceal goblet cell adenocarcinoma

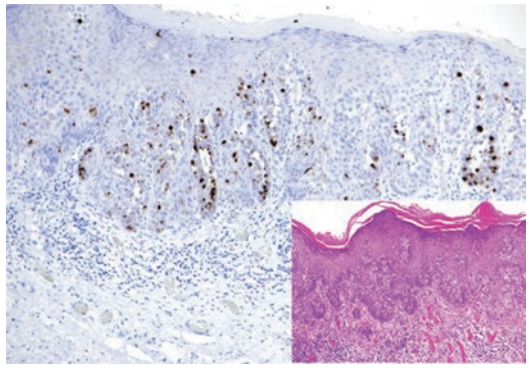


Fig. 7.7 Primary anal Paget's disease; intraepithelial neoplastic cells labeled by GCFP-15

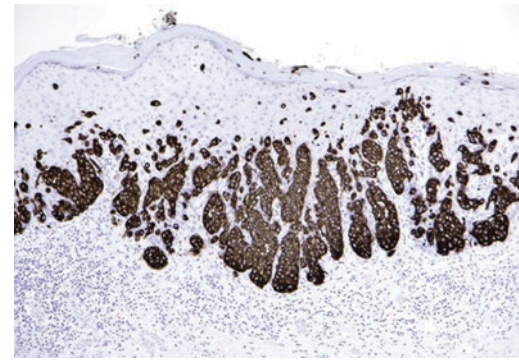


Fig. 7.8 Primary anal Paget's disease. Intraepithelial tumor cells with strong cytoplasmic CK7 expression

Differentiation neuroendocrine tumor G3 (NET G3) vs. neuroendocrine carcinoma (NEC) [15]										
Diagnosis	Morphology	Ki-67	P53	Rb1	P16	Islet-1	ATRX	SSTR2	DAXX	MSI
NET G3	Tumor with endocrine morphology	21–55%	–	+	–	+	–/+	+	–/+	–
NEC	Morphology of poorly differentiated tumor	21–90%	+/–	–/+	+	–	+	–	+	+ (MSI-H)

RB1 retinoblastoma 1, *Islet-1* human insulin gene enhancer binding protein 1, *SSTR2* somatostatin receptor type 2

Markers of dysplasia in Barrett's mucosa			
Barrett's esophageal mucosa (intestinal metaplasia)	Without dysplasia	Low-grade dysplasia	High-grade dysplasia
AMACR	Usually negative	20–40%	Up to 80%
P53	Expression in <10%	Expression in up to 30%	Expression in up to 60%
β-Catenin	Membranous expression	Negative/weak nuclear expression	Marked nuclear expression
Hep Par-1	Usually positive	Usually negative	Usually negative

7.2 Gastrointestinal Mesenchymal Tumors

7.2.1 Diagnostic Antibody Panel for Gastrointestinal Stromal Tumors (GIST)

CD34, CD117 (c-Kit), DOG-1, PDGFR-α, and SDHG.

7.2.2.1 CD117

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, neuroendocrine tumors, 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 the tyrosine kinase growth factor receptor type III family encoded on chromosome 4q11–12. This family includes c-Kit, platelet-derived growth factor receptor (PDGFR-α), macrophage colony-stimulating factor, and FMA-like tyrosine kinase 3. The CD117 molecule comprises an extracellular, transmembrane, 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 (See Chap. 18).

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 stromal tumors (GISTs), whereas single or multiple activating mutations of the c-Kit gene are found in about 80% of the GISTs, mainly in exon 11 and less frequently in exons 9, 13, and 17. The coexpression of CD34 and DOG-1 is a characteristic profile for the diagnosis of GIST (Fig. 7.9). 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 (See Chap. 17) [16].

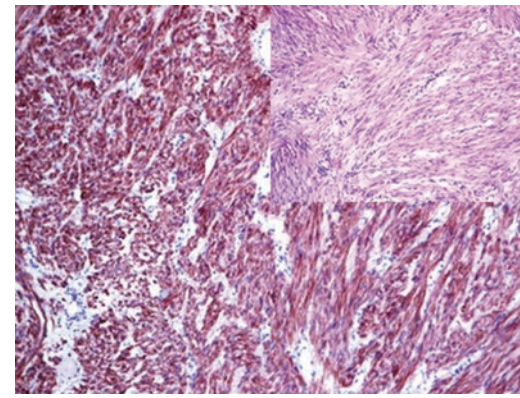


Fig. 7.9 Gastrointestinal stromal tumor showing strong CD117 expression

7.2.2 Diagnostic Antibody Panel for Miscellaneous Mesenchymal Gastrointestinal Tumors

sm-Actin, h-Caldesmon, Calponin, Smoothelin, Sox-10, CD34, and β-Catenin.

Diagnostic Pitfalls Five to eight percent of the GISTs are associated with mutations within the PDGFR-α gene (mainly in exon 18) and are usually negative for CD117. These tumors frequently show epithelioid morphology and are commonly positive for PDGFR-α and/or DOG-1 [15, 17].

7.2.3 Platelet-Derived Growth Factor Receptor α

Platelet-derived growth factor receptor α (PDGFR-α) is a tyrosine kinase receptor and a member of the type III tyrosine kinase receptor family involved in the 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 immunostaining, 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 [18, 19].

7.2.4 DOG-1

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, chondroblastoma, sebaceous adenoma/carcinoma	Cajal cells, gastric surface epithelium, salivary gland and pancreatic acini, gallbladder and bile duct epithelium, myoepithelial cells, basal cells of squamous epithelium, eccrine glands, luminal borders of epididymis and rete testis, spermatocytes, and spermatids
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 stromal tumors (GISTs) and reacts with more than 95% of this tumor identity (Fig. 7.10), including the extra-gastrointestinal stromal tumors of the peritoneum (E-GIST). The expression spectrum of DOG-1 differs from that of CD117, but there is a high concordance between the expression of both markers in GISTs [20–22]. 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 similar morphology as long as pancreatobiliary adenocarcinomas are not in the differential diagnosis (see tumors of salivary glands, Sect. 6.2).

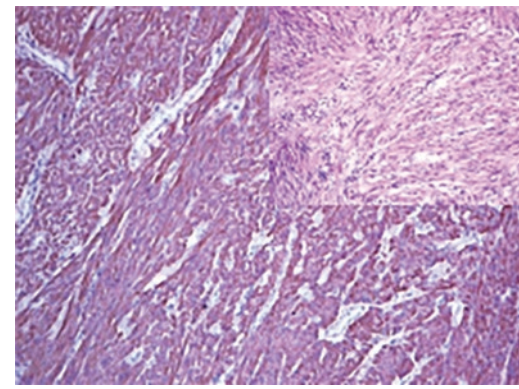


Fig. 7.10 Strong DOG-1 expression in the cells of the gastrointestinal stromal tumor

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

7.2.5 CD34

CD34 is a cell surface adhesion glycoprotein listed with endothelial markers (Chap. 25). CD34 labels the majority of GISTs but lacks specificity; consequently, it 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. 7.11).

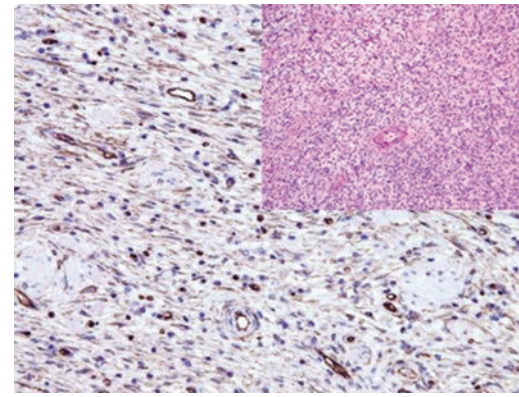


Fig. 7.11 CD34 labels the stromal cells of inflammatory fibroid polyp of the gastrointestinal tract

7.2.6 Succinate Dehydrogenase

Succinate dehydrogenase (SDHG) deficiency is found in ~15% of all GISTs, mainly pediatric tumors. SDHG is listed in detail in Sect. 12.1.

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
Malignant gastrointestinal neuroectodermal tumor/ Gastrointestinal clear cell sarcoma[c]:	**Sox-10**, S100, CD56	Synaptophysin, Chromogranin, Neurofilaments		**DOG-1**, **CD117**, **CD34**, **HMB45**, **Melan A**, **Actin**
Gastroblastoma[d]:	*Epithelial cells*: Pan-CK, CK7 *Mesenchymal cells*: Vimentin, CD10			CD117, DOG-1, CDX-2, CK20
Inflammatory fibroid polyp of the gastrointestinal tract:	*Stromal cells*: CD34, **PDGFRα**, Fascin, Cyclin D1	Calponin, CD35	Sm-Actin	DOG-1, CD117, S100, Desmin, h- Caldesmon, bcl-2
Granular cell tumor:	S100, **Sox-10**, CD56, NSE, Laminin, Nestin	Inhibin, TFE-3, CD68, PGP 9.5, Calretinin		Pan-CK, GFAP, Neurofilaments, EMA, Desmin, HMB45
Plexiform fibromyxoma:	Actin, CD10		Desmin	CD117, DOG-1
Calcifying fibrous tumor of the gastrointestinal tract:	Factor XIIIa, Vimentin		CD34	Actin, Desmin, h-Caldesmon, CD117, DOG-1, Pan-CK, β- Catenin
Mesenteric fibromatosis:	Vimentin, β- Catenin [d]	sm-Actin	Desmin, CD117	Calponin, CD34, CD117, DOG-1, Pan-CK, S100
Inflammatory myofibroblastic tumor (inflammatory pseudotumor):	Actin (in spindle cells), Vimentin	Cyclin D1, **ALK (p80)**	Desmin, bcl-2, ROS-1[e], RET[e]	Pan-CK, EMA, CD56

[a] GISTs with epithelioid morphology are frequently CD117 negative, mainly localized in the stomach wall
[b] PDGFR-α positive in CD117 negative GISTs
[c] Associated with the EWSR1-ATF1 or EWSR1-CREB1 gene rearrangement
[d] Nuclear and cytoplasmic staining pattern (Fig. 7.12)
[e] Can be positive in ALK-negative cases
[f] Associated with the MALAT1-GLI1 gene rearrangement

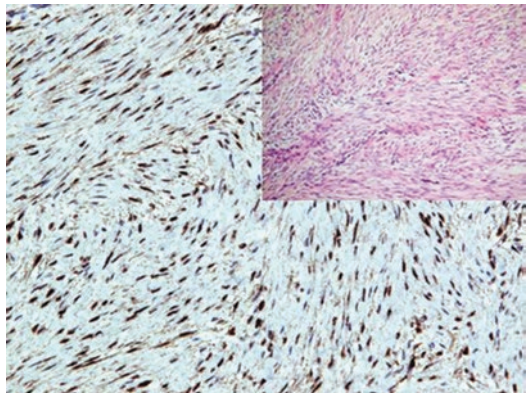


Fig. 7.12 Mesenteric fibromatosis with strong nuclear β-Catenin expression

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8 Markers and Immunoprofile of Pancreatic Tumors

Contents

8.1 Diagnostic Antibody Panel for Exocrine Pancreatic Tumors

Cytokeratin and MUC profile, E-Cadherin, PDX-1, Trypsin, Amylase, S100P, CA19.9, CEA, DOG-1, bcl-10, Mesothelin, and IMP3 [1–4].

8.2 Diagnostic Antibody Panel for Endocrine Pancreatic Tumors

Cytokeratin profile, general neuroendocrine markers [INSM-1, Chromogranin, Synaptophysin, CD56, Islet-1, Somatostatin

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receptor 2 (SSTR2); see markers for endocrine and neuroendocrine tumors in Chap. 14], PDX-1, PAX-6, PAX-8, Insulin, Gastrin, Glucagon, Vasoactive intestinal polypeptide (VIP), human pancreatic polypeptide (hPP), and proliferation index (Ki-67).

8.2.1 PDX-1

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 adenocarcinomas	Hepatocellular carcinoma, pancreatic acinic cell carcinoma, pulmonary mucinous adenocarcinoma, prostatic adenocarcinoma	Endocrine cells of the pancreas, pancreatic ductal epithelium and centroacinar cells, pyloroduodenal mucosa, Brunner's glands, enteroendocrine cells
Positive control: Pancreatic tissue		

Diagnostic Approach PDX-1 (**p**ancreatic and **d**uodenal homeobo**x** **1**; also known as insulin promotor factor 1, STF-1, IDX-1) [2] is a transcription factor involved in the early development of the pancreas, antral part of the stomach, gastro-duodenal junction, proximal duodenum and duodenal papilla, as well as bile ducts and maturation of the endocrine β-cells and enteroendocrine cells in addition to Brunner's glands. In adult tissue, PDX-1 is intensely expressed in endocrine cells of the upper gastrointestinal tract, pyloroduodenal and pancreatic duct mucosa (Fig. 8.1) while it is negative in normal acinic pancreatic cells. PDX-1 also strongly labels pancreatic endocrine tumors and pancreatobiliary adenocarcinomas, including adenocarcinoma of the gall bladder, extrahepatic bile ducts, and intrahepatic cholangiocarcinoma in addition to a small subset of hepatocellular carcinomas (Figs. 8.2 and 8.3). Weak PDX-1 expression is detected in pancreatic acinar cells and pancreatic acinar cell carcinoma. In the interpretation of PDX-1 positive metastases, it is important to consider that the duodenum and pancreas have a common embryologic origin and neither adenocarcinomas nor endocrine tumors arising from these organs are definitely distinguishable regarding the exact site of origin.

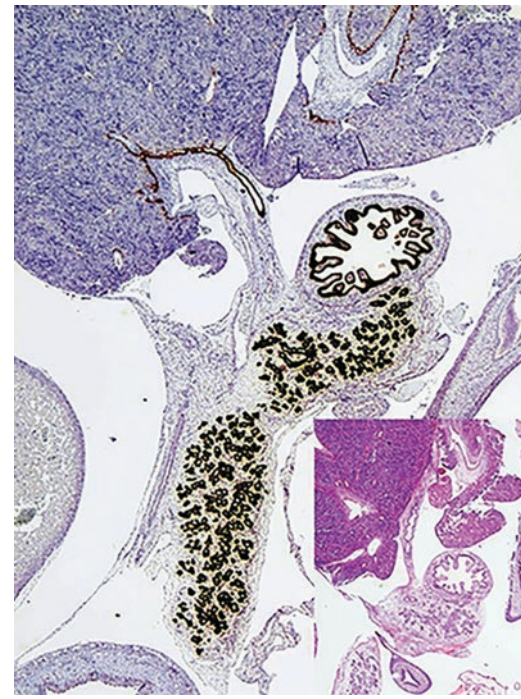

Fig. 8.1 Section through a 12-week embryo. The PDX-1 immunostaining highlights pancreatic ducts, duodenal mucosa, and mucosa of the bile ducts

Diagnostic Pitfalls Moderate to strong expression of PDX-1 is also found in colorectal and gastric adenocarcinomas beside intestinal-type mucinous ovarian carcinoma and primary pulmonary mucinous adenocarcinoma (Fig. 8.4). PDX-1 is a useful marker for the majority of poorly cohesive gastric carcinomas with signet ring cells (Fig. 8.5). Primary mucinous adenocar-

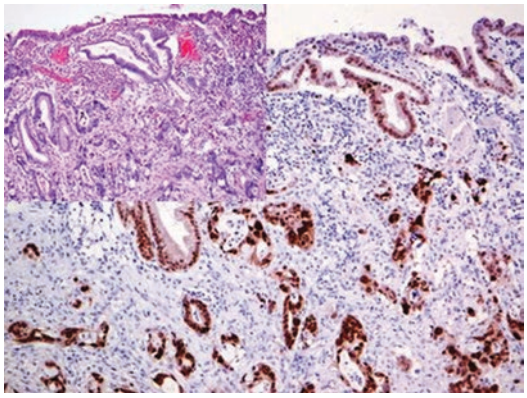

Fig. 8.2 Adenocarcinoma of the common bile duct. Tumor cells with strong nuclear PDX-1 expression

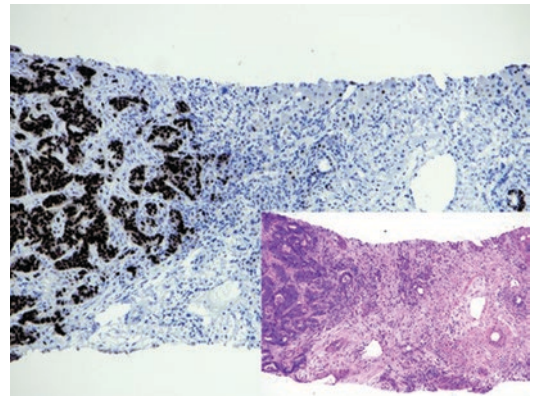

Fig. 8.3 Intrahepatic cholangiocarcinoma with strong nuclear PDX-1 expression. PDX-1 also labels the cells of normal intrahepatic bile ducts

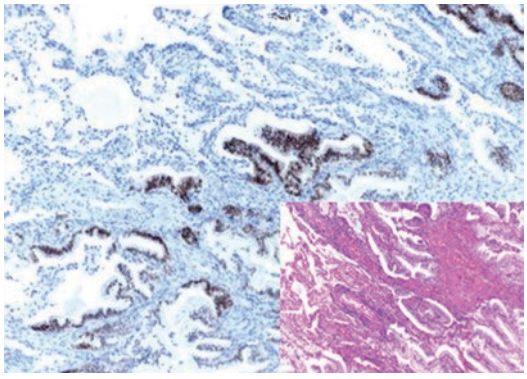

Fig. 8.4 Pulmonary mucinous adenocarcinoma with strong nuclear PDX-1 expression

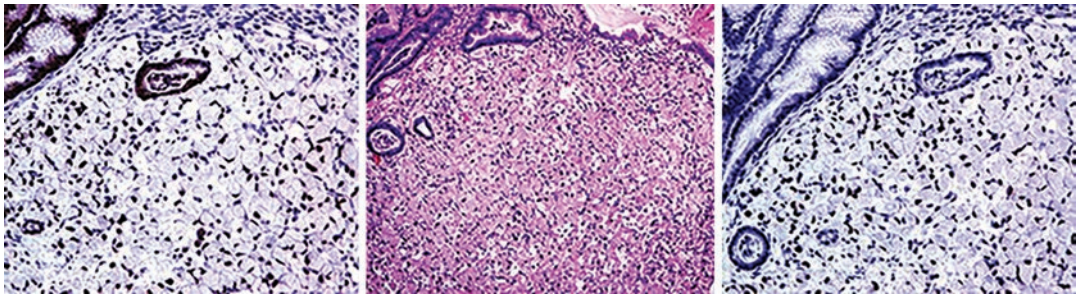

Fig. 8.5 Signet cell carcinoma of gastric mucosa, PDX-1 expression in normal mucosa and signet cells (***left***), CDX-2 expression in normal mucosa and signet cells (***right***)

cinomas of the lung are also positive for PDX-1. Focal weak PDX-1 expression may also be found in prostatic glands, breast epithelium, thyroid, liver, spleen, kidney, and skin but usually has no diagnostic significance.

8.2.2 Pancreatic Enzymes (Trypsin, Amylase, and Lipase)

8.2.2.1 Trypsin

Trypsin is a 24 kDa enzyme and a member of the serine proteinase family. It is synthesized by the pancreatic acinic cells as an inactive precursor, which is activated in the gastrointestinal tract. Antibodies to trypsin are used as specific markers for pancreatic acinar cell carcinomas that label more than 95% of this tumor type.

8.2.2.2 Amylases

These are enzymes that catalyze the cleavage of the glucoside bonds of large sugar molecules into oligosaccharides. Amylases are synthesized by acinic cells of salivary glands and pancreas, whereas salivary gland- and pancreatic amylase are encoded by different genes and have different amino acid sequences. Pancreatic amylase consists of a single polypeptide chain with a molecular weight of 54 kDa. In routine immunohistochemistry, antibodies to pancreatic amylase are rarely used as specific for pancreatic acinar cell carcinoma as it is usually negative or patchy weakly positive.

8.2.2.3 Lipase

Pancreatic lipase is an enzyme essential for the digestion of lipids secreted by pancreatic acinic cells. It hydrolyzes triglycerides into fatty acid and glycerol. Similar to other pancreatic enzymes, antibodies to pancreatic lipase are also specific markers for pancreatic acinar carcinoma.

8.2.2.4 CA19–9

CA19–9		
Expression pattern: Membranous/cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Pancreatic and gastrointestinal carcinoma – Ovarian carcinoma	Lung adenocarcinoma, carcinoma of the breast, renal cell carcinoma, transitional cell carcinoma, mucoepidermoid carcinoma	Epithelium of breast ducts, salivary and sweat glands, lung, gastrointestinal tract, hepatobiliary system
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 and salivary and sweat glands, besides the glands of the gastrointestinal mucosa. CA19–9 strongly stains pancreatic, hepatobiliary, and gastrointestinal adenocarcinomas but lacks the specificity for these carcinoma types. CA19–9 has a broad expression spectrum, as it is found in many other carcinomas of different origins. Consequently, the diagnosis of primary pancreatic carcinoma must be supported by a complete immunohistochemical panel.

8.2.2.5 Bcl-10

Bcl-10 is an apoptotic regulatory nuclear protein listed in detail in Sect. 16.2 (markers and immunoprofile of B-cell neoplasms). In pancreatic tissue, bcl-10 labels acinic cells and acinar cell carcinoma (See Sect. 16.2).

8.2.2.6 Placental S100 (S100P)

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 primarily isolated from the human placenta and consists of 95 amino acids [5]. Besides the placenta, S100P is also expressed in many other types of normal tissue, including myocardium and skeletal muscle, epithelial cells of the gastrointestinal tract and prostatic gland, as well as 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 [6–9]. Normal breast tissue and normal or inflamed pancreatic tissue lack the expression of S100P [10, 11]. This wide expression profile makes S100P a useful marker for the diagnosis of pancreatic and breast adenocarcinomas, especially on small biopsies and FNA. 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.

Besides S100P, IMP3 and Mesothelin are further informative markers for the diagnosis of pancreatic carcinoma, especially in small biopsies. Both markers show strong expression in pancreatic ductal adenocarcinoma but negative or patchy, very weak positive in normal or reactive pancreatic tissue (see Chap. 15; Fig. 8.7 and Fig. 15.4) [12].

8.2.2.7 SMAD-4 (DPC4)

SMAD-4 (Mothers against decapentaplegic homolog 4), also known as DPC-4 (Deleted in Pancreatic Cancer-4), is a tumor suppressor gene that encodes a member of the SMAD family of signal transduction proteins. The SMAD-4 protein acts as a tumor suppressor and inhibits cell proliferation.

Mutations in the SMAD-4 (DPC4) gene are associated with juvenile polyposis syndrome. Inactivation or deletion of this gene is found in ~50% of pancreatic adenocarcinoma, ~ 30% of extrahepatic cholangiocarcinoma and ampullary adenocarcinoma, and in ~20% of colorectal adenocarcinomas. The nuclear SMAD-4 protein can be detected by immunohistochemistry and is found in almost all normal cells. The loss of this protein is considered a surrogate for the presence of SMAD4 inactivating mutations or deletion [13].

8.2.2.8 Islet-1

Islet-1 (ISL-1)		
Expression pattern: Nuclear		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Pancreatic neuroendocrine tumors (NET-NEC) – Neuroblastoma	Neuroendocrine tumors (NET, small and large cell NEC) of different origins; endocrine tumors (medullary thyroid carcinoma, pheochromocytoma); Merkel cell carcinoma; rhabdomyosarcoma	Pancreatic islet cells, pineal body, smooth muscle
Positive control: Pancreatic tissue		

Diagnostic Approach The human insulin gene enhancer binding protein (Islet-1/ISL-1) is a transcription factor involved in the differentiation of sympathetic neurons and neuroblasts. Islet-1 is also a DNA transcriptional activator essential for the differentiation of the exo- and endocrine pancreas. Islet-1 is a sensitive marker for gastrointestinal and pancreatic neuroendocrine tumors in addition to Merkel cell carcinoma, neuroblastoma, pheochromocytoma, and medullary carcinoma of the thyroid [14, 15]. Islet-1 is usually used in a panel with other neuroendocrine markers to differentiate the origin of metastatic neuroendocrine tumors (see the chapter on neuroendocrine tumors).

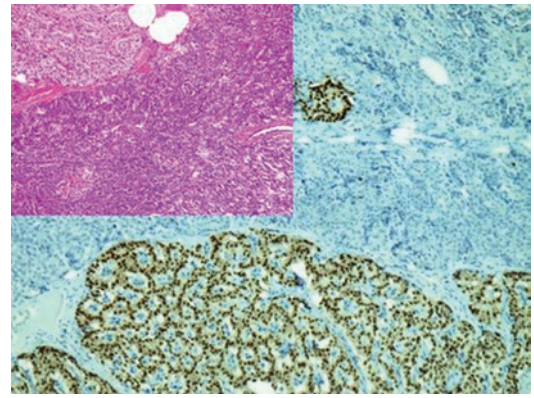

Fig. 8.6 Well-differentiated neuroendocrine tumor of the pancreas (NET G1). PAX-6 highlights the tumor cells and the endocrine cells of the pancreatic islets

Diagnostic Pitfalls Islet-1 may also be expressed in several neuroendocrine tumors of different origins, including pulmonary neuroendocrine tumors and small cell carcinoma, medullary thyroid carcinoma, and Merkel cell carcinoma. Islet-1 expression is found in more than 50% of rhabdomyosarcoma and in areas of rhabdomyoblastic differentiation which is to be considered in the differential diagnosis of metastatic lesions. Furthermore, the expression of Islet-1 is found in ~30% of B-cell lymphomas, mainly in diffuse large B-cell lymphoma (see Sect. 16.2, Fig. 16.1).

8.2.2.9 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 playing a role in the development of the central nervous system, endocrine glands, and sensory organs, including eye and olfactory tissue. Antibodies to PAX-6 stain neuroendocrine cells of different origins, mainly those of endocrine pancreas and tumors derived from these cells (Fig. 8.6). PAX-8 is also a further marker that labels pancreatic neuroendocrine tumors but less specific than PAX-6 [16]. PAX-8 is listed in detail in Sect. 12.1.

Additional neuroendocrine markers, including INSM-1, Chromogranin, Synaptophysin, and other differential diagnoses, are listed in detail in Chap. 14 (Immunoprofile of Tumors of Endocrine Organs and Neuroendocrine Tumors).

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, CK8/18, CK19, Inhibin, Glut-1		CA19.9, EMA, CK20	CEA, Trypsin, S100
Mucinous cystic neoplasms (with low-, intermediate, and high-grade dysplasia) and mucinous cystadenocarcinoma:	CK7, CK8/18 CK19, EMA, S100P, CEA, CA19.9 *Ovarian type stroma:* ER, PgR, CD10	CK20	DPC-4	CDX-2

Intraductal papillary mucinous neoplasm (IPMN): – Gastric type (Gt) – Pancreatobiliary type (Pt) – Intestinal type (It)	CK7, CK8/18, CA19.9, S100P *Gt:* MUC5AC *Pt:* MUC5AC, MUC6, EMA *It*: CDX-2, CK20, MUC2, MUC5AC	CK19, CA19.9, CEA, MUC4, MUC3		*Gt:* CDX-2, MUC1, MUC7 *Pt:* CDX-2, MUC2, MUC7 *It*: CDX-2, MUC1, MUC7
Oncocytic type IPMN:	CEA, CA19.9, MUC1, MUC6	Mesothelin, CK20,[a] CDX-2,[a] MUC5AC		
Intraductal tubulopapillary neoplasm:	CK7, CK19, CA19.9, DPC-4	MUC1, MUC6	CEA	CK20, CDX-2, MUC2, MUC5AC
Ductal adenocarcinoma:	CK7, CK8/18, CK13, CK 19, CEA, **S100P**, MUC-1, MUC-3, MUC-4, MUC5AC, Mesothelin, CA19.9, CA125, CEA, EMA, Claudin-4	**PDX-1**, Maspin, CK4, CK17, CDX-2, Fascin, IMP3, HER-2, E-Cadherin	**DPC4**, GATA-3, CK20,	MUC-2, Lipase, Trypsin, Calretinin, Thrombomodulin (CD141), Vimentin
Acinar cell carcinoma:	**Trypsin**, **Chymotrypsin**, **bcl-10**, CK8/18	PDX-1, CD56, EMA, Glypican-3, Pancreatic CEA, Vimentin	CK7, CK19, AFP, DOG-1, Lipase, Chromogranin, Synaptophysin	CK20, S100P, MUC1, MUC2, Amylase
Solid pseudopapillary neoplasm:	CD10, **LEF-1**, α-1 antitrypsin, PgR, β-catenin, CD56, NSE, Vimentin	Pan-CK, CD99[b], SOX11, Androgen receptor, Glutamine synthetase, FLI-1, Galectin-3, Cyclin-D1, CD200	S100, CK7, CK19, CD117, Synaptophysin,	Chromogranin, CA19.9, bcl-10, ER, CEA, AFP, Trypsin
Pancreatoblastoma:	*Acinar cells*: CK7, CK8/18, CK19, EMA, Trypsin, Lipase		AFP	NSE, CEA
	Squamoid nests: CK8/18, EMA, NSE		Synaptophysin, Chromogranin, CEA	CK5/6/14, CK7
	Ductal component: CK7, CK8/18, CK19, EMA, CEA *Solid component*: CK7, EMA			Trypsin

B. Immunophenotype of pancreatic neuroendocrine neoplasms				
Classification and screening markers for neuroendocrine pancreatic neoplasms: **Neuroendocrine tumors:** – NET G1 (carcinoid) – NET G2 (atypical carcinoid) – NET G3 **Neuroendocrine carcinoma:** [c] – NEC of small cell type – NEC of large cell type	Pan-CK, CK8/18, CK19, **INSM-1**, **Islet-1**, **Chromogranin**, **Synaptophysin**, **SSTR2**, **CD56**, NSE, PGP9.5, Leu7	**PAX-6, PDX-1**, PgR, S100		CK5/6, CK7, CK20, S100P
	Proliferation: NET G1 (carcinoid): Proliferation index (Ki-67): < 3%; mitotic rate: <2/2mm^2 NET G2 (atypical carcinoid): Proliferation index (Ki-67): 3–20%; mitotic rate: 2–20/2mm^2 NET G3 & NEC: Proliferation index (Ki-67): > 20%; mitotic rate: >20/2mm^2			
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] In goblet cells
[b] Dot-like paranuclear expression pattern
[c] See table below
[d] Enterochromaffin cells
[e] Human pancreatic polypeptide
[f] Vasoactive intestinal polypeptide

Differentiation neuroendocrine tumor G3 (NET G3) vs. neuroendocrine carcinoma (NEC) [17]											
Diagnosis	Morphology	Ki-67	P53	Rb1	P16	Islet-1	ATRX	SSTR2	DAXX	MSI	CXCR-4 (CD184)
NET G3	Tumor with preserved endocrine morphology	21–55%	–	+	–	+	∓	+	∓	–	–
NEC	Morphology of poorly differentiated tumor	21–90%	±	∓	+	–	+	∓	+	+(MSI-H)	+

+ expression in >90%; ± in 50–90%; ∓ in 10–50%; – in <10%
RB1 retinoblastoma 1, *Islet-1* human insulin gene enhancer binding protein 1, *SSTR2* somatostatin receptor type 2, *CXCR4* C-X-C chemokine receptor type 4

Immunohistochemical differentiation pancreatic ductal adenocarcinoma vs. chronic pancreatitis		
Marker	Ductal adenocarcinoma	Chronic pancreatitis
IMP-3	+	–
Maspin	+	–
pVHL	–	+
S100P	+	∓
CEA	+	∓
Mesothelin	+	–
P53	+	–
SMAD-4 (DPC4)	∓	+

+ expression in >90%; ± in 50–90%; ∓in 10–50%; – in <10%; see Fig. 8.7

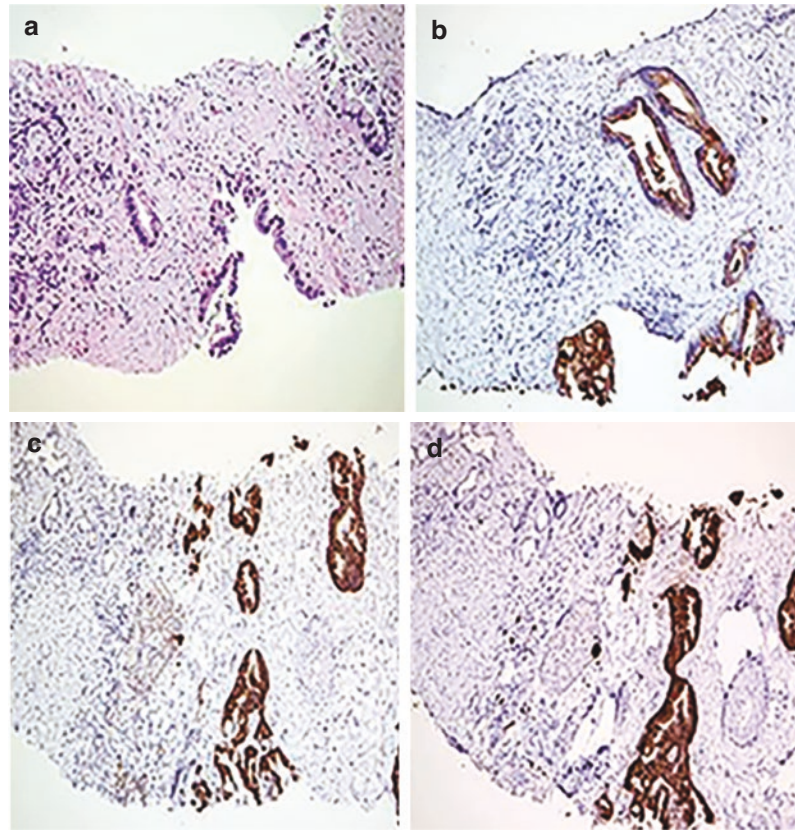

Fig. 8.7 (**a**) Pancreas core biopsy with ductal adenocarcinoma. (**b**) CEA highlighting malignant glands with extracellular luminal expression. (**c**) IMP3 highlights malignant glands, while pancreatic islet cells show only low expression intensity. (**d**) S100P highlighting malignant glands

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9 Markers and Immunoprofile of Hepatobiliary Tumors

Contents

9.1 Hepatocellular Tumors

9.1.1 Diagnostic Antibody Panel for Hepatocytes and Hepatocellular Tumors

Hep Par 1, Arginase-1, CD10, Alpha-fetoprotein, Glutamine synthetase, BSEP, MDR-3, Glypican-3, HSP70, CD34, and cytokeratin profile [1–3].

9.1.1.1 Hepatocyte Specific Antigen (Hep Par1)

Hepatocyte specific antigen (Hep Par1)		
Expression pattern: Cytoplasmic (granular)		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Hepatocellular carcinoma/ hepatoblastoma	Adrenal gland tumors, intestinal metaplasia and small intestinal adenocarcinoma, signet ring cell carcinoma, tumors with hepatoid differentiation, yolk sac tumor	Hepatocytes, intestinal enterocytes
Positive control: Liver tissue		

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https://doi.org/10.1007/978-3-031-45024-2_9

Diagnostic Approach Antibodies to Hep Par-1 (Hepatocyte paraffin-1) react with the carbamoyl-phosphate synthetase-1, a urea cycle enzyme located on the mitochondrial membrane of hepatocytes that is also found in the mitochondria of the intestinal epithelium and cells of renal tubules. Hep Par-1 is a specific marker for hepatocytes and hepatocellular tumors; however, it also labels the epithelium of small intestinal mucosa and small intestinal adenocarcinomas in addition to gastric and esophageal intestinal metaplasia, including Barrett's mucosa [4–8].

Diagnostic Pitfalls Generally, extrahepatic tumors with hepatoid differentiation have the same immunoprofile as hepatocellular tumors, being positive for Hep Par-1, AFP, and CD10 [9]. The expression of Hep Par1 is also reported in tumors of the adrenal cortex and adenocarcinomas of the stomach and small intestine, but these tumors are negative for Arginase [10].

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 biotin-free polymer detection system is recommended for immunohistochemistry on liver tissue.

9.1.1.2 Arginase-1

Arginase-1 is a manganese urea cycle metalloenzyme that catalyzes the conversion of arginine to ornithine and urea. In the hepatobiliary and gastrointestinal systems, the expression of Arginase-1 is limited to hepatocytes, while bile duct epithelium, sinusoidal endothelial cells, and gastrointestinal mucosa lack the expression of this enzyme. Arginase-1 is more specific for hepatocytes and hepatocellular carcinomas than Hep-Par-1 and is found in 85–100% of primary and metastatic hepatocellular carcinoma, whereas the expression intensity correlates with the differentiation grade of the tumor (Fig. 9.1) [11, 12].

Diagnostic Pitfalls Similar to Hep Par-1, hepatoid carcinomas of different origins may be positive for Arginase-1. Various expression levels of Arginase-1 are also found in myeloid cells and macrophages.

9.1.1.3 Alpha Fetoprotein

Alpha fetoprotein (AFP)		
Expression pattern: Cytoplasmic/membranous		
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		

Diagnostic Approach Alpha-fetoprotein (AFP) is an oncofetal glycoprotein found in the fetal liver, fetal gastrointestinal tract, 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 also found in germ cell tumors, that is, yolk sac tumor (Fig. 9.2).

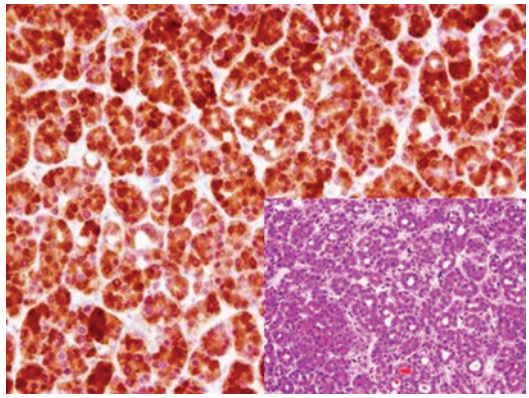

Fig. 9.1 Well-differentiated hepatocellular carcinoma. Arginase-1 staining the cytoplasm of neoplastic hepatocytes

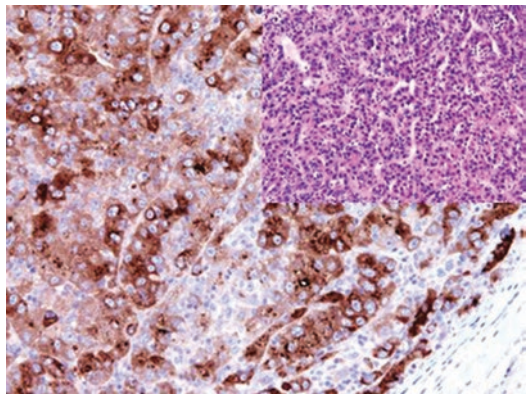

Fig. 9.2 Hepatocellular carcinoma showing strong cytoplasmic AFP expression in neoplastic hepatocytes

Diagnostic Pitfalls It is important to consider that about 5% of all hepatocellular carcinomas are negative for AFP. Due to formalin fixation and tissue processing, up to 50% of hepatocellular carcinoma turns negative for AFP in paraffin immunohistochemistry. The low expression level of AFP is reported in pancreatic acinar cell carcinoma, pancreatoblastoma, and renal cell carcinoma. AFP is also expressed by hepatoid tumors of different origins.

9.1.1.4 Bile Salt Export Pump and Multidrug-Resistance Protein 3

Bile salt export pump (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 canalicular microvilli and subcanalicular vesicles of hepatocytes and responsible for the transport of bile-conjugated salts out of hepatocytes into the canaliculus system [13].

The multidrug-resistance protein 3 (MDR-3) is another member of the same transporter family and a transmembrane protein 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 also be used to differentiate between hepatocellular and bile duct tumors [14].

9.1.1.5 Glypican-3

Glypican-3 is a membrane and extracellular heparan sulfate glycoprotein that regulates signaling during embryogenesis, acting as a receptor for several heparin-binding growth factors. Glypican-3 is normally expressed in fetal tissue and trophoblasts. In adult tissue, the expression of glypican-3 is restricted to a few tissue types, namely gastric glands and renal tubules. In neoplastic tissue, Glypican-3 is expressed in a wide range of epithelial and mesenchymal tumors, including pulmonary squamous cell carcinoma and small cell carcinoma, hepatocellular carcinoma and hepatoblastoma, acinar carcinoma of the pancreas, neuroblastoma, Wilms tumor, ovarian clear cell carcinoma, endometrial carcinoma, yolk sac tumor, choriocarcinoma, placental site nodule, liposarcoma, rhabdomyosarcoma, and undifferentiated pleomorphic sarcoma. Glypican-3 is a helpful marker to discriminate

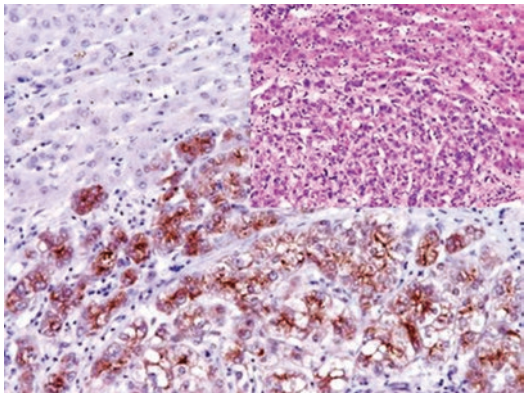

Fig. 9.3 Hepatocellular carcinoma exhibiting cytoplasmic Glypican-3 expression in neoplastic hepatocytes. Note negative reaction in non-neoplastic liver tissue

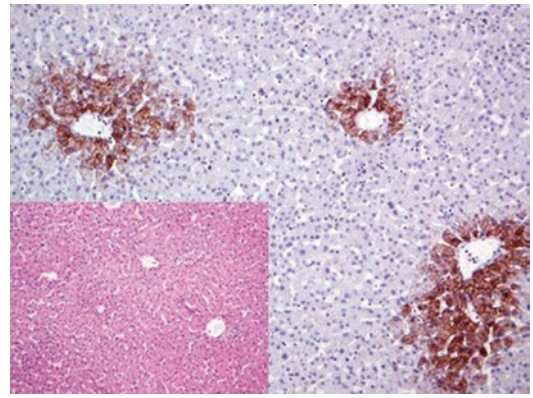

Fig. 9.4 Centrilobular expression of Glutamine synthetase in normal liver parenchyma

between hepatocellular carcinoma (overexpressed in 70–80% of the cases) and benign liver tissue, which is consistently negative for Glypican-3. Nevertheless, it is essential to consider that Glypican-3 may be focally positive in cirrhotic liver tissue, active chronic hepatitis C, and dysplastic liver nodules (Fig. 9.3). In germ cell tumors, embryonal carcinoma and seminoma lack the expression of Glypican-3.

9.1.1.6 Glutamine Synthetase

Glutamine synthetase (GS) is an enzyme that catalyzes the synthesis of glutamine from glutamate and ammonia in the hepatocytes. GS is involved in the regulation of pH and nitrogen balance in the liver. The expression of GS is activated by β-catenin, and mutations causing the activation of this transcriptional factor cause the overexpression of GS. Glutamine synthetase is normally expressed in hepatocytes, proximal renal tubules, and the brain, in addition to the solid pseudopapillary neoplasm of the pancreas [3, 15].

In normal and pathologic liver parenchyma, GS shows the following different expression patterns:

- In normal liver parenchyma, the expression of GS is limited to centrilobular hepatocytes around the central hepatic venules, whereas periportal and mid zones lack the expression of GS (Fig. 9.4).

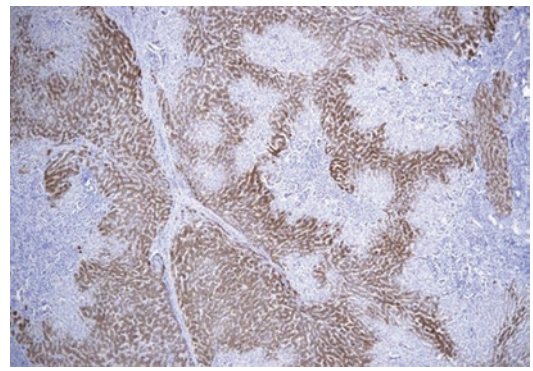

Fig. 9.5 Focal nodular hyperplasia (FNH) with centrilobular GS expression in an anastomosing pattern

- Focal nodular hyperplasia (FNH) shows centrilobular expression in an anastomosing pattern (Fig. 9.5).
- In hepatocellular adenoma (HCA), the GS expression pattern depends on the mutation associated with the adenoma type: In inflammatory and HNF-1α- inactivated HCA, the GS expression is similar to that in normal liver parenchyma with a centrilobular distribution pattern in addition to a weak patchy positivity at the periphery of the lobules. HCA associated with exon 3 mutation or β-catenin-activated HCA shows diffuse GS expression similar to hepatocellular carcinoma.
- High intracytoplasmic accumulation of GS with diffuse expression is characteristic for up to 70% of hepatocellular carcinoma (HCC), whereas the expression intensity correlates

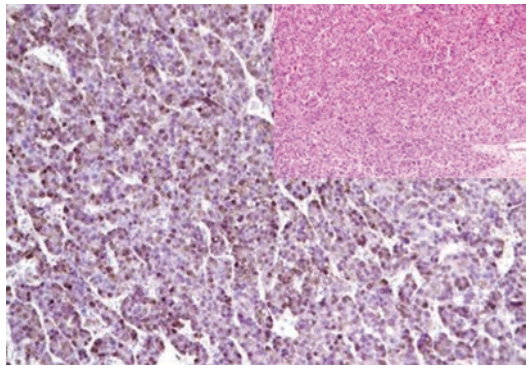

Fig. 9.6 Hepatocellular carcinoma with strong diffuse GS expression in the hepatocytes

with the differentiation grade of the HCC (Fig. 9.6).

– High-grade dysplastic nodules (HGDN) usually lack the expression of GS or show weak focal expression.

9.1.1.7 Heat-Shock Protein-70

Heat-shock protein-70 (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 stain pattern) and dysplastic nodules or hepatocellular adenoma negative for HSP70.

Diagnostic Pitfalls ~ 30% of hepatocellular carcinomas are negative for HSP70 and a subset of cholangiocarcinomas may also be positive for HSP70. Since HSP70 is expressed in different malignant tumors, including gastrointestinal adenocarcinomas, it cannot be used to discriminate between hepatocellular carcinoma and metastatic carcinoma [16, 17].

9.1.1.8 Immunoprofile of Liver Sinusoidal Endothelial Cells

In normal liver parenchyma, the sinusoidal cells are positive for CD4, CD14, CD16, and CD31

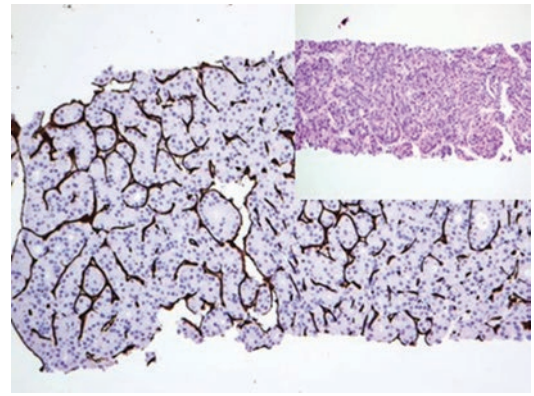

Fig. 9.7 Liver core biopsy. CD34 highlighting sinusoidal cells in hepatocellular carcinoma (sinusoidal capillarization)

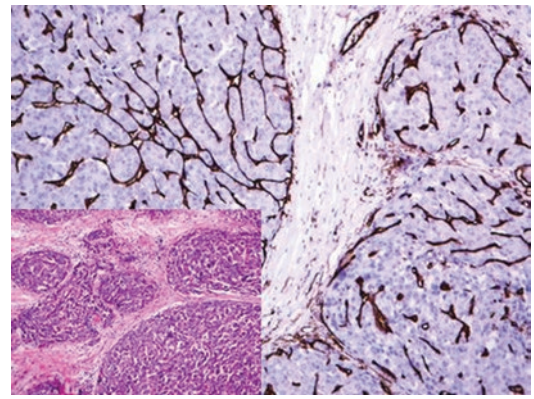

Fig. 9.8 Hepatocellular carcinoma with sinusoidal capillarization labeled by CD34

but negative for CD34. During malignant transformation and remodeling of the normal liver histological architecture, the sinusoidal cells undergo capillarization and exhibit the immunophenotype of vascular endothelial cells with strong CD31 and CD34 expression. This pattern is characteristic for hepatocellular carcinoma (Figs. 9.7 and 9.8).

Finally, a panel of Glypican-3, Glutamine synthetase, heat shock protein 70, BSEP, and CD34 is highly effective in differentiating between benign, dysplastic, and malignant liver nodules.

9.2 Cholangiocarcinoma

9.2.1 Diagnostic Antibody Panel for Intra- and Extrahepatic Cholangiocarcinoma and Gallbladder Carcinoma

Cytokeratin profile, hepatocellular markers, CEA, PDX-1, TTF-1, CD56, S100P, MUC-5 AC, and MUC-6 [18].

All the abovementioned markers are listed in detail in previous sections. PDX-1 is a specific marker for primary intra- and extrahepatic cholangiocarcinoma; nevertheless, it is also expressed in a subset of hepatocellular carcinomas. Although TTF-1 is a specific marker for pulmonary adenocarcinoma and thyroid carcinomas, a weak to moderate nuclear expression is also found in a subset of cholangiocarcinomas, which is to be considered in the differential diagnosis between primary and metastatic liver tumors [19]. The TTF-1 expression in cholangiocarcinoma is most characteristic for large duct-type cholangiocarcinoma and carcinomas of extrahepatic bile ducts (Fig. 9.9).

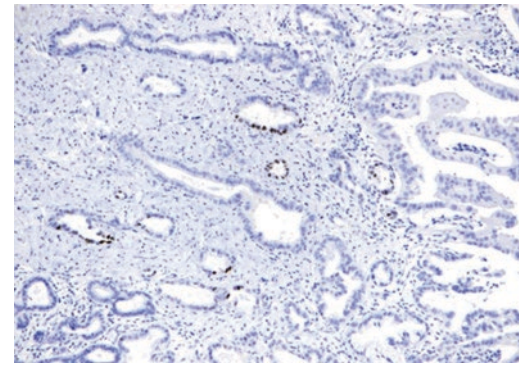

Fig. 9.9 Nuclear TTF-1 expression in the cholangiocarcinoma cells of the common bile duct

Immunoprofile of hepatobiliary tumors				
Tumor type	+ in > 90% (+)	+ in 50–90% (±)	+ in 10–50% (∓)	+ in < 10% (−)
A. Intrahepatic tumors				
Hepatocellular adenoma: – HNF-1α-inactivated HCA – Inflammatory HCA – β-catenin-activated HCA – β-catenin-activated inflammatory HCA – Unclassified HCA	Arginase-1, Hep Par-1, **CD34**[a]	ER, PgR	**Glutamine synthetase**, [b]	**Glypican-3**, **AFP**, HSP70
Hepatocellular carcinoma:	**Arginase-1**[c], Hep Par-1, **Glutamine synthetase**[d], **BSEP**, **MDR-3**, **CD34**[a], CK8/18	**Glypican-3**, **AFP**, Cadherin 17, SATB-2, EMA, CD138, CD10[e], CK7[f] CEA[g], HSP70, PDX-1, TTF-1, [h] ER, AR, MAGE-1, Osteonectin, CD66a, CD56, CD68[i]	CK19[j] CK20[j], BER-EP4, PgR, Vimentin	CK5/6, CK14, EMA, Inhibin, Melan A, EPCAM[k]
Hepatoblastoma:	**Hep Par-1**, Pan-CK, **Glypican-3**, β-catenin	CK18, AFP, CEA, CD34, CD56, EMA, Chromogranin, Vimentin	S100	
Intrahepatic Cholangiocarcinoma: – Small duct type	**CK7**, CK8, CK18, CK19, CEA, EMA, CK17	**PDX-1**, CDH17, N-Cadherin, CD56, CD5	CDX-2	AFP, CK5/6, CK20, TTF-1

Intrahepatic Cholangiocarcinoma: – Large duct type	**CK7**, CK8, CK18, CK19, CEA, EMA, CK17	**PDX-1**, CDH17, S100P, TTF-1; MUC-5AC, MUC-6	CDX-2, CK20, Vimentin	AFP, CK5/6
Angiomyolipoma:	**HMB45**, HMB50, **Melan-A**, Actin, CD63 (NK1-C3), Calponin, PgR	CD117	MIFT, ER	EMA, Pan-CK
B. Extrahepatic and gallbladder tumors				
Biliary intraepithelial neoplasia (BilIN- 1/2/3):	CK7, CK19	CK20, **Cyclin D1**[l], **p21**[l], **MUC5AC**[l], **MUC-6**, **S100p**[l], p16[m]		
Extrahepatic Cholangiocarcinoma	CK7, CK8, CK18, CK19, CEA, EMA, CK17, PDX-1, S100P, DPC-4	CK20, CDX-2, TTF-1		CD56
Biliary mucinous cystic neoplasm: – Cystadenoma – Cystadenocarcinoma	CK7, CA125, CA19.9, CEA *Ovarian type stroma*: ER, PgR, CD10	CK20		
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 (sinusoidal capillarization), which are absent or rare in normal liver parenchyma
[b]The diffuse expression only in β-catenin-activated type of HCA
[c] The intensity of arginase expression correlates with the differentiation of HCC
[d] In HCC, diffuse GS expression in the three zones of liver parenchyma
[e] Apical canalicular stain pattern
[f] CK7 strong expression in the majority of fibrolamellar hepatocellular carcinoma and in up to 30 in conventional hepatocellular carcinoma but is usually negative in normal hepatocytes
[g] Only polyclonal CEA antibodies show a canalicular staining pattern, whereas monoclonal antibodies are negative
[h] Granular cytoplasmic TTF-1 expression due to cross-reaction in hepatocellular carcinoma (Fig. 9.10)
[i] Positive in fibrolamellar hepatocellular carcinoma, negative in conventional HHC and normal hepatocytes.
[j] CK19/20 shows aberrant expression in a subset of neoplastic hepatocytes, usually negative in normal hepatocytes
[k] EPCAM (BerEp-4) is usually positive in hepatoid carcinomas but negative in hepatocellular carcinoma
[l] The expression increases with the grade of dysplasia
[m] The expression decreases with the grade of dysplasia

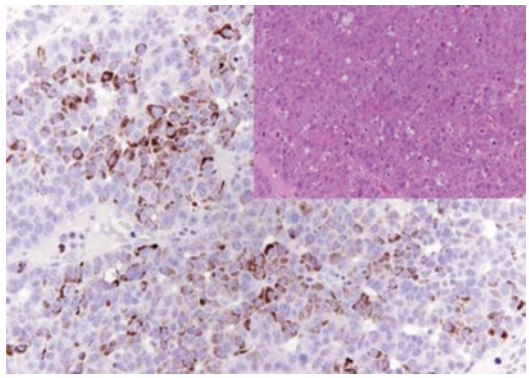

Fig. 9.10 Cytoplasmic TTF-1 expression in hepatocellular carcinoma

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Markers and Immunoprofile of Breast Tumors

10

Contents

Normal breast tissue comprises mesenchymal and epithelial components, which include luminal ductal and acinar (lobular) cells and myoepithelial/basal cells, each cell type with its characteristic immunoprofile. The immunoprofile of breast tumors depends on the origin of neoplastic cells.

10.1 Diagnostic Antibody Panel for Breast Carcinoma

10.1.1 Markers for Luminal Cells

Cytokeratin profile, E-Cadherin, GATA-3, estrogen and progesterone receptors, Mammaglobin, GCFPD-15, TRPS-1, and NY-BR-1.

M. S. A. Tuffaha et al., *Immunohistochemistry in Tumor Diagnostics*,
https://doi.org/10.1007/978-3-031-45024-2_10

10.1.2 Markers for Basal/Myoepithelial Cells

CK5/6/14, S100P, Sox-10, sm-Myosin, sm-Actin, and Calponin.

10.1.3 Therapy-Related Markers

Steroid hormone receptors (estrogen, progesterone, and androgen receptors); HER-2; NTRK; PD-L1; Trop-2; and Ki-67 [1].

10.2 Diagnostic Antibody Panel for Fibroepithelial Tumors

Cytokeratin profile, CD34, and proliferation index (Ki-67).

10.3 Diagnostic Antibody Panel for Mesenchymal Tumors

See panels of other mesenchymal tumors.

10.3.1 GATA-3

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 – Brenner tumor – Tumors of skin adnexa – Yolk sac tumor – Salivary gland tumors – Paraganglioma – Parathyroid adenoma/carcinoma – Clear cell papillary renal tumor	Endometrioid carcinoma, trophoblastic tumors/choriocarcinoma, cervical mesonephric carcinoma, basal cell carcinoma, mesothelioma, pancreatic ductal adenocarcinoma, colorectal adenocarcinoma, chromophobe renal cell carcinoma, bladder small cell carcinoma, neuroblastoma, pituitary adenoma (gonadotroph pituitary adenoma), pheochromocytoma, adrenal cortical carcinoma, squamous cell carcinoma of different locations, peripheral T-cell lymphoma, tumors of ceruminous glands	Luminal adult breast, terminal ducts of the parotid gland, distal renal tubules and renal pelvic and urinary bladder urothelium, prostatic basal cells and seminal vesicle epithelium, cortex and medulla of the adrenal gland, ductal epithelium of skin adnexa and salivary glands, trophoblasts (mainly intermediate), T-lymphocytes
Positive control: Normal breast tissue		

Diagnostic Approach GATA-3 (GATA-binding protein 3 to DNA sequence [A/T]GATA[A/G]), also known as endothelial transcription factor 3, is one of the six members of the GATA family of transcription factors divided into two groups. GATA-1, GATA-2, and GATA-3 are involved in the regulation of proliferation and differentiation of hematopoietic cells and the nervous system. The second group includes GATA-4, GATA-5, and GATA-6, participating in the regulation of mesoderm and endoderm, including the gastrointestinal tract, genitourinary, and respiratory system.

GATA-3 plays an essential role in the differentiation of T-lymphocytes and early erythropoiesis beside skin adnexa, breast and salivary glands, adrenal and parathyroid glands, neuronal cells, and placenta.

In diagnostic immunohistochemistry, GATA-3 is widely used as a marker for primary and metastatic breast carcinoma and transitional cell carcinoma (Figs. 10.1 and 10.2) [2, 3]. In breast carcinomas, the expression of GATA-3 strongly correlates with the expression of the estrogen receptors but lacks therapeutic and prognostic value. The expression of GATA-3 is found in up to 90% of breast carcinomas, while the lowest expression level is found in triple-negative breast carcinomas as well as metaplastic and sarcomatoid breast carcinomas (less than 70%). Only one-third of male breast carcinomas are positive for GATA-3 [4]. Generally, high expression lev-

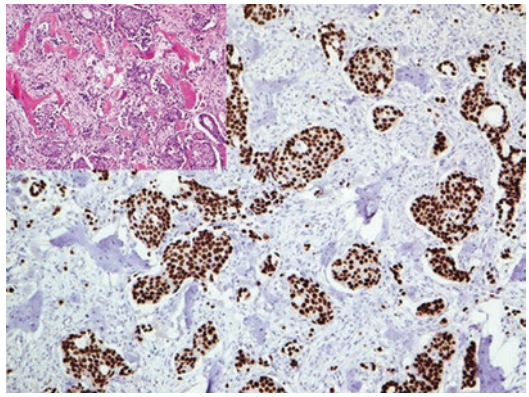

Fig. 10.1 Bone metastases of invasive ductal breast carcinoma. Tumor cells with strong nuclear GATA-3 expression

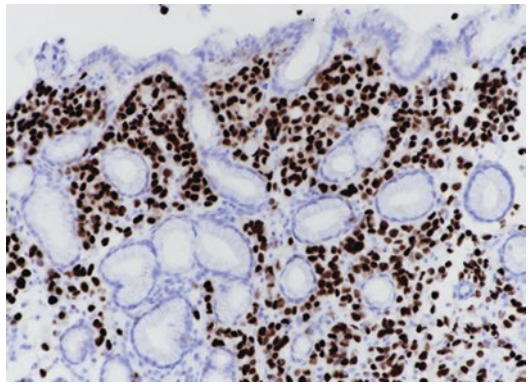

Fig. 10.2 Gastric mucosa infiltrated by metastatic breast carcinoma. Tumor cells with strong nuclear GATA-3 expression

els of GATA-3 in breast cancer predict a good prognostic outcome. GATA-3 as a marker for urothelial and other tumors is discussed in related chapters.

Diagnostic Pitfalls The expression of GATA-3 is not restricted to breast and urothelial tumors but is also found in a wide range of tissue and tumor types, which is to be considered in the interpretation of this marker [5]. Different expression intensity of GATA-3 is 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 [6, 7]. 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 a source of misinterpretation. Accordingly, GATA-3 is a multilineage marker that lacks specificity to breast and urothelial tumors, and the abovementioned notes must be considered in the interpretation of the GATA-3 stain.

10.3.2 Mammaglobin

Mammaglobin		
Expression pattern: Cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Breast carcinoma	Endometrioid adenocarcinoma; endocervical adenocarcinoma; salivary gland carcinoma (mammary analog secretory carcinoma, low-grade polymorphous adenocarcinoma, salivary duct carcinoma, adenoid cystic carcinoma and mucoepidermoid carcinoma); sweat 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 [8]. Mammaglobin is one of the most specific and sensitive markers for tumors of breast origin. The expression of mammaglobin is found in 80–90% of primary breast carcinoma and lymph node metastases [9, 10].

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 several types of salivary gland tumors, mainly mammary analog secretory carcinoma and low-grade polymorphous adenocarcinoma, in addition to endometrioid carcinoma, sweat gland carcinoma, and in a small subset of gastrointestinal cholangiocellular and pulmonary adenocarcinomas. Mesothelioma constantly lacks the expression of mammaglobin.

10.3.3 Gross Cystic Disease Fluid Protein 15

Gross cystic disease fluid protein 15 (GCDFP-15)		
Expression pattern: Cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Breast carcinoma – Primary Paget disease of the vulva	Salivary gland tumors, apocrine skin adnexal tumors, apocrine tumors, pulmonary adenocarcinoma, renal cell carcinoma, and 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 **G**ross **c**ystic **d**isease **f**luid **p**rotein **15** (GCDFP-15, also known as BRST-2) is a prolactin-inducible glycoprotein initially isolated from the fluid of fibrocystic disease of the human breast. GCDFP-15 is expressed by apocrine cells or cells with apocrine metaplasia, regulated by the androgen receptor, and can be inhibited by anti-androgens [11]. In normal breast, ductal and lobular cells lack the expression of GCDFP-15. Antibody to GCDFP-15 reacts with apocrine cells of different origins and tumors arising from these cells. According to different reports, 30–90% of primary and metastatic breast carcinomas are positive for GCDFP-15. Triple-negative breast carcinoma is usually negative for GCDFP-15.

Diagnostic Pitfalls GCDFP-15 is one of the most specific markers for breast carcinoma; nevertheless, it is also expressed in other apocrine, eccrine, and serous glandular epithelium and carcinomas derived from these glands, including tumors of skin adnexa, which is to be considered in the differential diagnosis between primary skin tumors and metastases of breast carcinoma [12].

10.3.4 Tricho-Rhino-Phalangeal Syndrome 1 Protein (TRPS-1):

Tricho-rhino-phalangeal syndrome 1 protein (TRPS-1; also known as transcriptional repressor GATA binding 1) is a GATA-like zinc finger transcription factor encoded on 8q23.3 that binds to the GATA sequences and suppresses the transcriptional activity of GATA-regulated genes. TRPS-1 is an important regulator for the differentiation and proliferation of chondrocytes. Defects in this gene cause the autosomal dominant tricho-rhino-phalangeal syndrome (TRPS) type III characterized by craniofacial and skeletal abnormalities.

In normal tissue, nuclear TRPS-1 expression is found in squamous epithelium, ductal epithelial luminal cells of sweat glands and breast, gall bladder mucosa, glandular cells of the endometrium, prostatic glands, thyroid gland, and a subset of glial cells. In diagnostic immunohistochemistry, TRPS-1 is found to be more specific and sensitive than GATA-3 for breast carcinomas. Strong nuclear TRPS1 expression is found in more than 90% of receptor (ER/PR)-positive, HER2-positive, and triple-negative breast carcinomas, including different types of metaplastic breast carcinoma (Figs. 10.3 and 10.4). Contrary to GATA-3,

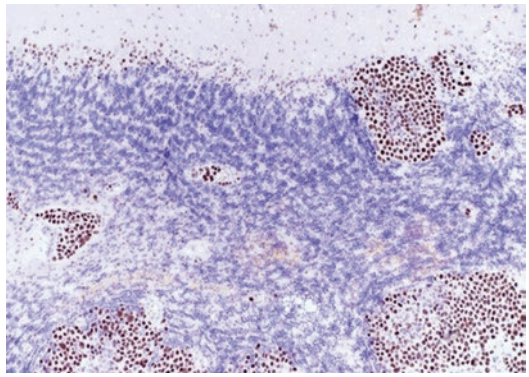

Fig. 10.3 Cerebellar metastasis of ductal breast carcinoma. Tumor cells with strong nuclear TRPS-1 expression

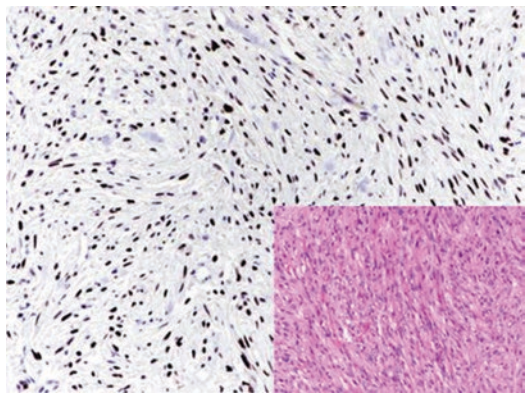

Fig. 10.4 Metaplastic breast carcinoma showing strong nuclear TRPS-1 expression in the tumor cells

TRPS-1 is usually negative or weakly positive in urothelial carcinoma. Furthermore, TRPS-1 is also a marker for mammary and extramammary Paget disease. Other adenocarcinoma types, including pulmonary, gastrointestinal, and pancreatic adenocarcinomas and ovarian and renal cell carcinomas, are also negative or very weakly positive for TRPS-1 [13–15].

Diagnostic Pitfalls TRPS-1 is reported to be negative in breast carcinomas with apocrine differentiation and in cutaneous apocrine carcinomas; both carcinoma types are usually positive for GATA-3 [16]. Similar to GATA-3, TRPS-1 is also expressed in squamous cell carcinomas of different origins, different salivary gland and sweat gland tumors and a subset of lymphocytes.

10.3.5 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 patterns, 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.

10.3.6 Estrogen Receptor

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

Diagnostic Approach The estrogen receptor (ER) is a member of the steroid family of ligand-dependent transcription factors that include the estrogen, progesterone, and glucocorticoid receptors, in addition to the mineralocorticoid receptor. There are two types of nuclear estrogen receptors encoded by two different genes located on different chromosomes, the alpha type (ER-α) and beta type (ER-β), and each type includes different splice variants. Both types have different distributions in different organs and tissue types, whereas many tissue types show the expression of both receptor types [18].

The ER-α type, encoded by the ESR1 gene on chromosome 6q25.1, is mainly expressed in both epithelial and stromal cells of the breast, uterus, placenta, liver, hypothalamus, some types of pituitary adenoma, endothelium, and bone.

The ER-β type is encoded by the ESR2 gene on chromosome 14q23.2 and is mainly expressed in the prostate, testes, granulosa cells, spleen, thymus, skin, and endocrine glands, including the thyroid and parathyroid glands, adrenal glands, and pancreas.

The expression of estrogen receptors -α (ER-α) is a diagnostic marker for the majority of breast carcinomas in addition to tumors of uterine and ovarian origin; however, the expression of estrogen receptors may be found in other tumors such as hepatocellular carcinoma and tumors of the skin.

For the immunohistochemical stain, 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. 10.5) [19].

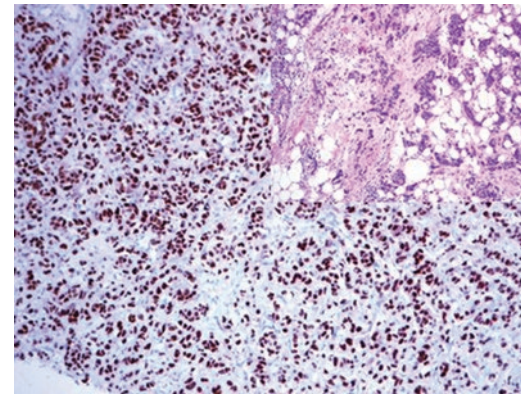

Fig. 10.5 Strong nuclear expression of estrogen receptors in breast carcinoma

During tumor progression, mutations can arise within the ESR1 gene causing resistance to aromatase inhibitors. These mutations usually appear in the ligand-binding domain and can be detected by molecular sequencing of the ESR1 gene.

Few scoring systems were suggested for semi-quantitative estimation of estrogen and progesterone receptors required to predict the response of different breast carcinoma types to specific endocrine therapy, including selective estrogen receptor modulators and aromatase inhibition. The modified scoring system introduced 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 the evaluation of the nuclear stain intensity and the percentage of positive tumor cells.

10.3.6.1 Remmele Scoring System

This simple scoring system has a 12-point scale (0–12) [19, 20]. 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 (3x4) [21]. Tumors with a score of less than 3 usually respond poorly to anti-estrogen therapy.

10.3.6.2 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 of more than 80%		

10.3.6.3 McCarty Scoring System

This scoring system has a 300-point scale (0–300) [22]. 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 (+++).

10.3.6.4 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). Tumors with a score of less than 3 usually respond poorly to anti-estrogen therapy.

10.3.6.5 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 Pitfalls The expression of ER depends on the histological type and differentiation grade of the breast tumor. The expression of ER is not specific to breast and uterine tumors and also can be found in many others, such as hepatocellular carcinoma, gastric adenocarcinoma, and transitional cell carcinoma. Additional markers such as GATA-3, TRPS-1, mammaglobin, GCDFP15, and progesterone receptors, as well as the cytokeratin profile, help to confirm the diagnosis of primary breast carcinoma.

10.3.7 Progesterone Receptor

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 the pancreas, stroma of mixed epithelial stromal tumor of the kidney, stromal tumors of the prostate, neuroendocrine tumors (mainly pancreatic)	Breast and endometrial cells, endometrial 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. The progesterone receptor (PgR) is a member of the steroid hormone receptor superfamily and estrogen-induced proteins that mediate the effect of the progesterone hormone expressed in different tissue types. PgR has three isoforms, A, B and C, all encoded by the same gene located on chromosome 11q22. PgR is a good marker for breast carcinomas and is more specific than estrogen receptors as it is expressed only in a limited number of tumors such as endometrial carcinoma, skin adnexal tumors, and meningiomas. The pro-

gesterone receptor status is one of the important prognostic factors for the management of breast, endometrial, and ovarian cancers [19]. A high expression level of both estrogen and progesterone hormone receptors is a positive prognostic factor for breast and endometrial cancers and predicts a good response to anti-estrogenic therapy.

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

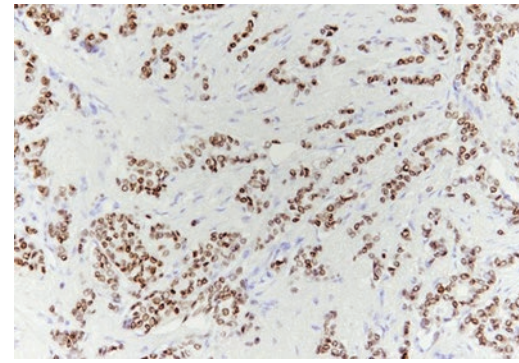

Fig. 10.6 Strong nuclear expression of androgen receptors in the neoplastic cells of invasive ductal carcinoma

10.3.8 Androgen Receptor

The androgen receptor (AR) is a nuclear receptor and a member of the steroid hormone receptor family, closely related to the progesterone receptor and activated by binding any of the androgenic hormones (testosterone and dihydrotestosterone). AR is variously expressed in different breast carcinoma types, and different expression levels are found in estrogen/progesterone/HER-2 positive and triple-negative breast carcinomas (luminal androgen receptor type) and is considered as one of the prognostic factors of breast carcinomas (Fig. 10.6) [23]. The strong expression of AR is one of the diagnostic characteristics of apocrine breast carcinoma and apocrine metaplasia. Metaplastic and mucinous carcinomas, in addition to basal-like and mesenchymal subtypes of triple-negative breast carcinomas, usually lack the expression of AR. AR is a potential therapeutic target in the luminal androgen receptor subtype of triple-negative breast carcinoma. The androgen receptor is also detailed in a later chapter (see Sect. 13.1).

10.3.9 Human Epidermal Growth Factor Receptor-2

HER-2 (c-erb-2)		
Expression pattern: Membranous		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Breast carcinoma – Assessment of targeted HER-2 immunotherapy in different tumors	Gastrointestinal adenocarcinomas, carcinomas of the salivary glands, ovarian and endometrial carcinomas, a 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, encoded on chromosome 17 (17q12). 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 occurs by its dimerization. The HER-2 molecule is a part of the membrane of normal epithelial cells and $20x10^3$ to $50x10^3$ receptors are generally found on the surface of normal breast epithelial cells. During carcinogenesis,

the amplification of the HER-2 gene may occur, causing the overexpression of the HER-2 receptor, and up to $3x10^6$ receptors may be expressed on the membrane of these tumor cells. The HER-2 molecule is the therapeutic target in various tumors exhibiting the expression or overexpression of this receptor using specific antibodies or drug-conjugated antibodies. The overexpression of HER-2 is characteristic for various types of human carcinomas, mainly breast and gastric adenocarcinomas (Fig. 10.6), in addition to a subset of other carcinoma types such as ovarian carcinoma, non-small cell carcinoma of the lung, salivary gland carcinoma, and urinary bladder transitional cell carcinoma [24]. The amplification of the HER-2 gene can be detected by the FISH or CISH assay. A good alternative is semiquantitative detection using specific antibodies. Immunohistochemistry is an easy technique to estimate the corresponding overexpression of the HER-2 molecules on the membrane of the tumor cells. The immunohistochemical expression score is an important parameter for the 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 a cold ischemic time of less than 1 hour and optimal fixation (6–48 hours fixation)—preferably preoperative biopsies—is to be used for the HER-2 immunostaining.
- The interpretation of the immunostaining 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 stains must be neglected. Staining caused by edge artifacts should also be ignored.
- Only invasive tumor components should be considered. The intraductal component may show a stronger signal than the invasive component, which can skew the evaluation.

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 vary and depend on the specimen type (see also HER-2 in gastric adenocarcinoma, Chap. 7).

10.3.9.1 Scoring of HER-2 Expression in Breast Cancer

IHC score	HER-2 overexpression	Staining result
0	Negative No gene amplification	No detectable staining (negative) 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+	Equivocal with uncertain gene amplification (see text below)	A weak to moderate staining of the entire membrane in more than 10% of tumor cells
3+	Positive High gene amplification	Strong staining of the entire membrane in more than 10% of tumor cells

According to the ASCO/CAP HER2 testing guidelines update 2018, breast carcinomas with the IHC HER-2 score 3+ are always classified as HER-2 positive and usually show a good response to the specific antibody therapy. Carcinomas with the scores IHC 1+ or 0 are to be classified as HER-2 negative with no evidence for gene amplification and are not sensitive to specific immunotherapy. However, score 1+ and non-amplified carcinomas with score 2+ can be classified as HER-2 low and maybe sensitive to drug-conjugated antibodies. Carcinomas with IHC HER-2 score 2+ need further confirmation by FISH/CISH assay or one of the molecular methods (real-time PCR or NGS). According to the ASCO/CAP HER-2 testing guidelines, the results of the FISH/CISH assays can be categorized into five groups: [25, 26] (Figs. 10.7 and 10.8)

- Group 1: HER-2/CEP17 ratio ≥ 2.0; average HER-2 gene copy number is ≥4.0/tumor cell. HER-2 status is categorized as HER-2 positive.
- Group 2: HER-2/CEP17 ratio ≥ 2.0; average HER-2 gene copy number < 4.0/tumor cell. HER-2 status is categorized as HER-2 negative unless IHC score 3 + .
- Group 3: HER-2/CEP17 ratio < 2.0; average HER-2 gene copy number ≥ 6.0 per tumor cell, probably due to trisomy or polysomy of chromosome 17, categorized as HER-2 negative unless IHC score 3 + .
- Group 4: HER-2/CEP17 ratio < 2.0; average HER-2 gene copy number > 4.0 and < 6.0/tumor cell, categorized as HER-2 negative unless IHC score 3 + .
- Group 5: HER-2/CEP17 ratio < 2.0; average HER-2 gene copy number is <4.0/tumor cell, categorized as HER-2 negative.

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

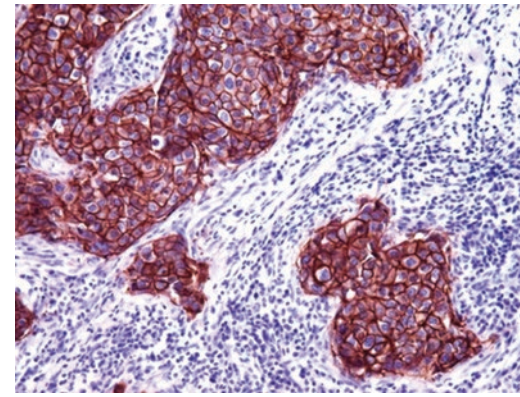

Fig. 10.7 Breast carcinoma with a strong membranous expression of HER-2 in all tumor cells (score 3+)

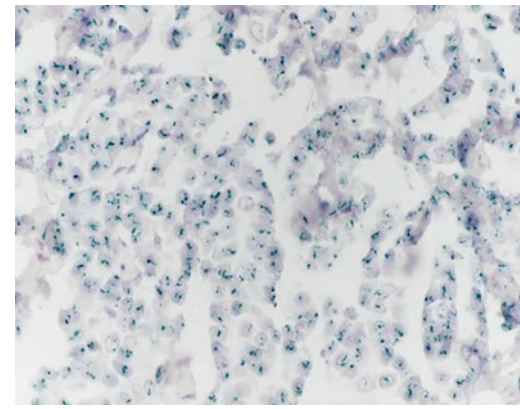

Fig. 10.8 CISH for HER-2 with high-level gene amplification with the formation of HER-2 gene clusters

10.3.10 Trophoblast Cell Surface Antigen 2

Trophoblast cell surface antigen 2 (Trop-2), also known as tumor-associated calcium signal transducer 2, is a type 1 transmembrane glycoprotein functioning as a calcium signal transducer. Low baseline Top-2 expression is found in different normal tissue types such as the breast, ovaries, pancreas, lungs, and kidney. During malignant transformation, the expression of Trop-2 is upregulated, and overexpression of Trop-2 is noticed in different carcinoma types, including gastrointestinal, pulmonary, genitourinary, and breast carcinomas. In most tumors, the overexpression of Trop-2 correlates with aggressive behavior and poor prognosis.

As a cell surface protein, Trop-2 is an interesting target for specific humanized therapeutic antibodies and specific inhibitors used to treat different carcinoma types exhibiting Trop-2 overexpression, such as triple-negative breast carcinoma (see also Sect. 14.3).

10.3.11 E-Cadherin

E-cadherin is a transmembrane glycoprotein and a member of the cadherin superfamily functioning as an adhesion molecule listed in detail in a previous section (see Sect. 2.4).

In routine histopathology, E-cadherin is a helpful marker to discriminate between ductal and lobular breast neoplasms as lobular breast carcinomas, including lobular carcinoma in situ,

lack the expression of E-cadherin due to mutations within the gene encoding E-cadherin (Fig. 10.9). These mutations cause the synthesis of anomalous E-cadherin molecule without cohesiveness properties, which also cannot be detected by the standard antibodies or might show atypical intracytoplasmic or perinuclear expression pattern. The absence of normal E-cadherin in the cells of lobular neoplasms leads to the intracytoplasmic accumulation of δ1- Catenin (also known as p120), making it a further interesting marker for lobular carcinomas of the breast with intense cytoplasmic stain. Myoepithelial cells surrounding non-invasive carcinomas are generally positive for E-cadherin [27] (Fig. 10.10). E-cadherin is also a prognostic marker for various carcinoma types such as breast, poorly cohesive gastric adenocarcinoma, and transitional carcinoma, as the loss of E-cadherin expression is found to be associated with aggressive behavior.

Diagnostic Pitfalls The correlation with the tumor morphology is essential as the loss of E-cadherin expression may be found in a subset of poorly differentiated carcinomas. It is also important to consider that up to 15% of invasive lobular breast carcinomas may show E-cadherin expression in different intensities.

10.3.12 Smooth Muscle Myosin Heavy Chain

Smooth muscle myosin heavy chain (SMMHC) is a structural contractile protein listed with the markers of smooth muscle tumors (Sect. 24.2). In breast pathology, SMMHC is a helpful marker that selectively highlights the myoepithelial cells usually preserved as a continuous layer surrounding benign breast lesions (Fig. 10.11). This assay is important to discriminate between invasive breast carcinomas (tubu-

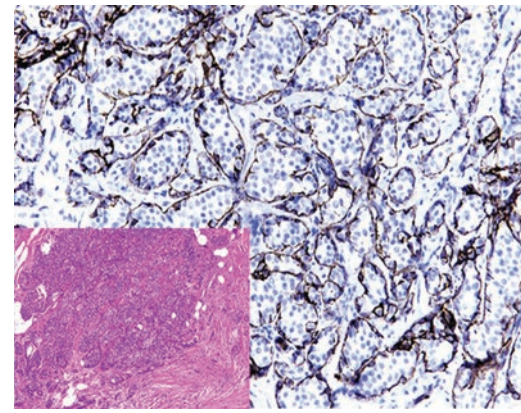

Fig. 10.10 Lobular carcinoma in situ surrounded by E-cadherin positive myoepithelial cells. Luminal neoplastic cells are negative for E-cadherin

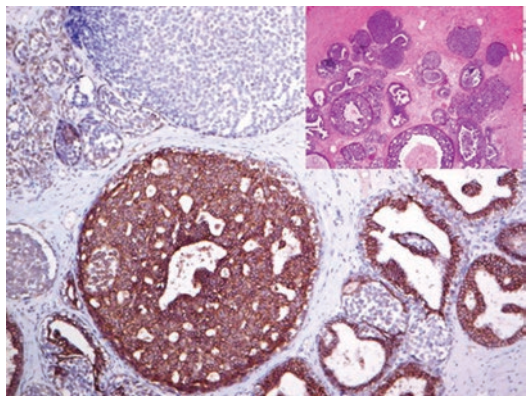

Fig. 10.9 E-cadherin highlighting the neoplastic cells of ductal carcinoma in situ (DCIS), whereas the cells of lobular carcinoma in situ (LCIS) lack the E-cadherin expression

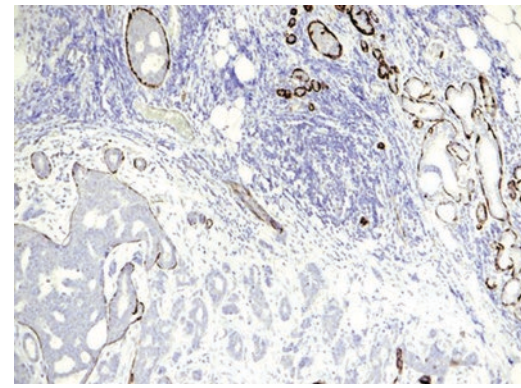

Fig. 10.11 Myosin stain of breast tissue. Benign areas and non-invasive (DCIS) structures with retained myoepithelial cells in the periphery and invasive portions lacking the myoepithelial cell layer

lar, papillary, and cribriform carcinoma) lacking the myoepithelial cell layer and other non-invasive, benign, or reactive lesions such as ductal carcinoma in situ (DCIS), radial scar, benign hyperplasia, and adenosis besides papilloma and nipple adenoma typically with intact myoepithelial cell component.

10.3.13 Assessment of Triple-Negative Breast Carcinoma

Triple-negative breast carcinoma is a heterogeneous group of breast tumors that lacks the expression of HER-2 and both estrogen and progesterone receptors and accounts for 10–15% of all breast tumors. This tumor group includes low-grade and high-grade carcinoma identities. The high-grade group contains several subtypes, including carcinoma with basal-like phenotype, carcinoma with apocrine differentiation, and metaplastic carcinoma. The low-grade carcinoma identities include secretory carcinoma, breast carcinomas of salivary gland type, and tall cell carcinoma with reversed polarity. The triple-negative immunophenotype is suspicious of BRCA1 mutation as ~60% of breast carcinomas associated with BRCA1 are triple-negative.

Several molecular subtypes of triple-negative breast carcinoma of no special type are described and can be assessed using special immunohistochemical markers. As reported in several studies, these subtypes have different biological and clinical behavior, different responses to therapy protocols, and include the following identities [28–31]:

- Basal-like immune suppressed type: carcinoma with high genetic instability.
- Immunomodulatory type (basal-like immune activated): carcinoma with high genetic instability. Tumor tissue enriched by tumor-infiltrating inflammatory cells, including CD8 positive T-lymphocytes and CD20 B- lymphocytes.
- Mesenchymal type: carcinoma with intermediate genetic instability.
- Luminal androgen receptor type: hormonally regulated carcinoma with strong expression of the androgen receptors and low genetic instability.

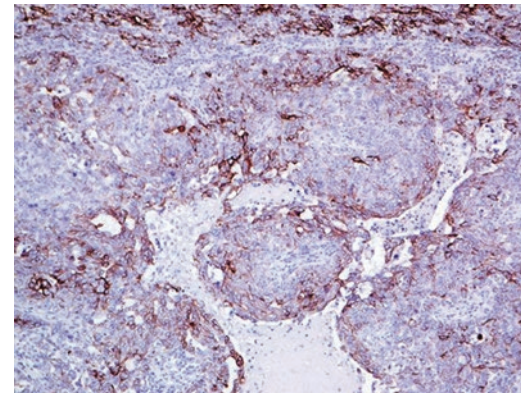

Fig. 10.12 Strong cytoplasmic CK 5/14 expression in triple-negative basal type breast carcinoma

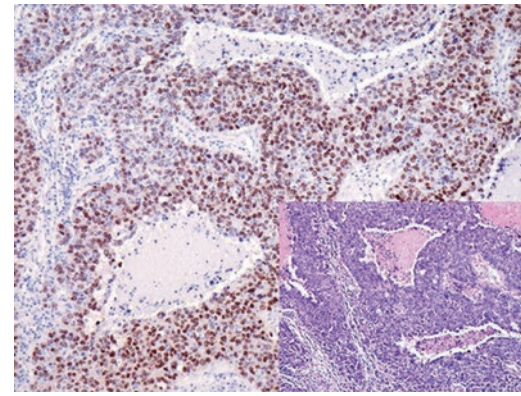

Fig. 10.13 Nuclear Sox-10 expression in neoplastic cells of triple negative basal-type breast carcinoma

Triple-negative breast carcinoma with basal-like phenotype is the most common triple-negative carcinoma type characterized by the expression of high molecular weight Cytokeratins (CK5/6/14/17), besides EGFR, Sox-10, and FOXC1 and usually associated with p53 accumulation (Figs. 10.12 and 10.13). It is also important to consider that high molecular weight Cytokeratins also stain metaplastic carcinoma and Sox-10 stains secretory carcinoma.

TRPS1 and GATA-3 are expressed in the majority of triple-negative breast carcinomas, while p40 and p63 have only a little diagnostic value as they are frequently negative. p16 and FOXC1 are strongly expressed in the basal-like immune-suppressed subtype.

Immunoprofile of breast tumors				
Tumor types	+ in > 90% (+)	+ in 50–90% (±)	+ in 10–50% (∓)	+ in < 10% (−)
Ductal hyperplasia (usual ductal hyperplasia; UDH):	UDH is 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. Intact continuous layer of 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, and CK19 2. No luminal or only rare residual CK5/6/14, p63 positive intermediate myoepithelial cells 3. Intact continuous layer of myoepithelial cells positive for CK5/6, CK14, p63, Actin and Myosin 4. Intact basement membrane positive for Laminin Luminal glandular cells are positive for Estrogen (ER) and Progesterone (PgR) receptors and negative for HER-2 and p53			
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%		CK20, p53, **E-cadherin**[b], HER-2 Cyclin D1
Invasive carcinoma of no special type (NST) A. Invasive ductal carcinoma NOS.	CK7, CK8, CK18, CK19, EMA[c], CD44, **GATA-3**, **E-Cadherin**, β-Catenin, p120 Catenin (m)[d]	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%	CK14, CK17, CK20
Invasive carcinoma of no special type B. Oncocytic carcinoma.	CK8, CK18, EMA	CK7, ER expression in 70–80% PgR in 60–70%	HER-2 overexpression in ~25%	HER2, GCDFP-15
Invasive carcinoma of no special type C. Lipid-rich carcinoma.	Adipophilin	HER-2 overexpression in 75–100%		CK5/6/14, ER, PgR
Invasive carcinoma of no special type D. Glycogen-rich carcinoma.			ER expression in 35–50%	PgR

Invasive carcinoma of no special type E. Sebaceous carcinoma.	ER, PgR and HER-2 expression in ~60%			
Invasive carcinoma of no special type F. Basal-like phenotype.	CK5/6/14, p40, p63, **Sox-10**, EGFR	Vimentin, CK17		ER, PgR, HER-2
Invasive carcinoma of no special type G. Medullary carcinoma and carcinoma with medullary features.	CK 8, CK 18	p53, EGFR	Vimentin, S100, CK5/6, CK14	HER-2, CK7, CK19, CK20, GCDFP15 ER and PgR[e] expression in 0–10%
Invasive lobular carcinoma:	**CK7**, CK8, CK18, CK19, **GATA-3**, **p120 Catenin**[d]	GCPF15, CEA, Cyclin D1, Maspin, ER expression in 80–95% PgR in 80–90% AR in 80%	NKX3.1, EGFR	**E-Cadherin**, HER-2 (<5%), CK5/6, CK14, CK20
Tubular carcinoma:	**CK7**, CK18, CK19, **GATA-3** Absence of basal myoepithelial cell layer positive for myoepithelial markers (p63, CK5/6/14, Actin, Myosin)	ER and PgR expression in 90–100%		HER-2, CK5/6, CK20
Cribriform carcinoma:	**CK7**, CK8, CK18, CK19, **GATA-3**	Human milk fat globule ER expression in 90–100%, PgR expression in ~70%	CK10/13	HER-2, CK14, CK20
Mucinous carcinoma:	CK7, CK18, CK19, MUC-2, CEA	WT-1, androgen receptors, NSE, chromogranin, Synaptophysin, ER expression in ~90%, PgR in 70–80%	EGFR	HER-2, CK20, CDX-2
Mucinous cystadenocarcinoma:	CK7, CK18		CK5/6/14 ER and PgR expression in <20%	HER-2, CK20, CDX-2
Invasive micropapillary carcinoma:	CK7, CK18, CK19, **EMA**[e] ER and PgR expression in ~90–100%	LEF-1		
Invasive papillary carcinoma:	CK7, CK18, CK19, CEA			CK5/6, CK14
Solid papillary carcinoma:	CK7, CK18 ER and PgR expression in ~90–100%	Chromogranin, Synaptophysin		HER-2, CK5/6/14
Carcinoma with apocrine differentiation:	CK8, CK18, CK19, **Androgen receptors**	**GCDFP15**, CEA, ER-β	HER-2 (amplification in 30–60%)	ER-α, PgR, S100
Metaplastic carcinoma:	Vimentin, Pan-CK	CK7, CD44	EMA, Actin, S100, GATA-3	ER, PgR

Breast tumors of salivary gland type: – Adenoid cystic carcinoma – Acinic cell carcinoma – Mucoepidermoid carcinoma – Polymorphous carcinoma	See salivary gland tumors (Sect. 6.2) Usually triple negative carcinomas			
Secretory carcinoma:	CK8, CK18, CK19, EMA, Lactalbumin, **S100**, **Sox-10**, **NTRK**[f]	CK5/6/14, S100, GATA-3, CEA, EGFR, Vimentin	CD117	ER and PgR expression in <10% HER-2
Tall cell carcinoma with reversed polarity:	CK5/6/14, CK7, **IDH-2$_{R172S}$**	GATA-3, GCDFP-15	AR	ER and PgR expression in <10%
Neuroendocrine tumors of the breast: – Carcinoid (NET G1 and G2) – Small cell carcinoma (NET G3/NEC) – Large cell neuroendocrine carcinoma (NEC)	See neuroendocrine tumors and neuroendocrine carcinomas (Chap. 14) Usually triple negative carcinomas			
Phyllodes tumor:	*Stromal cells*: Vimentin *Epithelial cells*: CK 5/6, CK14, CK8/18, Pan-CK, EMA Proliferation index (Ki-67) in stromal cells: In benign type usually <20% In malignant type usually >20%	CD34, bcl-2, CD117, p53 CEA	CD34, actin, Desmin, CD10, CD117	S100, pan-CK, EMA
Myofibroblastoma of the breast:	Desmin, CD34, CD99, bcl2, Vimentin	CD10, Actin, Androgen receptors	PgR, ER	Pan-CK, S100
Tumors of the nipple				
Paget's disease of the nipple:	**CK7**, [g] CK8, CK18, EMA (MUC-1), CD63 (NK1-C3)	CEA, GCDFP15, **HER-2**[g]	ER, PgR, AR	CK5/6, CK20, MUC-2
Syringomatous tumor:	CK5/6/14, CK8, CK18, p40, p63		ER	PgR, HER-2

[a] No luminal or only residual of CK5/6/14 positive intermediate myoepithelial cells. An 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 non-neoplastic breast lobular cells

[c] In invasive micropapillary carcinoma, the reverse cell polarity with inverted EMA expression on the basal surface is characteristic; other breast tumors show the EMA expression on the apical surface of tumor cells (see Fig.10.14)

[d] P120 Catenin shows a membranous stain in invasive ductal carcinoma but a characteristic cytoplasmic stain in lobular carcinoma

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

[f] Secretory carcinoma is usually associated with the t(12;15)(p13;q25) translocation generating the ETV6-NTRK gene fusion

[g] See Figs. 10.15 and 10.16

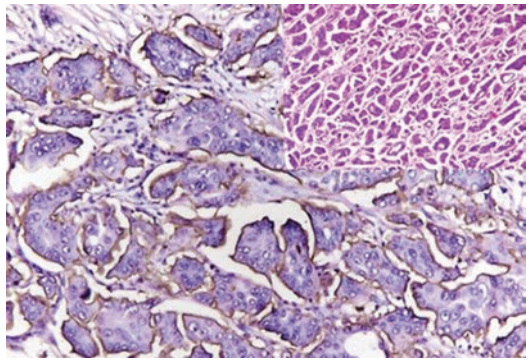

Fig. 10.14 Characteristic inverted (basal) EMA expression in invasive micropapillary carcinoma

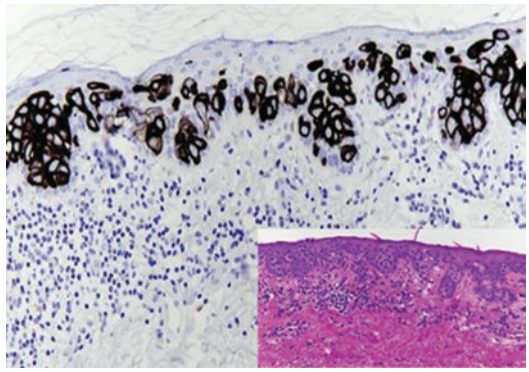

Fig. 10.15 Paget's disease of the nipple. Intraepidermal tumor cells with strong cytoplasmic CK7 expression

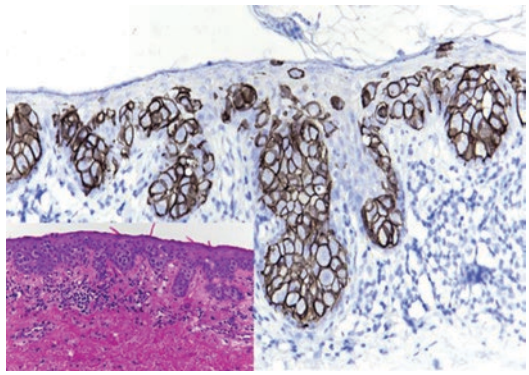

Fig. 10.16 Paget's disease of the nipple. Intraepidermal tumor cells with strong membranous HER-2 expression

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11 Markers and Immunoprofile of Tumors of Female Reproductive Organs

Contents

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11.1 Diagnostic Antibody Panel for Tumors of the Vulva and Vagina

Cytokeratin profile, p40, p63, CEA, p16, Ki-67, HPV, Desmin, Myogenin, and melanoma markers.

11.2 Diagnostic Antibody Panel for Epithelial Tumors of the Uterine Cervix

Cytokeratin profile, p40, p63, CEA, PAX-8, PAX-2, p16, p53, Ki-67, HPV, and Steroid hormone receptors [1].

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

Cytokeratin profile; CEA; PAX-8; p16; HNF-1β; WT-1; Steroid hormone receptors (ER, PgR); DNA mismatch repair proteins (MLH1, PMS2, MSH2, MSH3, MSH6); p53; and Ki-67.

11.4 Diagnostic Antibody Panel for Uterine Mesenchymal Tumors

Smooth muscle markers (Actin, Smoothelin, Caldesmon, Calponin); IFTIM (CD225); CD10; and Steroid hormone receptors (ER, PgR).

11.5 Diagnostic Antibody Panel for Gestational Trophoblastic Disease

Cytokeratin profile, PLAP, human leukocyte antigen G (HLA-G), human placental lactogen (hPL), GATA-3, and Inhibin.

11.5.1 p16

p16 (CDKN2A)		
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, leiomyosarcoma, and smooth muscle tumors of uncertain malignant potential	
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 $p16^{INK4a}$ (CDKN2) suppressor gene. p16 inhibits the cyclin-dependent kinases (4 and 6) involved in cell cycle regulation and progression (G1 to S). p16 plays a role in the pathogenesis of different malignancies. The expression of p16 is regulated by the activity of the retinoblastoma gene (Rb), 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 patterns and the intensity of the stain correlates with the grade of HPV infection and the grade of associated dysplasia. The so-called block staining pattern is characteristic for HPV-associated high-grade dysplasia (Fig. 11.1) and HPV-associated squamous cell carcinoma. p16 is

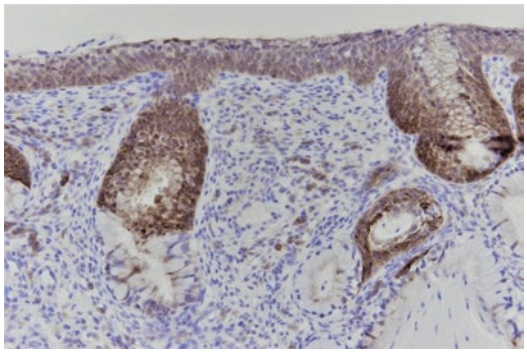


Fig. 11.1 Cervical biopsy with HPV-associated high-grade dysplasia. Dysplastic cells exhibit a strong block-like p16 expression

usually negative in HPV-independent squamous cell carcinomas, which in turn are frequently positive for p53.

p16 is also highly expressed in serous uterine carcinoma and is a helpful marker that labels the cells of serous tubal intraepithelial carcinoma (STIC) [2].

p16 is also a helpful marker to discriminate between benign and malignant adipocytic tumors and between benign nevi and malignant melanocytic tumors (see related chapters) [3, 4].

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 one of the best markers for endometrial adenocarcinoma and a subset of endocervical adenocarcinomas. This marker is listed in detail in the following chapter (see Chap. 12).

11.5.2 Hepatocyte Nuclear Factor-1 β

Hepatocyte nuclear factor-1 β (HNF-1 β) 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 is also variously found in other organs, including the pancreas, kidney, prostate, and female genital system [5]. HNF-1 β is used in diagnostic immunohistochemistry to differentiate between different types of ovarian and endometrial carcinomas. The strong nuclear HNF-1 β expression is characteristic for both endometrial and ovarian clear cell carcinomas but is usually negative in reactive lesions with clear cell appearance such as clear cell metaplasia and Arias-Stella phenomenon [6]. However, we must consider that a focal weak to moderate HNF-1 β expression can also be found in other endometrial and ovarian carcinoma types, such as endometrioid and serous carcinomas [7]. Additionally, a different HNF-1 β expression intensity is also found in other carcinomas of different origins, including colorectal, pancreatobiliary, prostatic, and renal cell carcinomas.

11.5.3 Phosphatase and Tensin Homolog

Phosphatase and **ten**sin homologue (PTEN) is a tumor suppressor gene located on 10q23 and encoding a widely expressed enzyme in mammalian cells that catalyzes the dephosphorylation of the 3'phosphate of the inositol ring, which is an essential reaction that causes the inhibition of the protein kinase (AKT) signaling pathway involved in the regulation of apoptosis. Deletions or mutations that inactivate the PTEN gene cause the inhibition of the apoptotic cascade and increase cell proliferation, mainly by the upregulation of the mammalian target of rapamycin (mTOR). Inactivating mutations within the PTEN are commonly seen in different human neoplasia such as urogenital, breast, and lung carcinomas in addition to melanoma and glial tumors [8]. The immunohistochemical staining of PTEN (cytoplasmic or nuclear stain pattern) is a simple way to detect the loss of this enzyme but may be difficult if the immunohistochemistry signal in normal glands is weak; careful titration of the primary antibody is key. The loss of PTEN expression is found in 35–55% of endometrioid carcinoma and in up to 65% of atypical endometrial hyperplasia/endometrioid intraepithelial neoplasia, which indicates that the loss of PTEN is not a specific marker of malignant transformation [9, 10]. Normal proliferative endometrium usually shows strong PTEN expression. The loss of PTEN expression is also found in a subset of endometrioid ovarian carcinoma (~20%), high-

grade serous carcinoma, and clear cell carcinoma.

Prostatic adenocarcinoma is also commonly associated with PTEN loss (see markers of prostatic carcinoma, Sect. 13.1) [8]. PTEN mutations with a loss of protein expression are also found in a subset of thyroid adenomas and different breast carcinoma types in addition to primary glioblastoma but rare in secondary glioblastoma.

11.5.4 Steroid Hormone Receptors

Both estrogen and progesterone receptors, in addition to androgen receptors, were discussed in detail with the markers of breast tumors. Endometroid adenocarcinoma and serous endometrial carcinoma are sex hormone-dependent tumors, and the expression of estrogen and progesterone is characteristic for both carcinoma types in various degrees [11]. More than 90% of grade 1 and 2 and ~ 50% of grade 3 endometroid carcinoma are positive for both hormone receptors and generally endometroid carcinomas, with the strong expression of steroid hormone receptors rarely associated with the overexpression of p53. The myometrium is also a target tissue for steroid hormone receptors; accordingly, the majority of uterine leiomyomas and leiomyosarcomas are positive for estrogen or progesterone receptors or for both. This characteristic feature can be used to differentiate between uterine and soft tissue leiomyosarcoma [12]. Squamous cell carcinoma and adenocarcinoma of the uterine cervix usually lack the expression of both receptors [13].

11.5.5 Mismatch Repair Proteins, Microsatellite Instability, and Molecular Classification of Endometrioid Carcinoma

Mismatch repair proteins and detection methods, including immunohistochemistry on paraffin fixed tissue, are discussed in detail in Chap. 35. In gynecological pathology, the analysis of the mismatch repair proteins is essential for the diagnosis and classification of uterine and ovarian carcinomas.

Microsatellite instability (MSI-H/MMRd) is detected in up to 30% of endometrial and ovarian endometrioid carcinomas as well as clear cell ovarian carcinoma and can show different expression patterns of the MMR proteins.

- Normal nuclear expression of the four MLH1, PMS2, MSH2, and MSH6 proteins. This normal immunohistochemical pattern indicates no evidence of mismatch repair deficiency.
- Loss of MLH1 and PMS2: This type is found in 90–95% of endometrioid carcinomas with microsatellite instability. The majority of these carcinomas are associated with the hypermethylation of the MLH1 promoter region and are considered sporadic tumors. The absence of promoter hypermethylation is found in 3–5% of endometrioid carcinomas and is generally due to a germline mutation/Lynch syndrome.
- Single loss of PMS2 (or rarely MLH1): This type is mostly associated with germline mutations in PMS2/MLH1 genes with a high probability for Lynch syndrome.
- Loss of MSH2 and MSH6: Mainly caused by a germline mutation in the MSH2 gene and the evaluation of EPCAM expression is indicated.
- Single loss of MSH6: mostly caused by germline mutations in the MSH6 gene, usually with a high probability for Lynch syndrome.

Four distinct molecular groups of endometrioid carcinomas of the endometrium are described and have different prognoses and therapy management: [14, 15]

- Group 1: ultramutated carcinomas with mutations in the exonuclease domain of the DNA polymerase epsilon (POLE) gene. This gene is responsible for a low mutation rate in DNA replication. This group includes all of POLE mutated endometrium carcinomas regardless

of mismatch repair status or p53 mutations and is usually associated with a good prognosis.
- Group 2: hypermutated carcinomas with microsatellite instability (MSI-H/MMRd). This group is associated with an intermediate prognosis.
- Group 3: carcinomas with low-copy-number alterations. These carcinomas are microsatellite stable and lack TP53 or POLE mutations. These carcinomas are classified as carcinomas with a nonspecific molecular profile and usually have an intermediate prognosis.

Group 4: carcinomas with high-copy-number alterations and recurrent TP53 mutations with a strong p53 expression. These carcinomas are classified as serous-like carcinomas and have a poor prognosis (see Chap. 36).

11.5.6 p53

p53 is a tumor suppressor protein that binds to DNA, inducing the synthesis of the p21 protein. Mutations within the TP53 gene cause the abnormal expression or the absence of the p53 protein, resulting in an uncontrolled proliferation of the involved cells. The p53 expression pattern is an important criterion for the classification of endometrial and ovarian carcinomas and the detection of premalignant tubal lesions (serous tubal intraepithelial carcinoma, STIC; see Fig. 11.2). p53 is listed in detail in Chaps. 33, 36.

11.5.7 Interferon-Inducible Transmembrane Protein-1

Interferon-inducible transmembrane protein-1 (IFITM-1, clustered as CD225) is a member of

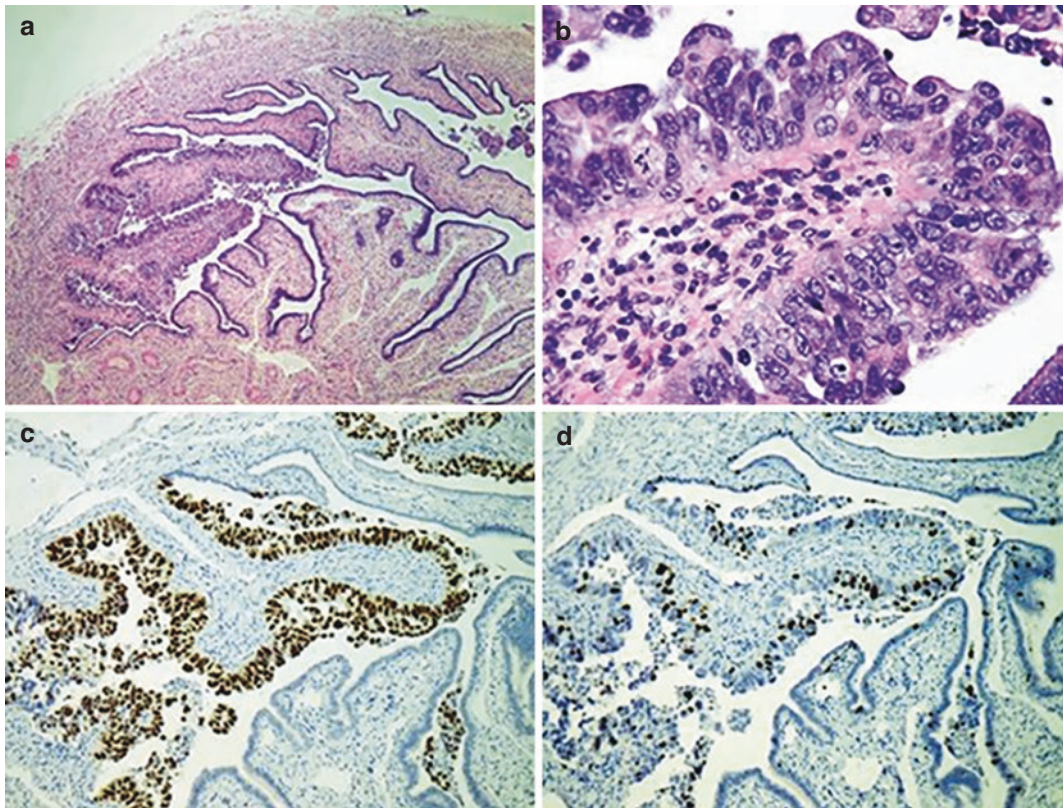


Fig. 11.2 Serous tubal intraepithelial carcinoma (STIC). (**a**, **b**) H&E 40X and 200X showing fallopian tube with marked atypia of tubal epithelium, (**c**) same section with strong diffuse nuclear p53 accumulation, and (**d**) Ki-67 expression in ~15% of epithelial cells

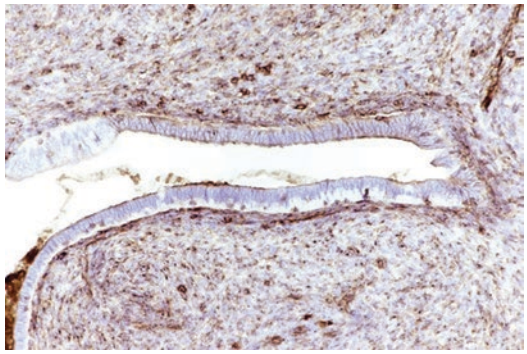


Fig. 11.3 Low-grade endometrial stromal sarcomas with IFITM-1 expression in tumor cells

the IFITM family functioning as a surface receptor regulating the CD19 phosphorylation. IFITM-1 is also described as a novel marker for endometrial stromal differentiation and is considered a specific marker for endometrial stromal cells. IFITM-1 is a sensitive marker for endometrial stromal nodules and low-grade endometrial stromal sarcomas but is negative in uterine smooth muscle tumors (Fig. 11.3) [16, 17].

11.5.8 GATA-3

GATA-3 is a transcription factor listed in detail in other chapters (see Chap. 10 and Sect. 12.2). In uterine, ovarian, and testicular germ cell tumors, GATA-3 is also used as a pan-trophoblastic marker that labels cytotrophoblasts, intermediate trophoblasts, and syncytiotrophoblasts.

11.5.9 Human Placental Lactogen

Human placental lactogen (hPL), also known as human chorionic somatomammotropin, is a placental hormone involved in the regulation of maternal and fetal metabolism and expressed by the placental syncytiotrophoblasts. In routine immunohistochemistry, hPL is a marker for intermediate trophoblasts, syncytiotrophoblastic cells of choriocarcinoma, placental site trophoblastic tumors, and exaggerated placental sites and usually negative in placental site nodules and epithelioid trophoblastic tumors.

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 disease of the vulva:	CK7, EMA (MUC1), TRPS-1, CEA, androgen receptors	ER, GCFP-15		CK5/6/14, CK20
Squamous cell carcinoma, HPV-associated:	**CK5/6**, CK19, p63, **p16**			CK7, CK20
Squamous cell carcinoma HPV-independent:	CK5/6, CK18, CK19, p63	p53		CK7, CK20, **p16**
Mucinous carcinoma, gastric type:	CK7, CDX-2, PDX-1, CEA		PAX-8, CK20, p53	p16, ER
Mucinous carcinoma, intestinal type:	CK20, CDX-2, PDX-1	SATB-2	CK7	ER
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, NKX3.1, PSA			PAX-8

Clear cell carcinoma:	CK7, EMA, CEA	HNF1-β, Napsin A		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
Fibroepithelial stromal polyp:	Desmin, ER, PgR		Actin, Myogenin	
Cellular angiofibroma:		CD34, ER, PgR		Actin, Desmin
Prepubertal fibroma:	CD34		ER, PgR	Actin, S100, Desmin
Superficial myofibroblastoma:	CD34, Desmin, ER, PgR			S100
Superficial angiomyxoma:	CD34			Actin, Desmin, S100
Deep aggressive angiomyxoma:	Desmin, actin, HMGA2	CD34, ER, PgR,	FXIIIa	Myogenin, MyoD1, Smoothelin, S100
Epithelioid sarcoma:	See miscellaneous soft tissue tumors			
Rhabdomyosarcoma:	See soft tissue rhabdomyosarcoma			
B. Tumors of the uterine cervix				
Squamous cell carcinoma, HPV-associated:	**CK5/6**, CK19, p63, p40, **p16**	CK14		CK7, CK20, ER, PgR
Squamous cell carcinoma HPV-independent:	**CK5/6**, CK19, p63, p40	p53		CK7, CK20, ER, PgR, **p16**
Mesonephric remnants/ hyperplasia/carcinoma	CD10, **GATA-3**	PAX-8, TTF-1		ER, PgR, p16
Endocervical adenocarcinoma, HPV-associated: – Usual type – Mucinous type (mucinous NOS, intestinal adenocarcinoma, signet ring cell adenocarcinoma, and stratified mucin-producing carcinoma)	**CK7**, CK8/18, CK19, **CEA**, EMA, **p16**	**PAX-8**	CK20, PDX-1[n], CDX-2[n], Vimentin	**ER**, PgR, CK5/6, p63, p40, WT-1, PAX-2[l], GFAP
Endocervical adenocarcinoma, HPV-independent, gastric type:	CK7, PAX-8	PDX-1	CK20, CDX-2, HNF1-β	PAX-2, ER, PgR, p16
Endocervical adenocarcinoma, HPV-independent, clear cell type:	CK 7, EMA, CA125, PAX-8, **HNF1-β**, AMACR	Napsin A, CD15, p16, Vimentin	AFP, CEA, p53, sox-2	ER, PgR, WT-1, GATA-3, mammaglobin, CK20, CD10
Endocervical adenocarcinoma, HPV-independent, mesonephric type:	CK5/6, CK7, CK8/18, GATA-3, PAX-2, PAX-8, EMA, CD10, CD15	CD10, p16, PAX-8, bcl-2, Vimentin	Androgen receptors, TTF-1, Calretinin, inhibin	PgR, ER, CK20, Napsin A, CEA, p53
Endometrioid adenocarcinoma of endocervix:	**CK7**, CK8, CK18, CK19, EMA, PAX-8, Vimentin,	**ER**, PgR, GFAP	p16, CD56, PAX-2	CK20, CK5/6, **CEA**, CDX-2
Serous carcinoma:	**CK7**, CK8, CK18, CK19, EMA, CA125, **p16**, **p53**, **PAX-8**, Vimentin, β catenin	**IMP3**	ER, PgR, sox-2, WT-1, Napsin A,	CK5/6, CK20, mammaglobin, HNF1-β
Adenosquamous carcinoma (glassy cell carcinoma):	CK7[c], PAX-8[c], CK5/6/14[b], p16			ER, PgR

Adenoid basal carcinoma:	CK5/14, CK7, p63, p40	p16		CD117
Neuroendocrine tumors: – NET G1[c] – NET[d] G2 – NET G3 and NEC (small cell carcinoma)[e]	Pan-CK, **CD56**, Insm-1, NSE, PGP9.5 Proliferation index (Ki-67) in: NET G1: < 2% NET G2: 3–20% NET G3: > 20% NEC G3: > 20%	**Synaptophysin**, **chromogranin**, SSTR2	TTF-1	CK7, CK20
C. Epithelial tumors of the uterine corpus				
Endometrioid adenocarcinoma:	**CK7**, CK8/18, CK19, **PAX-8**, **sox-17**, EMA, **Vimentin**, CA125	**ER**, PgR, GFAP	Mammaglobin, p16, CD56, p53	CK5/6, CK20, CEA, WT-1, **IMP3**, CDX-2[f]
Serous endometrial carcinoma:	**CK7**, CK8/18, CK19, EMA, CA125, **p16**, **p53**, **PAX-8**, **sox-17**, **IMP3**, Vimentin, β catenin Proliferation index (Ki-67): >75%		ER, PgR, sox-2, WT-1	CK5/6, CK20, mammaglobin, HNF1-β, PTEN
Clear cell carcinoma:	CK 7, EMA, CA125, **PAX-8**, **HNF1-β**, **sox-17**, **Napsin A**	p504s (AMACR), CD15, p16, Vimentin	ER, AFP, CEA, sox-2	PgR, WT-1, mammaglobin, CK20, CD10, p53 (wild type)
Undifferentiated/ dedifferentiated carcinoma:	EMA, Vimentin	Pan-CK, CK8/18, p53	PAX-8, Synaptophysin, chromogranin	ER, PgR, E cadherin
Mesonephric adenocarcinoma/ mesonephric-like adenocarcinoma:	Pan-CK, CK7, EMA, PAX-8, GATA-3	TTF-1, CD10, Calretinin	ER, Napsin A	PgR, p53, PAX-2, HNF1-β, WT-1
D. Mesenchymal tumors of the uterine corpus				
Low-grade endometrial stromal sarcoma:	**CD10**[m], **CD225** (IFITM1), β-catenin, Vimentin Proliferation index (Ki-67): ~5%	ERα, PgR, bcl-2, WT-1, TLE-1	Cyclin D1, androgen receptors, actin, Desmin, CD34, pan-CK	**h- Caldesmon**, **Calponin**, EMA, inhibin, oxytocin receptor
High-grade endometrial stromal sarcoma: (YWHAE-NUTM2A/B associated)	**Cyclin D1**, BCOR	CD56, CD117	CD117	ER, PgR, CD10, DOG1
High-grade endometrial stromal sarcoma: (ZC3H7B-BCOR associated)	**Cyclin D1**, CD10	BCOR	ER, PgR, actin, pan-TRK	Desmin, DOG-1

Uterine leiomyoma/ leiomyosarcoma:	Desmin, **actin**, **Smoothelin**, **Calponin**, **h-Caldesmon**, oxytocin receptor, p16[h], p53[g], Vimentin Proliferation index (Ki-67) in: – Uterine leiomyoma: <5% – Atypical uterine smooth muscle tumors: 5–10% – Uterine leiomyosarcoma: >15%	**PgR**, WT-1, CD56	Pan-CK, ER, CD10	EMA
Undifferentiated uterine sarcoma:		p16, p53	CD10, ER, PgR	
Perivascular epithelioid tumor of the uterus:	**HMB45**, **Melan A**, Tyrosinase, MITF[h], CD63 (NK1-C3)		Actin, Desmin, CD117	CD10, CD34, DOG-1, pan-CK, S100
E. Gestational trophoblastic disease				
Exaggerated placental site:	Human leukocyte antigen G (HLA-G), human placental lactogen (hPL), GATA-3 Proliferation index (Ki-67): < 10%			P63
Placental site nodule and plaque/atypical placental site nodule:	Pan-CK, HLA-G, **PLAP**, **GATA-3**, inhibin, **p63** CD10 Proliferation index (Ki-67): 5–15%[i]	p16		hPL
Epithelioid trophoblastic tumor:	Pan-CK, **PLAP**, **GATA-3**, **p63**, inhibin Proliferation index (Ki-67): >10%			hPL, HLA-G, p16
Placental site trophoblastic tumor:	Pan-CK, HLA-G, hPL, CD146, MUC-4 Proliferation index (Ki-67): >10–30%	Inhibin	βhCG	p63
Gestational choriocarcinoma:	See choriocarcinoma of the ovary (Sect. 16.2)			
F. Tumors of the fallopian tube				
Serous tubal intraepithelial carcinoma (STIC)[j]:	**p53**, p16, Stathmin-1[k] Ki-67 > 15%			
Serous carcinoma:	CK7, CK8, CK18, CK19, EMA, WT-1, **p53**, p16	ER, PgR		CK5/6, CK20

Endometrioid adenocarcinoma:	**CK7**, CK8/18, CK19, **PAX-8**, EMA, **ER**	PgR, GFAP, Vimentin	CD56	WT-1, p53, p16, CK20, CK5/6, CEA, CDX-2
Undifferentiated carcinoma:	EMA, Vimentin	Pan-cytokeratin, CK8/18	Synaptophysin, chromogranin	ER, PgR
Adenomatoid tumor:	Pan-CK, CK5/6, CK7, **WT-1**,[o] **Calretinin**, **BAP1**, Mesothelin, Podoplanin (D2–40)[p]	Thrombomodulin (CD141)		EPCAM, CK20, p63, CEA, CDX-2, TTF-1, PAX-8, Smoothelin
G. Tumors of uterine ligaments				
Wolffian tumor:	Pan-CK, CK7, androgen receptors, Vimentin	Calretinin, CD10, Melan A	Inhibin	EMA, GATA-3, CK5/6, CK20, PAX-8, SF-1
Epithelial tumors of Müllerian type:	See uterine equivalents			
Leiomyoma/leiomyosarcoma:	See uterine equivalents			

[a] CK7 is positive in glandular components
[b] CK5/6/14 is positive in squamous components
[c] Well-differentiated neuroendocrine tumor (carcinoid)
[d] Well-differentiated neuroendocrine carcinoma (atypical carcinoid)
[e] Poorly differentiated neuroendocrine carcinoma
[f] CDX-2 may be positive in mucinous-type endometrioid adenocarcinoma
[g] p16 and p53 are markers for leiomyosarcoma, negative in benign tumors
[h] Microphthalmia transcription factor
[i] Proliferation index (Ki-67) in placental site nodule and exaggerated placental site <5%; >5% in atypical placental site nodule; 10-30% in epithelioid trophoblastic tumor and placental site trophoblastic tumor and > 70% in choriocarcinoma
[j] See Fig. 11.2
[k] Diffuse expression in STIC lesions, but only a few scattered cells in normal fallopian mucosa are present [2]
[l] PAX-2 is usually expressed in benign proliferating endocervical glands
[m] See Fig 11.4
[n] CDX-2 and PDX-1 positive in intestinal- and signet ring cell adenocarcinoma
[o] See Fig 11.5
[p] See Fig 11.6

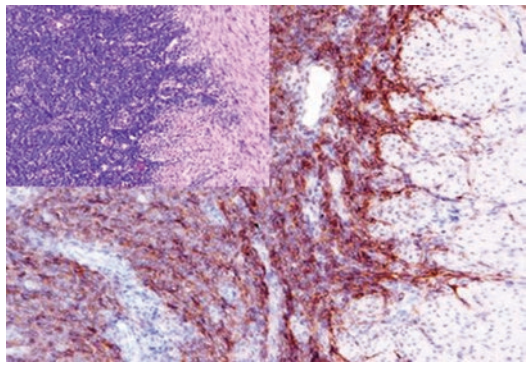


Fig. 11.4 Low-grade endometrial stromal sarcoma. Tumor cells with strong CD10 expression

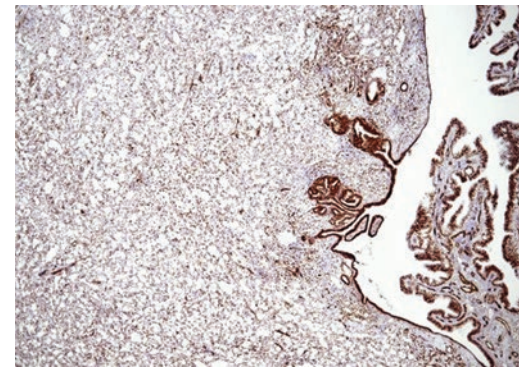


Fig. 11.5 Adenomatoid tumor of the fallopian tube with nuclear WT-1 expression in the tumor cells and the epithelial cells of the tubal mucosa

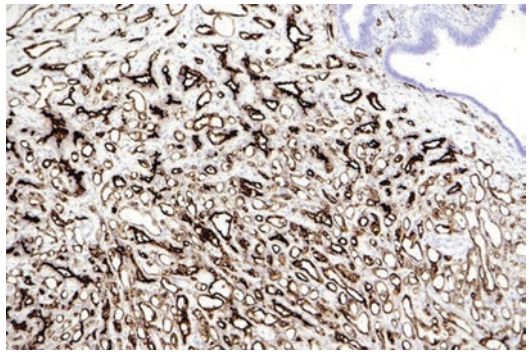


Fig. 11.6 Adenomatoid tumor of the fallopian tube with D2–40 expression in the tumor cells, whereas epithelial cells of tubal mucosa negative for D2 40

11.6 Tumors of the Ovary

11.6.1 Diagnostic Antibody Panel for Ovarian Epithelial Tumors

Cytokeratin profile, CEA, CA125, PAX-8, WT-1, Sox-17, p53, p16, GATA-3, S100P, steroid hormone receptors, and HNF-1β.

11.6.2 Diagnostic Antibody Panel for Ovarian Germ Cell Tumors

Oct-4, SALL-4, CD117, PLAP, GATA-3, Sox-2, Sox-17, AFP, CD30, βhcG, and cytokeratin profile (see also markers of testicular germ cell tumors, Sect. 13.2).

11.6.3 Diagnostic Antibody Panel for Ovarian Sex Cord-Stromal Tumors

Inhibin, anti-Müllerian hormone, Adrenal 4 binding protein (SF-1), FOXL-2, Melan A, CD56, and CD99 (see testicular sex cord-stromal tumors).

11.7 Therapy-Related Markers

Steroid hormone receptors (ER, PgR); Mismatch repair proteins (MLH1, PMS2, MSH2, MSH3, MSH6); PD-L1; p53; HER-2; folate receptor alfa (FRα); L1CAM (CD171); and Ki-67.

11.7.1 Wilms Tumor Protein-1

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 – Mucinous breast carcinoma	Acute myeloid leukemia, Burkitt lymphoma and a 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, liposarcoma, angiosarcoma, osteosarcoma	Renal tissue (glomerular podocytes), myoepithelial cells, mesothelial cells, granulosa cells, Sertoli cells, mucosa of the 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 Wilms tumor gene 1 on chromosome 11p13 with 4 isoforms. WT-1 plays an important role in the regulation of growth factors and the development of tissues from the inner layer of the 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. 11.7). Second, a cytoplasmic staining pattern found in endothelium and vascular tumors in addition to some carcinoma types such as pulmonary adenocarcinoma [18]. The cytoplasmic expression pattern appears to result from 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 weak focal positivity. WT-1 immunohistochemistry helps differentiate between WT-1 positive tumors and many other WT-1 negative tumors with similar morphology, such as neuroblastoma and the PNET tumor group.

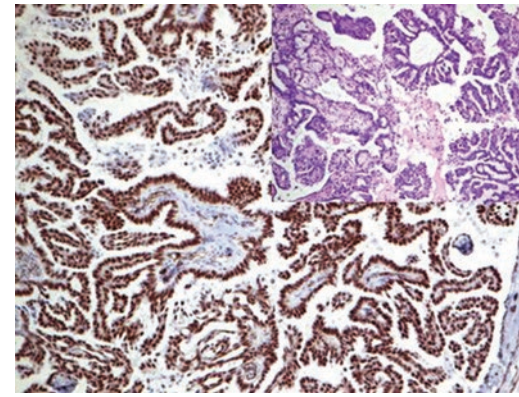


Fig. 11.7 Serous ovarian carcinoma with strong nuclear WT-1 expression

WT-1 is also a helpful marker to discriminate between malignant melanoma cells (WT-1 +) and benign nevus cells (WT-1 -) and between neoplastic endothelial cells (hemangioma) (WT-1 +) and reactive endothelial cells or vascular malformation (WT-1 -) (see Chaps. 19, 22 and 25).

Diagnostic Pitfalls WT-1 labels a high percentage of epithelioid mesotheliomas, which are to be considered in the differential diagnosis between ovarian peritoneal carcinosis and primary peritoneal mesotheliomas. Other antibodies such as PAX-8, Ber-Ep4, and Calretinin are helpful for differential diagnosis.

11.7.2 Carbohydrate Antigen 125

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) – Endometrium carcinoma – Adenocarcinoma of the uterine cervix – Pancreatic adenocarcinoma	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 lung, gastrointestinal tract, biliary system, the pancreas, female genital tract and apocrine glands, mesothelial cells
Positive control: Serous ovarian carcinoma		

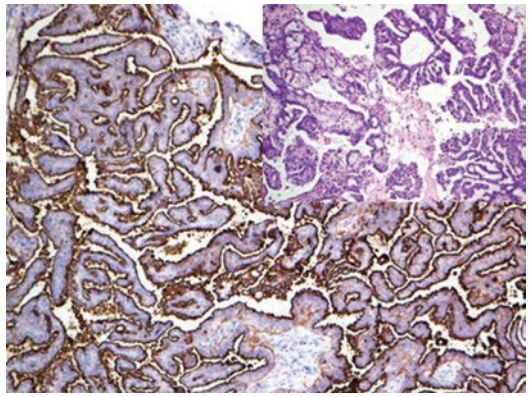


Fig. 11.8 Serous ovarian carcinoma with strong membranous CA125 expression

Diagnostic Approach Carbohydrate antigen 125 (CA125) is a high molecular weight glycoprotein classified as mucin 16 (MUC-16). CA125 is normally expressed by the glandular epithelium of different organs and is highly expressed in ovarian serous and clear cell carcinomas (Fig. 11.8). Serum CA125 is also used to monitor the progression of ovarian carcinoma.

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

11.7.3 Hepatocyte Nuclear Factor-1 β

HNF-1 β is a member of the hepatocyte nuclear factor family listed in detail in the previous chapter. HNF-1 β is used to differentiate between different types of ovarian and endometrial carcinomas.

11.7.4 PAX-8

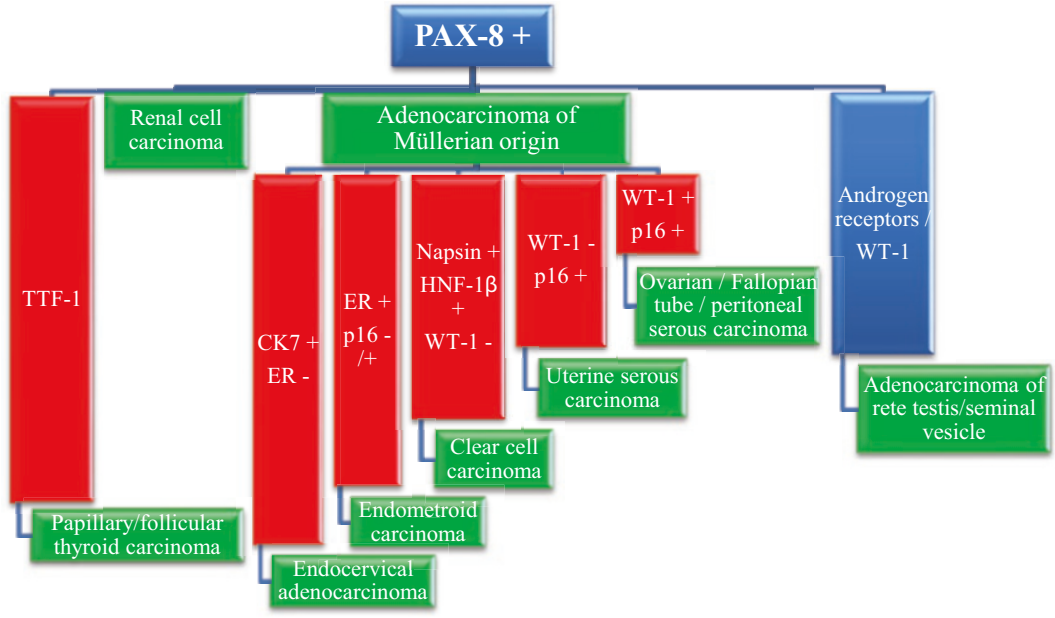
PAX-8 is a transcriptional factor and a member of the paired box (PAX) family listed in detail with the markers of renal cell tumors (Sect. 12.1). PAX-8 is highly expressed in Müllerian glandular epithelia, renal tubules, and the upper urinary system in addition to thyroid follicular cells. PAX-8 strongly labels all endocervical, uterine, and ovarian tumors of Müllerian origin, including serous, clear cell, and endometrioid carcinomas (see Algorithm 11.1). Mucinous ovarian carcinomas express PAX-8 in about 40% of the cases.

11.7.5 Sox-17

Sox-17		
Expression pattern: Nuclear		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
Endometrial and ovarian – Low and high-grade serous carcinoma – Endometrioid carcinomas – Clear-cell carcinomas	Yolk sac tumor, seminoma, dysgerminoma	Epithelium of the fallopian tube, endometrium and endocervical glands, endothelium
Positive control: Normal endothelium		

Diagnostic Approach Sox-17 (SRY-box transcription factor 17) is a member of the SOX family of transcription factors, involved in the regulation of embryonic development, including differentiation of endoderm, formation of vascular endothelium, and maintenance of fetal and neonatal hematopoietic stem cells. Sox-17 is normally expressed in the epithelium of the fallopian tube, endometrium, endocervical glands, and vascular endothelial cells.

Sox-17 is highly expressed in different ovarian and endometrium carcinomas, including serous, endometrioid, and clear cell carcinomas, in addition to germ cell tumors, including Yolk sac tumor, dysgerminoma, and seminoma but negative in ovarian mucinous carcinoma and sex cord-stromal tumors. Sox-17 is also expressed in endothelial tumors, including angiosarcoma (Figs.11.9 and 11.10) [19].



Algorithm 11.1 PAX-8 positive tumors

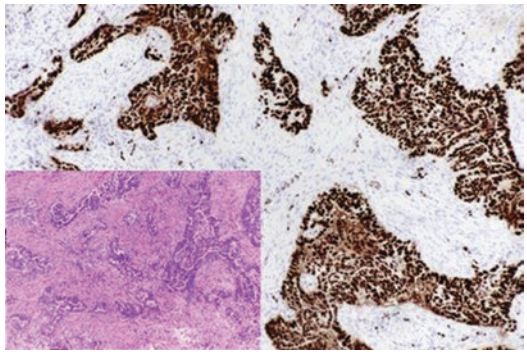


Fig. 11.9 Peritoneal biopsy infiltrated by high-grade serous ovarian carcinoma. Tumor cells exhibit strong nuclear Sox-17 expression; Sox-17 also stains the nuclei of endothelial cells

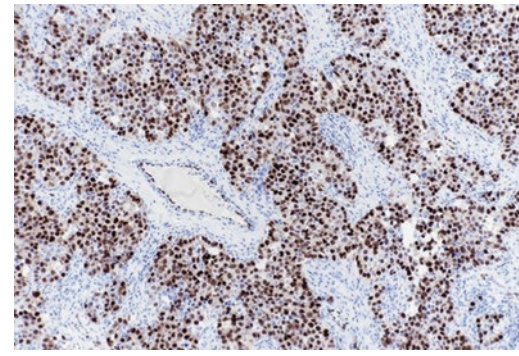


Fig. 11.10 Ovarian dysgerminoma with strong nuclear Sox-17 expression in the tumor cells. Endothelial cells also show strong nuclear Sox-17 expression

Sox-17 is not expressed in normal thyroid tissue and cells of renal tubules. Thyroid, renal, breast, bladder, colorectal, and squamous cell carcinomas are usually negative for Sox-17.

Diagnostic Pitfalls Sox-17 may be weakly positive in a subset of other carcinoma types, such as endocervical adenocarcinoma, hepatocellular carcinoma, and cholangiocarcinoma.

11.7.6 Folate Receptor

Folate receptors (FR) are a receptor family that includes four isoforms, FRα (adult), FRβ (fetal), FRγ, and FRδ, which are cell-surface glycoproteins except for FRγ, found as a secreted protein. The folate receptors are encoded on chromosome 11q13.3–14.1, bind to folic acid (vitamin B9) and its derivatives, and transport them inside the cells

essential for the biosynthesis of purines and thymidine required for DNA synthesis, methylation, and repair. In modern oncology, FRα is the therapeutic target for specific antibodies and drug-conjugated antibodies. FRα has low expression levels in limited normal tissue types with an apical membranous expression pattern. It is normally expressed in the mucosa of fallopian tubes, cells of proximal renal tubules, pneumocytes type I and II, bronchial glands, submandibular salivary glands, choroid plexus, and placental trophoblasts. FRα is overexpressed in different tumor types, including ovarian, endometrial, triple-negative breast, and lung carcinomas, in addition to mesothelioma [20, 21]. For therapeutic purposes, the expression of FRα can be detected in tumor tissue by immunohistochemistry using specific antibodies. Only membranous stained cells are considered for the interpretation of stained tumor slides, and only tumors exhibiting moderate to strong membranous expression in more than 75% of the tumor cell population are considered positive. FRβ is the therapeutic target for some acute myeloid leukemia (AML) types.

11.7.7 FOXL2

FOXL2		
Expression pattern: Nuclear		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Sex cord-stromal tumors	Squamous cell carcinoma of the cervix, breast carcinoma, pituitary gland adenoma (gonadotropin producing and null cell adenoma)	Granulosa cells, a subset of pituitary cells, eyelid
Positive control: Ovarian tissue (granulosa cells)		

Diagnostic Approach **F**orkhead b**ox** transcription factor **L2** (FOXL2) is a transcriptional factor involved in the development of the ovaries as it is essential for the maturation of ovarian follicles, maintenance of ovarian function, and normal development of the female genital tract. FOXL2 is also essential for the endocrine function of the pituitary gland.

Mutations within the FOXL2 gene associated with the strong FOXL2 expression are found in the majority of adult granulosa cell tumors; nevertheless, the expression of FOXL2 is also found in all other sex cord-stromal tumors also lacking the FOXL2 gene mutations. FOXL2 is highly expressed in testicular and ovarian sex cord-stromal tumors, including adult granulosa cell tumors, thecoma/fibroma but less common in Sertoli/Leydig cell tumors and sclerosing stromal tumors. A subset of pituitary gland adenomas is also positive for FOXL2, namely gonadotropins-producing adenomas and the majority of null cell adenomas [22–24]. Ovarian surface epithelial tumors and germ cell tumors are negative for FOXL2.

11.7.8 Adrenal 4 Binding Protein (SF-1)

This marker is listed with the markers of adrenal cortex tumors (Sect. 14.6). SF-1 is one of the best markers for sex cord-stromal tumors as it is expressed in the vast majority of adult granulosa cell tumors, Sertoli and Leydig cell tumors, and steroid cell tumors, in addition to ovarian fibroma and fibrothecoma.

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 tumor – 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%	PAX-2, p63[b], Glut-1[b], Mesothelin	Vimentin, ER, PgR, Calretinin, S100, CK5/6, TTF-1, CD99	Villin, CK20, **CEA**, MUC-2, CDX-2, inhibin
Mucinous ovarian neoplasms (adenoma, borderline tumor and carcinoma):	CK7, CK8, CK18, CK19, EMA	CK20[c], CDX-2[c], MUC-2, MUC5AC, **CEA**, p53[d]	PAX-8, villin	**WT-1**, **p16**, **sox-17**, ER, PgR, CA125, Vimentin, inhibin, TTF-1
Endometrioid carcinoma:	CK7, CK8, CK18, CK19, EMA, **PAX-8**, **sox-17**, ER, CA125	Vimentin, Mesothelin, CD99	p16, PAX-2, CK5	CK20, **WT-1**, CEA, inhibin, TTF-1
Clear cell adenocarcinoma:	**Hepatocyte nuclear factor 1-β (HNF1-β)**, **PAX-8**, **sox-17**, CK7, EMA	**Napsin A**, Vimentin, CD15, CA125	AFP, CEA, p53, PAX-2	**WT-1**, **p16**, ER, PgR, CK20, CD10
Brenner tumor (benign/ malignant):	*Epithelial components*: EMA, **CK7**, p63, CEA, **CK5/6/14**[e], **GATA-3**, CA125, **Uroplakin III** *Fibrous stroma*: Vimentin	**WT-1**, AR, S100P, bcl-2		PAX-8, CK19, CK20, Thrombomodulin (CD141), CDX-2, p16, ER, PgR, Vimentin Pan-CK
Mesonephric-like adenocarcinoma:	CK7, PAX-8, TTF-1, GATA-3		CD10	WT-1, ER, PgR
Undifferentiated/ dedifferentiated carcinoma	Pan-CK		PAX-8	ER, PgR
B. Sex cord-stromal tumors				
Adult granulosa cell tumor:	**FOXL2**, **adrenal 4 binding protein (SF-1)**, **inhibin**, Vimentin	Calretinin, **CD99**, actin, S100, CD56, **WT-1**, Melan- A, ER-β, PgR	Pan-CK, CK8, CK18, ER-γ	CK7, EMA, CEA, anti-Müllerian hormone, PAX-8, β-catenin, Desmin
Juvenile granulosa cell tumor:	**Inhibin**, Calretinin, CD99, WT-1	EMA		β-Catenin
Thecoma/fibroma/ fibrosarcoma:	**Inhibin**, Calretinin, **FOXL2**, **adrenal 4 binding protein (SF-1)**, **WT-1**, CD56, Vimentin	Sm-actin, Calretinin	ER, PgR	Pan-CK, CD10
Sclerosing stromal tumor:	Sm-actin, PgR, **FOXL2**, Vimentin	**Inhibin**, Calretinin, Desmin	ER, TFE-3	Pan-CK, EMA
Leydig cell tumor:	**Inhibin, adrenal 4 binding protein (SF-1)**, Melan-A, Calretinin, Vimentin	CD99, CD56	Pan-CK, S100, actin, Desmin, Synaptophysin, chromogranin, EMA	PLAP, **β-catenin**, AFP, CEA, Oct-4, SALL-4
Sertoli cell tumor:	**Inhibin, adrenal 4 binding protein (SF-1)**, **β-catenin**, **anti-Müllerian hormone**, WT-1, Melan A, Vimentin	AFP, **FOXL2**, CD56, CD99, pan-CK, Calretinin, NSE, S100	Synaptophysin, chromogranin	EMA, CK7, PLAP, PAX-8, GATA-3, Oct-4, SALL-4, CEA

Microcystic stromal tumor:	WT-1, β-catenin[f], CD10, FOXL2, SF-1, cyclin D1		AR	Inhibin, Calretinin, ER, PgR, EMA
Signet-ring stromal tumor:	SF-1, Calretinin, β-catenin, CyclinD1, actin		Pan-CK	Inhibin, EMA
Steroid cell tumor:	Inhibin, Melan A, Calretinin			FOXL2
Sex cord tumor with annular tubules:	**Inhibin**, **adrenal 4 binding protein (SF-1)**, WT-1, Calretinin	CD56	Pan-CK	EMA
C. Germ cell tumors				
Dysgerminoma:	**SALL4**, **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	GATA-3, PLAP, CDX-2	HepPar1	**EMA**, CD30, ßhcG, Oct-4, **sox-2**, CK7, Vimentin
Choriocarcinoma:	*Syncytiotrophoblastic cells*: **ßhcG**, inhibin, **GATA-3**, CD10, SALL-4, pan-CK, CK8/18, CK19, p63, EGFR	**PLAP**, human placental lactogen, EMA, CEA	Vimentin	CD30, AFP, Oct-4
	Cytotrophoblastic cells: CD10, pan-CK, CK8/18, CK19, CEA	PLAP		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. Mesenchymal tumors				
Endometrioid stromal sarcoma:	CD10, ER, PgR	WT-1		
Ovarian myxoma:	Vimentin	Actin		Pan-CK, Desmin, S100
E. Miscellaneous tumors				
Female adnexal tumor of probable Wolffian origin (ovarian Wolffian tumor):	Pan-CK, CK7, **androgen receptors**, Vimentin	Calretinin, CD10, Melan A	Inhibin	EMA, GATA-3, CK5/6, CK20, PAX-8, SF-1
Solid pseudopapillary tumor:	CD10, CD56, CD99, WT-1, β-catenin	PgR,	CD117	Inhibin, Calretinin
Small cell carcinoma, hypercalcemic type:	EMA, **WT-1**, p16, p53	Calretinin, CD56, SALL-4	Synaptophysin, chromogranin	CD10, inhibin, TTF-1
Small cell carcinoma, pulmonary type:	NSE, **CD56**	Pan-CK, TTF-1	Synaptophysin, chromogranin	

[a] High expression levels of p16 and p53 are characteristic for high-grade serous carcinoma and low expression levels or negativity characteristic for low-grade carcinoma. p53 is constantly negative in the case of nonsense-type mutations
[b] Stain intensity correlates with the grade of malignancy [25]
[c] CDX-2 and CK20 are positive in mucinous adenocarcinoma and intestinal-type adenoma
[d] Usually negative in adenoma and borderline tumors
[e] CK5/6/14 positive in basal epithelial cells
[f] Nuclear and cytoplasmic

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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.

12.1.1 Markers for Renal Cell Tumors

PAX-8, PAX-2, RCC, GATA-3, CD10, CD117, AMACR, Napsin, human kidney injury molecule-1 (KIM-1), Cadherin 16 (Ksp-cadherin), Carbonic anhydrase IX (CAIX), TFE-3, Succinate dehydrogenase, Fumarate hydratase, FOXI-1, SMARBCB-1 (INI-1), DOG-1, WT-1, cytokeratin profile, and Vimentin [1, 2].

12.1.2 Markers for Tumors of the Renal Pelvis

Cytokeratin profile, p40, p63, GATA-3, PAX-8, and Thrombomodulin (CD141).

M. S. A. Tuffaha et al., *Immunohistochemistry in Tumor Diagnostics*,
https://doi.org/10.1007/978-3-031-45024-2_12

12.1.3 Therapy-Related Markers

PD-L1.

12.1.3.1 PAX-8

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, anaplastic, and squamous) – Ovarian carcinoma (serous, clear cell, and endometrioid carcinoma) – Adenocarcinoma of seminal vesicle and rete testis – Well-differentiated papillary mesothelial tumor	Pancreatic neuroendocrine tumors and other NET of the gastrointestinal tract, parathyroid adenoma and carcinoma, endocervical adenocarcinoma, mesonephric and mesonephric-like adenocarcinoma, thymoma (type A and B and thymic carcinoma), seminoma, yolk sac tumor, Merkel cell carcinoma, B-cell lymphomas, medulloblastoma	Thyroid follicular cells, parathyroid cells, thymus cells, renal tubules, endocervical and endometrial epithelial cells, non-ciliated epithelium of fallopian tubes, rete testis, epididymis and seminal vesicles, and a subset of B-lymphocytes
Positive control: thyroid tissue		

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, non-ciliated cells of fallopian tubes mucosa, and renal tubules; consequently, tumors developed from these tissue types are generally positive for PAX-8.

Renal tumors including clear cell, papillary and chromophobe renal cell carcinomas, in addition to nephroblastoma and the majority of collecting duct carcinoma, oncocytomas and about 50% of sarcomatoid renal cell carcinoma are positive for PAX-8 (Figs. 12.1 and 12.2). PAX-8 is also a diagnostic marker for tumors of Müllerian origin including serous, endometrioid, and clear cell ovarian carcinomas, while the majority of mucinous carcinomas of the female reproductive system is usually negative. Follicular and papillary thyroid carcinomas show high

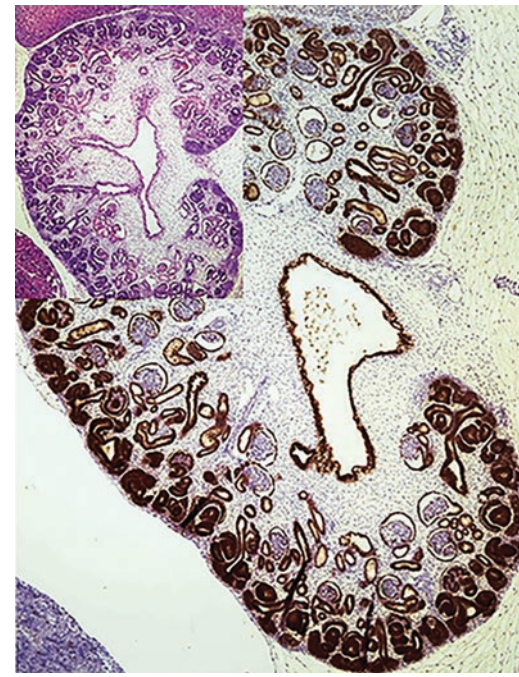

Fig. 12.1 Kidney of a 12-week-old embryo; PAX-8 highlighting the renal collecting system and the urothelium of the renal pelvis

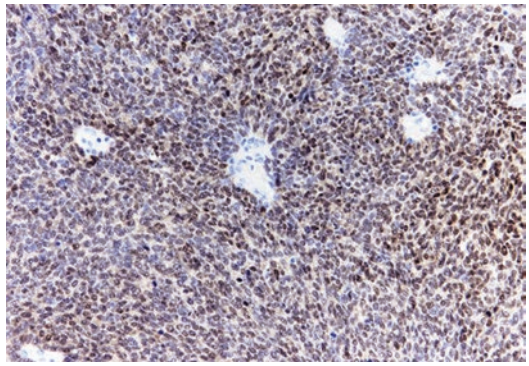

Fig. 12.2 Nephroblastoma, cells in the blastema part with strong nuclear PAX-8 expression

expression levels of PAX-8 but not medullary thyroid carcinoma.

The expression of PAX-8 is also reported in different percentages of well-differentiated neuroendocrine tumors of pancreatic, gastroduodenal, appendicular, and rectal origin [4].

Diagnostic Pitfalls As mentioned, PAX-8 is expressed in a wide range of tumors and must be used as a part of a diagnostic panel. A diagnostic panel composed of PAX-8, WT-1 and 2 different 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 helps 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 also be considered in the interpretation of the PAX-8 stain, which is also a good positive internal control.

12.1.3.2 PAX-2

PAX-2 is a further member of the paired box family of transcription factors analogous to PAX-8, also involved in renal development, and appears slightly later than PAX-8. PAX-2 has a broad 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 carcinoma. 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 is constantly negative in thyroid tissue and thyroid carcinomas. Similar to PAX-8, PAX-2 is also positive in B-lymphocytes and related lymphoma types.

12.1.3.3 Renal Cell Carcinoma Marker

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, namely clear cell, chromophobe, and papillary renal cell carcinomas, 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 is to be considered in the differential diagnosis.

12.1.3.4 CD10

CD10 is listed in detail with the lymphoma markers (Sect. 16.1). CD10 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 pattern but negative in chromophobe renal cell carcinoma, which is usually positive for CD117 (Fig. 12.3) [7, 8].

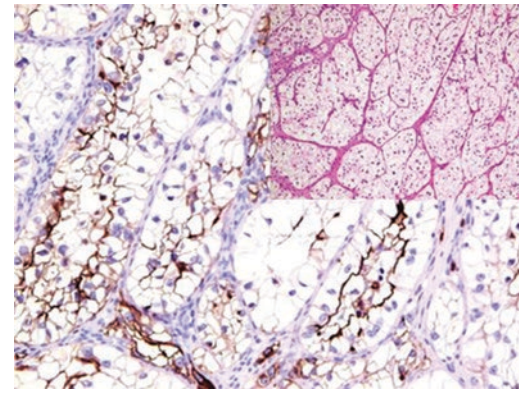

Fig. 12.3 Clear cell renal cell carcinoma stained with CD10; CD10 expression accentuated on the apical side of the cell membrane

Diagnostic Pitfalls CD10 is also expressed in tumors with similar morphology, such as tumors of the adrenal cortex and hepatocellular carcinoma; the latter lacks the expression of PAX-8 that can be used to discriminate between both tumors.

12.1.3.5 Paxillin

Paxillin is a cytoskeletal protein involved in the formation of focal adhesion complexes between F-actin and integrin and is 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.

12.1.3.6 Carbonic Anhydrase

Carbonic anhydrase IX (CA IX)		
Expression pattern: membranous/membranous basolateral/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 a member of the carbonic anhydrases family, zinc metalloenzymes catalyzing the hydration of carbon dioxide. CA IX is a transmembrane isoenzyme taking part in 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 normal renal tissue lacks the expression of CA IX. The expression of CA IX is activated during malignant transformation, and CA IX is markedly expressed in clear cell renal cell carcinoma, whereas the intensity of the expression correlates with the differentiation grade of the tumor. Less and various CA IX stain intensity is also characteristic for papillary renal cell carcinoma, clear cell papillary renal cell carcinoma, and Xp11.2 translocation renal cell carcinoma. Chromophobe cell carcinoma and renal oncocytoma usually lack the expression of CA IX.

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 positive for this marker (Fig. 12.4) [10].

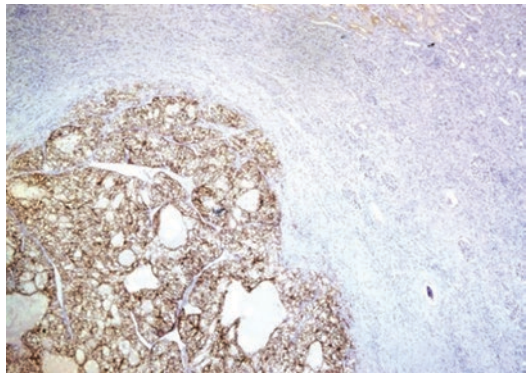

Fig. 12.4 CA IX expression in clear cell carcinoma. The expression is restricted to tumor areas, and normal renal tissue is negative

Diagnostic Pitfalls In the interpretation of the CA IX stain, only the expression in well-preserved viable tumor areas can be considered positive, as the focal expression may also be found in perinecrotic ischemic portions of other renal tumors. CA IX is not a specific marker for renal cell tumors, and different expression levels are found in various tumors of different origins, including pulmonary carcinoma, esophageal carcinoma, renal transitional/urothelial cell carcinoma, breast carcinoma, neuroendocrine tumors, cervical squamous cell carcinoma and high-grade intraepithelial neoplasia, endometrial carcinoma, embryonal carcinoma, mesothelioma, Sertoli cell tumor, and adrenocortical carcinoma [11].

12.1.3.7 Human Kidney Injury Molecule-1

KIM-1 (also known as hepatitis A virus cellular receptor 1) is a type 1 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 extra-renal tumors, KIM-1 is positive in ovarian and uterine clear cell carcinomas, hepatocellular carcinoma, and a subset of colorectal carcinoma in addition to germ cell tumors, which may have a similar morphology to clear cell renal cell carcinoma [13].

Similar to KIM-1, hypoxia-induced factor 1α (HIF-1α) is another hypoxia-induced molecule expressed in different types of renal cell carcinoma.

12.1.3.8 Transcription Factor-E3

TFE-3 is a transcription factor for the Ig heavy chain enhancer region 3 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 the TFE-3 transcriptional factor, which is considered a specific immunohistochemical marker for the Xp11.2 translocation-associated renal cell carcinoma [14]. The expression of TFE-3 is also characteristic for alveolar soft part sarcoma due to another equivalent (X;17)(p11.2;q25) translocation.

The TFE-3 expression is found in other tumors, including granular cell tumor and a subset of angiomyolipoma, clear cell sarcoma, and melanoma [15, 16].

12.1.3.9 Succinate Dehydrogenase

Succinate dehydrogenase (SDHG) is an enzyme complex located in the inner mitochondrial membrane involved in the Krebs cycle, catalyzing the conversion of succinate to fumarate. SDHG is composed of four subunits A, B, C, and D. Mutations within the SDHG gene causing the loss or inactivation of SDHG or its subunits lead to the accumulation of succinate, and the decrease of fumarate levels is found in different tumor types, such as SDHG-deficient renal cell carcinoma and a subset of gastrointestinal stromal tumors in addition to pituitary adenoma. The SDHG deficiency is also associated with ~15% of pheochromocytomas and paragangliomas. SDHG can be detected in formalin-fixed tissue by immunohistochemistry (mainly SDHA and SDHB) and shows a granular cytoplasmic expression pattern (Fig. 12.5). The negative immunohistochemical reaction for SDHG in tumor tissue is a surrogate marker for inactivating mutations of the SDHG gene and can be used as a marker for SDHG-deficient tumors [17, 18].

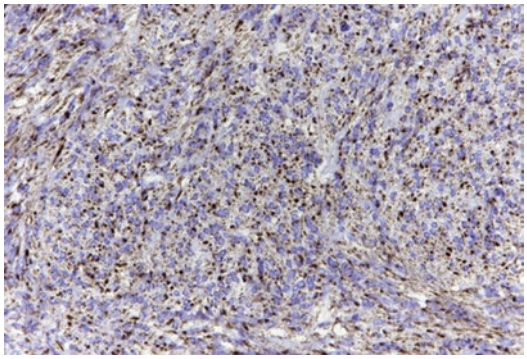

Fig. 12.5 Granular cytoplasmic SDHG expression in the gastrointestinal stromal tumor

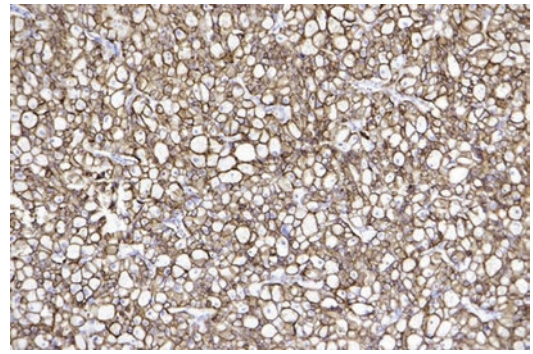

Fig. 12.6 Chromophobe renal cell carcinoma exhibiting strong membranous Cadherin-16 expression

12.1.3.10 Fumarate Hydratase

Fumarate hydratase (Fumarase, FH) is an enzyme that catalyzes the reversible hydration/dehydration of fumaric acid to I-malic acid and immediately follows the succinate dehydrogenase in the Krebs cycle [19]. Alterations of the fumarate hydratase gene causing the inactivation of this gene leads to the elevation of intracytoplasmic levels of fumarate. These genetic alterations are found in association with a certain type of high-grade renal cell carcinoma with characteristic morphological features and poor prognosis. Furthermore, it is also found in association with hereditary leiomyomatosis with cutaneous and uterine leiomyomas—mainly atypical leiomyomas with bizarre nuclei—and can appear as the manifestation of the rare autosomal dominant hereditary leiomyomatosis and renal cancer syndrome (HLRCC) [20, 21]. Fumarate hydratase-deficient renal cell carcinomas can occur both in the germline and acquired somatic setting. The expression of fumarate hydratase can be detected by immunohistochemistry with cytoplasmic expression pattern. Lack of fumarate hydratase in the context of a matching characteristic morphology is diagnostic for fumarate hydratase-deficient renal cell carcinoma or leiomyoma [22].

12.1.3.11 Alpha-Methylacyl-CoA Racemase

Alpha-methylacyl-CoA racemase (AMACR, p504S) is a member of the isomerases enzyme family listed in detail as a helpful marker for diagnosing prostatic adenocarcinoma (Sect. 13.1). In renal cell carcinoma, AMACR is a marker for papillary renal cell carcinoma and is also expressed in Xp11 translocation renal cell carcinoma in addition to mucinous tubular and spindle cell carcinoma. Clear cell renal cell carcinoma may also show a weak expression in a small subset of tumor cells, whereas clear cell papillary renal cell carcinoma lacks the expression of AMACR.

12.1.3.12 Cadherin-16

Cadherin-16 is a member of the cadherin superfamily encoded on chromosome 16q22.1. Cadherin 16 is exclusively expressed in the kidney and is known as kidney-specific cadherin (Ksp-cadherin). In normal renal tissue, Cadherin-16 is expressed in the basolateral membrane of renal tubular and collecting duct epithelium, whereas glomerular and interstitial cells lack the expression of Cadherin-16. In kidney tumors, Cadherin-16 is positive in chromophobe renal cell carcinoma (Fig. 12.6) and, to a lesser degree, in oncocytoma. If the antibody is titrated carefully, it can be helpful to discriminate chromophobe renal cell carcinoma (strongly positive) from oncocytoma (mostly negative). Clear cell- and papillary renal cell carcinomas usually lack the expression of Cadherin-16 or show only weak focal expression.

12.1.3.13 FOXI-1

FOXI-1 is a member of the forkhead family of transcription factors that plays a role in the tran-

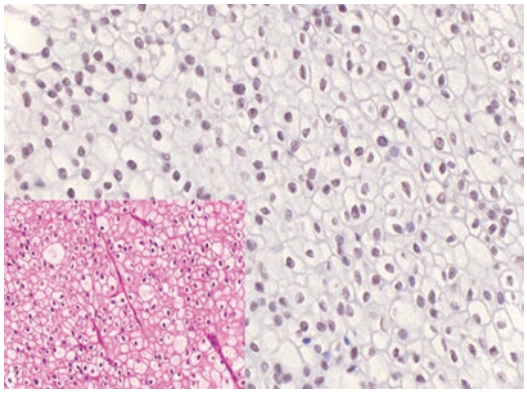

Fig. 12.7 Chromophobe renal cell carcinoma exhibiting nuclear FOXI-1 expression

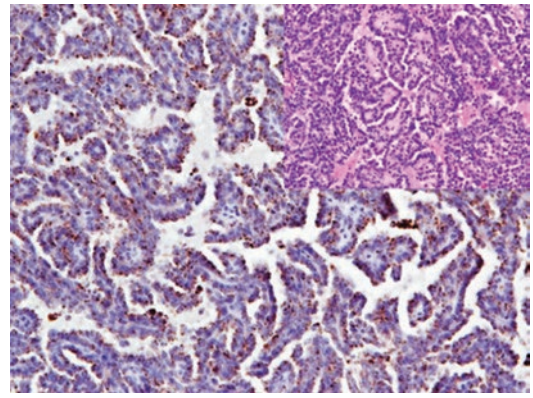

Fig. 12.8 Napsin expression in the cells of papillary renal cell carcinoma

scription of four subunits of the proton pump required for the differentiation of renal intercalated cells in the distal segments of the kidney tubules in addition to the epididymal cells and endolymphatic cells in the inner ear. The mentioned cells show a nuclear FOXI-1 expression. In diagnostic immunohistochemistry, FOXI-1 labels >90% of chromophobe renal cell carcinoma, renal oncocytoma, and oncocytic renal cell tumors (NOS) (Fig. 12.7). FOXI-1 expression is also found in a subset of translocation renal cell carcinomas. All other renal cell carcinoma types were reported to be negative for FOXI-1. The FOXI-1 expression is also lost in sarcomatoid chromophobe renal cell carcinoma [23, 24].

12.1.3.14 Napsin A

Napsin A is a pepsin-like aspartic proteinase listed in detail with the markers of lung tumors (Chap. 3). It is normally expressed in Type 2 pneumocytes, respiratory epithelium, alveolar macrophages, and renal tubular epithelial cells, in addition to plasma cells and a subset of lymphocytes. Besides pulmonary adenocarcinomas, Napsin A is expressed in about 80% of papillary renal cell carcinomas and rarely in clear cell renal cell carcinoma (Fig. 12.8). Chromophobe renal cell carcinoma lacks the expression of Napsin A.

12.1.3.15 SMARCB-1 (INI-1)

SMARCB1, also known as INI-1, is a nuclear protein involved in chromatin remodeling and regulation of the cell cycle listed in detail in Chap. 34. The loss of INI-1 expression occurs due to biallelic mutations or deletions within the encoding gene, which is characteristic for malignant rhabdoid tumor, atypical teratoid/rhabdoid tumor of the brain, and other tumors of different origins.

Immunoprofile of renal tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Oncocytoma	Pan-CK, **E-cadherin**, **CD117**, **FOXI-1**, Claudin-8, S100A, MSH-2[a]	PAX-2, PAX-8, EMA, **CD15**, CK20, DOG-1, cyclin D1, S100A1	CK7[e]	Vimentin, RCC38, PAX-2, CD10, KIM-1, CA IX, Cadherin-16 (Ksp-cadherin)
Papillary adenoma	Pan-CK, CK5/14, PAX-8, EMA	P504S (AMACR)		
Metanephric adenoma	Pan-CK, **PAX-2**, **PAX-8**, **WT-1**, S100, vimentin	CD56, **CD57**, cadherin 17, BRAF-$_{V600E}$		CK7, CK19, EMA, p504S (AMACR)

Multilocular cystic renal neoplasm of low malignant potential	PAX-8, **CA IX**			
Clear cell renal cell carcinoma	Pan-CK, **PAX-2**, **PAX-8**, CA IX, KIM-1, MOC31, α B-Crystallin	Vimentin, **RCC** (gp200), CD10, SATB-2, CK19, EMA	p504S (AMACR), CD9	CK5/14, CK7, CK13, CK19, CK20, p63, E-cadherin, FOXI-1, cadherin 16, CD117, inhibin, MSH-2, GATA-3
Chromophobe renal cell carcinoma	Pan-CK, **CK7**[f], EMA, **PAX-8**, **CD117**, **FOXI-1**, SDHG, fumarate hydratase, Cadherin-16, Paxillin	CK19, CD9, MSH-2[b], DOG-1, E-cadherin, Ki-67 (MIB-1 clone)[b], Parvalbumin	RCC (gp200), PAX-2, CD10[c]	Vimentin, **CD15**, p504S (AMACR), cyclin D1, **CA IX**, **S100A**, KIM-1, Claudin-8, CK13, **CK20**, GATA-3
Low-grade oncocytic tumor (LOT)	Pan-CK, **CK7**, **PAX-8**	FOXI-1	CD10, AMACR	**CD117**, CAIX, vimentin, HMB45
Eosinophilic vacuolated tumor (EVT)	Pan-CK, **PAX-8**, CD10, **CD117**	AMACR	CK7	**CK20**, vimentin
Eosinophilic, solid and cystic renal cell carcinoma	Pan-CK, **CK20**, PAX-8, SDHG, fumarate hydratase			**CD117, CK7**
Papillary (chromophile) renal cell carcinoma	Pan-CK, **PAX-8**, **p504S (AMACR)**[g], SDHG, fumarate hydratase	EMA, **PAX-2**, **Napsin**, **RCC** (pg200), CK19, CD9, CD10, **CK7**, CK19, vimentin	CA IX	CK5/6, CK13, CK20, FOXI-1, E-cadherin, cadherin 16, CD57, CD117, WT-1, GATA-3
Clear cell papillary renal cell tumor	Pan-CK, **CK7**, CA IX (basolateral), HIF-1α, SDHG, fumarate hydratase, Glut-1	**PAX-8**, PAX-2,	GATA-3, CD10	**p504S (AMACR)**, E-cadherin, CD10, FOXI-1
Collecting duct carcinoma (Bellini duct carcinoma)	Pan-CK, CK7, CK19, p63, **PAX-8**, UEA-1, E-cadherin, SDHG, fumarate hydratase, SMRCB-1 (INI-1), lectin	EMA, **PAX-2**, CK7, CD15, HER-2, vimentin	CD117	RCC, CD10, GATA-3, CK5/6, CK13, CK20, OCT-4
Succinate dehydrogenase-deficient renal cell carcinoma	Pan-CK, **PAX-8**, fumarate hydratase, vimentin			**SDHG**, CK7, CD117, OCT-4
Fumarate hydratase-deficient renal cell carcinoma	Pan-CK, **PAX-8**, SDHG, vimentin		GATA-3	**Fumarate hydratase**, CD117, OCT-4, CK7
TFE3-rearranged renal cell carcinoma (Xp11.2 translocation associated RCC)	**TFE-3**, **PAX-8**, **Cathepsin-K**, SDHG, fumarate hydratase, vimentin	Pan-CK, CD10, p504S (AMACR), RCC	CK7, PAX-2, HMB45, Melan A	
TFEB-rearranged renal cell carcinoma (t(6;11) associated RCC)	**TFEB**, **PAX-8**, Melan-A, HMB45, SDHG, fumarate hydratase	Pan-CK, CD117, CD10, **Cathepsin-K**, HMB45, vimentin		CA IX
ELOC (TCEB1)-mutated renal cell carcinoma	CK7, CAIX, CD10			
ALK-rearranged renal cell carcinoma	Pan-CK, PAX-8, **ALK**		CD10, AMACR	CD117

SMARCB-1-deficient renal cell carcinoma (renal medullary carcinoma)	Pan-CK, PAX-8, SDHG, fumarate hydratase, EMA	CK20, CK7, PAX-8, OCT-4, CEA		**SMARCB-1 (INI-1)**
Acquired cystic disease-associated renal cell carcinoma	PAX8, CD10, P504S (AMACR)			CK7, GATA-3, CD117
Tubulocystic renal cell carcinoma	Pan-CK, CK7, CK19, p504S (AMACR)	CD10		
Mucinous tubular and spindle cell carcinoma (loopoma)	Pan-CK, PAX-8, CK7, p504S (AMACR), EMA, vimentin			CD10, RCC
Spindle cell (sarcomatoid) carcinoma	CK8, CK18, vimentin	EMA	CD10, PAX-8	RCC
Neuroendocrine carcinoma	Pan-CK, CD56, S100, chromogranin, Synaptophysin, NSE			CK19
Juxtaglomerular cell tumor	**Renin**, CD31, CD34, actin, CD117	Calponin		Pan-CK, PAX-8, Desmin, Synaptophysin, chromogranin, S100
Nephroblastoma (Wilms tumor)	**WT-1**[h], CD56, vimentin	Myogenin, PAX-2, PAX-8, S100, Pan-CK, NSE		CD57, CK19
Congenital mesoblastic nephroma	**NTRK**[i]	WT-1, actin		PAX-8
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, BCOR**	NGFR, SATB-2, Bcl-2		CK7, Pan-CK, EMA, PAX-8, RCC, CD34, CD56, CD99, WT-1, Desmin, S100
Rhabdoid tumor	Pan-CK, vimentin	CK8, EMA, CD99, NSE	Synaptophysin, SALL-4, actin, Desmin	PAX-2, PAX-8, myoglobin, **INI-1** CD34, S100
Mixed epithelial and stromal tumor	*Epithelial components*: Pan-CK, EMA *Stromal components*: Actin	CEA Desmin, ER, PgR		HMB45, CD34
Metanephric stromal tumor	CD34			PAX-8. Pan-CK, Desmin, S100
Renomedullary interstitial cell tumor (medullary fibroma)		Actin, Calponin	CD34, ER, PgR	
Transitional cell (urothelial) carcinoma of the renal pelvis	Pan-CK, CK5, CK7, CK13, CK17, 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. 12.9
[e] Only scattered CK7 positive cells, see Fig. 12.10
[f] Diffuse CK7 expression
[g] See Fig. 12.12
[h] See Fig. 12.11
[i] Neurotrophic tyrosine receptor kinase, see Fig. 12.13

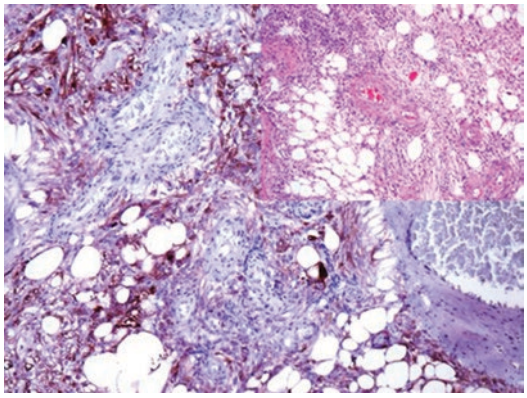

Fig. 12.9 HMB45 staining the perivascular epithelioid tumor cells in angiomyolipoma

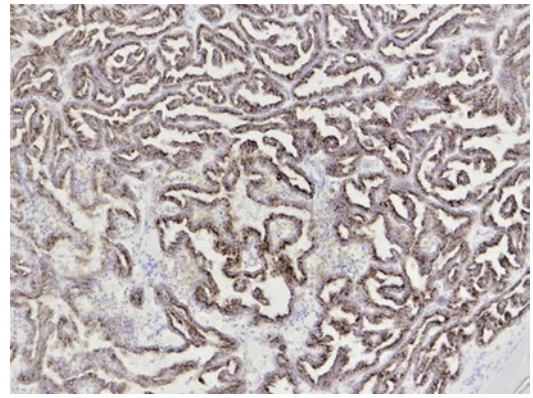

Fig. 12.12 Strong AMACR expression in papillary renal cell carcinoma

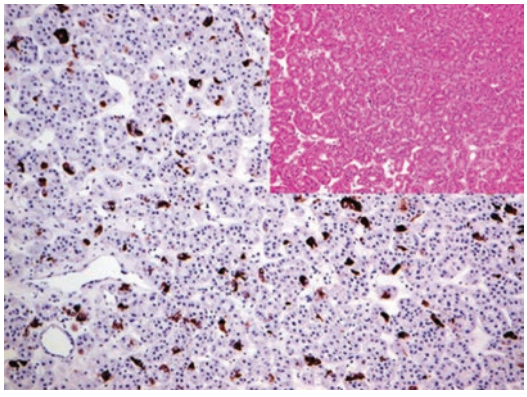

Fig. 12.10 Renal oncocytoma with scattered CK 7 positive cells

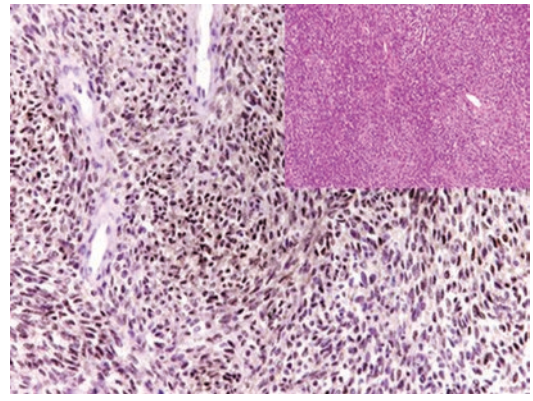

Fig. 12.13 NTRK staining tumor cells of congenital mesoblastic nephroma

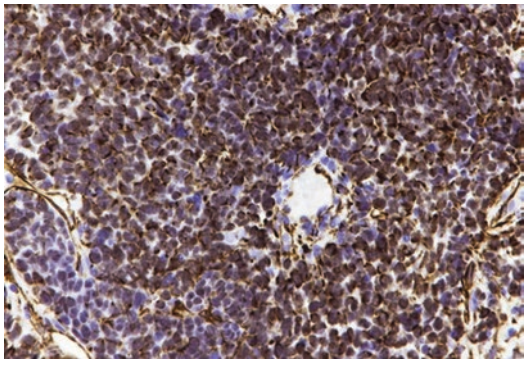

Fig. 12.11 Nephroblastoma with strong nuclear WT-1 expression

Differential diagnosis of clear cell renal carcinoma vs. tumors with clear cell appearance								
	Pan-CK	PAX-8	CD10	P16	Inhibin	Arginase	HMB45 SOX10	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

12.2.1 Diagnostic Antibody Panel for Transitional Cell Carcinoma

Cytokeratin profile (CK5/6/7/14/20), p63, GATA-3, Uroplakin, S100P, p16, p53, CD44, and Thrombomodulin (CD141).

12.2.2.1 Uroplakins

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 the mammalian urothelium and play a role in the strengthening of the urothelial apical surface during distention of the urinary bladder and urinary tract [25, 26]. 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. 12.14). Both Uroplakins are also absent in primary squamous cell carcinoma and adenocarcinoma of the urinary bladder [27]. 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 the 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 [28].

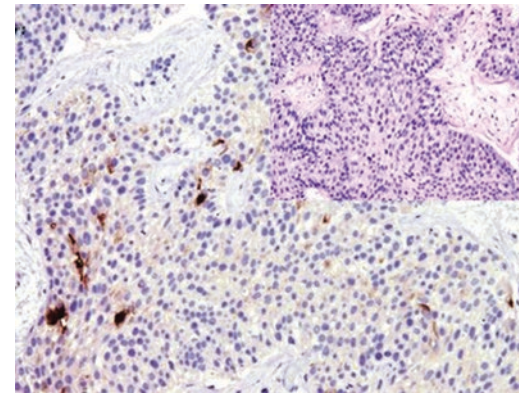

Fig. 12.14 Uroplakin highlights the cell membrane of transitional cell carcinoma

12.2.2 Therapy-Related Markers

PD-L1.

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 as they disappear in poorly differentiated carcinomas and a complete panel including the Cytokeratins CK5/6/7/20, p63, GATA-3, and Thrombomodulin is required for the appropriate diagnosis of metastatic tumors suspect of transitional cell carcinoma. Uroplakin II 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 also be considered in the differential diagnosis.

12.2.2.2 GATA-3

GATA-3 is a transcription factor involved in the differentiation and proliferation of breast luminal epithelium, urothelium, and subsets of T-lymphocytes (GATA-3 listed in detail with the markers of breast tumors, Chap. 10). GATA-3 is a useful screening marker to characterize metastases of unknown primary. Because of the broad 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. 12.15) [29, 30]. Besides the membranous expression of β-Catenin, the co-expression of GATA-3, CDX-2, and CK7 is characteristic for primary adenocarcinoma of the bladder [31, 32].

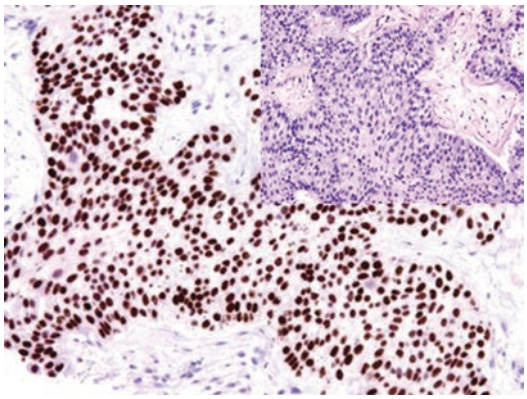

Fig. 12.15 Nuclear GATA-3 expression in transitional cell carcinoma

12.2.2.3 Placental S100

S100P is one of the members of the S100 protein family listed in detail in Chap. 8. 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.

12.2.2.4 Thrombomodulin (CD141)

Thrombomodulin is a transmembrane glycoprotein functioning as an endothelial anticoagulant protein clustered CD141. Thrombomodulin is 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 helpful screening marker expressed in mesothelioma, squamous cell carcinoma, and vascular tumors, in addition to the majority of transitional cell carcinomas (Fig. 12.16). Thrombomodulin is listed in detail with the mesothelioma markers (Chap. 15).

12.2.2.5 Cytokeratin 20 and CD44

CK20 and CD44 are important markers to differentiate between different urothelial lesions, including reactive urothelial atypia, atypia of unknown significance, different grades of dysplasia, and carcinoma in situ [33].

In normal urothelium, reactive urothelial atypia/hyperplasia, and atypia of unknown significance, the expression of CK20 is limited to umbrella cells on the surface. In mild dysplasia, the expression of CK20 is found in the deep layer of the urothelium. In high-grade dysplasia and CIS, the expression of CK20 is observed through all cell layers of the urothelium (Fig. 12.17).

In normal urothelium and reactive urothelial hyperplasia, the expression of CD44 is limited to the basal cells. In non-neoplastic urothelium or reactive urothelial atypia, the expression of CD44 is observed in all layers of the urothelium. The expression of CD44 is lost in high-grade dysplasia and CIS (Fig. 12.18) [34]. p53 and p16 are further markers expressed in dysplastic and malignant urothelium but negative in the normal and reactive urothelium (Fig. 12.19).

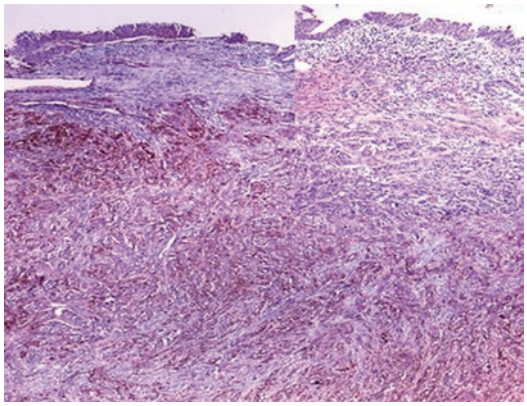

Fig. 12.16 Thrombomodulin expression in high-grade transitional cell carcinoma of the urinary bladder

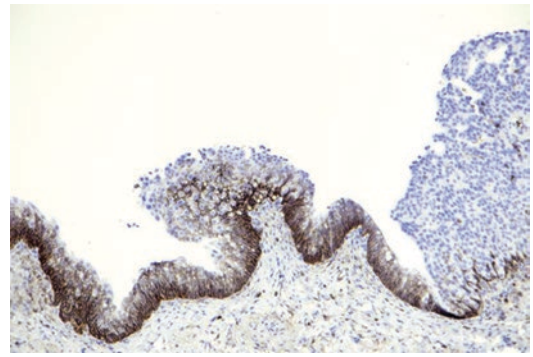

Fig. 12.18 CD44 expression through all the cell layers of the normal urothelium (***left*** side). In normal urothelium, the CD44 expression is restricted to the basal cell layers

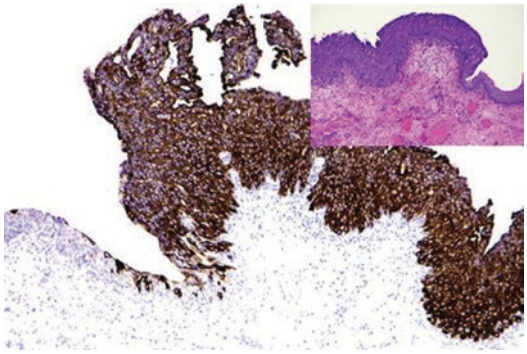

Fig. 12.17 Strong CK20 expression through all the cell layers of the dysplastic urothelium. In normal urothelium, the CK20 expression is restricted to umbrella cells (***left*** side)

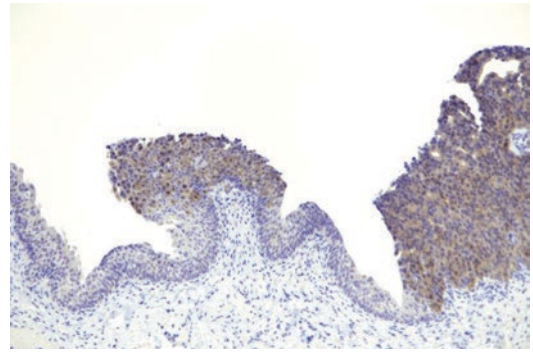

Fig. 12.19 Strong p16 expression in the dysplastic urothelium

Immunoprofile of the urinary bladder and urinary tract tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Urothelial carcinoma in situ	CK20[a]	**p53**[b], p16, CEA		CD44[c]
Transitional cell (urothelial) carcinoma	CK5, CK7, CK8, CK13, CK17, CK18, CK19, E-cadherin, p63, **GATA-3**, S100P, **Thrombomodulin**	**Uroplakin** (Ia, II, and III), CEA, Fascin[d], CK20[e]	Calretinin, androgen receptors	**PAX-8**[f], PAX-2, NKX3.1, WT-1, vimentin
Adenocarcinoma of the urinary bladder – Enteric type – Mucinous type – Signet ring cell type – Mixed type – Nos	CK8, CK18, CK19, β-catenin[g]	**Thrombomodulin**, CK7, CK20, CDX-2, CEA, CD15	GATA-3	CK5, PAP, PSA, PAX-8, NKX3.1

Urachal carcinoma	CK20, β-catenin, CD15	CDX-2[h], PDX-1, CEA, CK7	CK5/14	p63
Clear cell adenocarcinoma (tumors of Müllerian type)	CK7, **PAX-8**, CA125, HNF1β, **p53** Proliferation index (Ki-67): >15%	PAX-2, p504S (AMACR), CEA	CD10, CK20	GATA-3, PSA, NKX3.1
Squamous cell carcinoma of the 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**, **p504S (racemase)** Proliferation index (Ki-67): <3%	Satb-2	CK5/14, p63, p53	GATA-3, NKX3.1
Botryoid fibroepithelial polyp of the urinary tract	Desmin, vimentin	ER, PR, actin, CD34		Pan-CK, S100, CD68, Myogenin, Myo D1, CD 68, CD117
Littre gland carcinoma of the urethra	CK7			NKX3.1, PSA
Skene gland adenocarcinoma of the urethra	Pan-CK, NKX3.1, PSA			PAX-8, CK20
Cowper gland adenocarcinoma of the urethra	CK7			NKX3.1, PSA

[a] In urothelial CIS, trans-epithelial CK20 expression is noted. In moderate dysplasia, CK20 expression is limited to the deep layer. In normal urothelium, CK20 expression is limited to umbrella cells

[b] p53 is absent in normal urothelium, and in moderate dysplasia p53 is found in <10% of urothelial cells. In CIS, the p53 expression is seen through all cell layers of the neoplastic urothelium

[c] In normal urothelium, the CD44 expression is limited to basal cells but absent in urothelial CIS (see Fig. 12.18)

[d] Normal urothelium lacks the expression Fascin

[e] CK20 is absent in high-grade carcinoma and inverted papilloma

[f] PAX-8 may be positive in transitional cell carcinoma of the renal pelvis

[g] Membranous stain of β-Catenin in bladder adenocarcinoma and nuclear stain in colorectal adenocarcinoma

[h] See Fig. 12.20

Differential diagnosis reactive urothelial atypia vs. urothelial carcinoma in situ		
	Normal/reactive urothelium	Urothelial carcinoma in situ
CK20	Limited to the umbrella cells	Transepithelial expression
CD44	Expression in basal cells or transepithelial	Loss of expression ~70%
P53	Few very weak positive cells	Strong diffuse expression in ~50%/completely negative (null type)
P16	Weak expression	Strong expression
Fascin	Negative	Moderate/strong expression

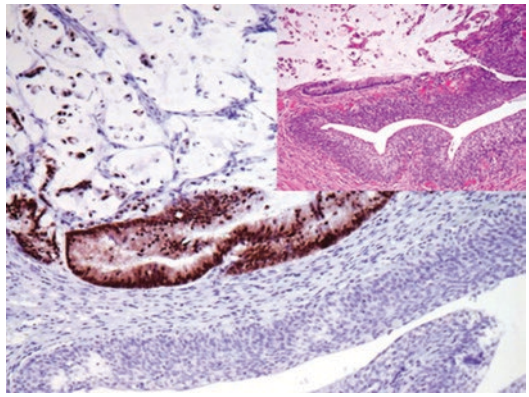

Fig. 12.20 Urachal carcinoma with nuclear CDX-2 expression, adjacent normal bladder mucosa negative for CDX-2

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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.

13.1.1 Markers for Prostatic Epithelium

PSA, PAP, NKX3.1, Prostein, Androgen receptor, ERG, Human glandular Kallikrein-2 (hK2), AMACR (p504S).

13.1.2 Markers for Basal Cells

High molecular weight Cytokeratins (CK5, CK6, CK14, CK34βE12), p40, p63, EGFR.

M. S. A. Tuffaha et al., *Immunohistochemistry in Tumor Diagnostics*,
https://doi.org/10.1007/978-3-031-45024-2_13

13.1.2.1 Prostate-Specific Antigen

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, salivary glands
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 acini and prostatic ducts and secreted into prostatic ducts playing a role in the liquefaction of seminal fluid. Normally, protease inhibitors rapidly inactivate PSA portion that enters the blood circulation. PSA is one of the most specific markers for prostatic parenchyma and prostatic carcinoma. Metastatic carcinoma positive for Pan-Cytokeratin but negative for Cytokeratins 5/7/14 and 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 carcinoma is 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. A 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.

13.1.2.2 Prostein

Prostein (SLC45A3, P501S)		
Expression pattern: cytoplasmic, perinuclear 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 in determining the prostatic origin than PSA and slightly more sensitive and is usually still preserved in poorly differentiated prostatic carcinoma. Prostein can thus be successfully used in a panel with NKX3.1 and PSA to classify metastases of unknown primary or to discriminate between prostatic, urothelial, and colorectal carcinomas [1]. Additionally, the expression of prostein is found in about 30% of small cell carcinoma of the prostate. The loss of prostein expression is associated with an unfavorable clinical course [2].

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

13.1.2.3 Prostatic Acid Phosphatase

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	Acinic and ductal epithelium of the prostate, 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 the 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 tumors.

Diagnostic Pitfalls Similar to PSA, PAP can also be expressed in neuroendocrine carcinomas of different origins. This feature is important for the differentiation between poorly differentiated prostatic carcinoma, prostatic carcinoma with neuroendocrine differentiation, and other neuroendocrine tumors. The expression of PAP is one of the immunohistochemical characteristics of the primary neuroendocrine tumors of the rectum.

13.1.2.4 Prostate-Specific Membrane Antigen (PSMA)

Glutamate carboxypeptidase II (also known as prostate-specific membrane antigen; PSMA) is a class II membrane glycoprotein and an enzyme that catalyzes the hydrolysis of *N*-acetylaspartylglutamate to glutamate and *N*-acetylaspartate. Despite its name as prostate-specific membrane antigen, PSMA is not a prostate-specific marker, and besides normal and malignant prostatic glandular epithelium, it is expressed in other different tissue types such as salivary glands, intestinal mucosa, epithelium of proximal renal tubules, and ganglion cells of the nervous system in addition to the apical surface of neovascular endothelial cells associated with different tumors [3–6]. The expression of PSMA is strongly upregulated in high-grade PIN and prostatic adenocarcinoma and correlates with the Gleason score and disease progression with high expressed levels in hormone-refractory high-grade carcinoma.

PSMA is also highly expressed in other tumors, such as adenoid cystic carcinoma.

PSMA is now the therapeutic target for neoplasia-associated angiogenesis and some tumor types exhibiting PSMA overexpression.

13.1.2.5 Androgen Receptor

Androgen receptor		
Expression pattern: nuclear		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Carcinoma of the prostate – Breast carcinoma with apocrine differentiation/ different receptor-positive and triple-negative breast carcinoma	Bladder transitional cell carcinoma, endometrioid carcinoma, salivary duct carcinoma, sebaceous carcinoma, basal cell and squamous cell carcinoma, Paget's disease, papillary thyroid carcinoma, mesonephric adenocarcinoma, spindle cell lipoma and well-differentiated liposarcoma, osteosarcoma, meningioma	Prostatic epithelium, urinary bladder urothelium, Sertoli cells, Leydig cells, rete testis, epididymis and seminal vesicles, apocrine and sebaceous glands, skin, oral mucosa, hepatocytes, erythroid precursors
Positive control: prostatic tissue		

Diagnostic Approach The androgen receptor (AR) is a nuclear receptor and a member of the steroid hormone receptor family that includes the estrogen receptor, progesterone receptor, glucocorticoid receptor, and mineralocorticoid receptor. The androgen receptor is activated by binding to testosterone or dihydrotestosterone and takes part in the development and differentiation of both male and female reproductive organs and musculoskeletal, cardiovascular, immune, neural, and hemopoietic systems [3, 7]. The AR is expressed in different tissue types, including the prostatic gland, bone, 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 [8]. AR is also positive in neuroendocrine tumors of the prostate.

Diagnostic Pitfalls The expression of AR is not restricted to prostatic carcinoma and can be found in other carcinoma types occasionally with similar morphology, such as transitional cell carcinoma of the urinary bladder and urethra, endometrioid carcinoma, salivary duct carcinoma, breast carcinoma, and breast carcinoma with apocrine differentiation.

13.1.2.6 NKX3.1

NKX3.1		
Expression pattern: nuclear		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Prostatic acinar adenocarcinoma – Prostatic ductal adenocarcinoma	GCNIS, lobular breast carcinoma, a subset of T-ALL, rare sarcoma types (EWSR1-NFATC2 Ewing-like sarcoma and mesenchymal chondrosarcoma)	Prostatic tissue, salivary glands, mucinous bronchial glands, Sertoli cells
Positive control: prostatic tissue		

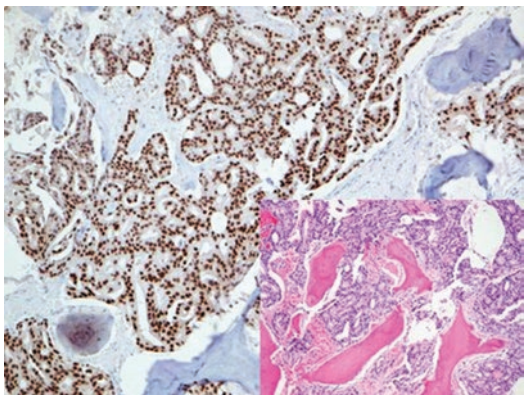


Fig. 13.1 Metastatic acinar prostatic carcinoma with strong nuclear NKX3.1 expression

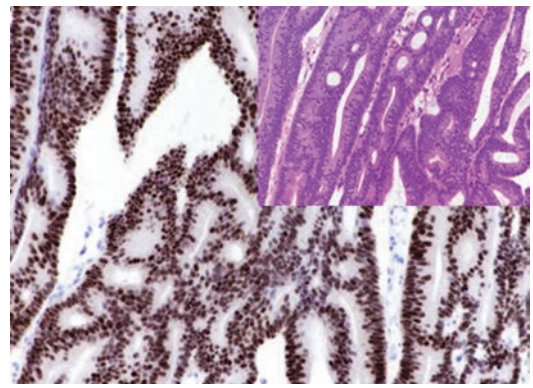


Fig. 13.2 Prostatic ductal adenocarcinoma with strong nuclear NKX3.1 expression

Diagnostic Approach NKX3.1 (also known as KX3–1, BAPX-2) is encoded by an androgen-regulated tumor suppressor gene located on chromosome 8p221.1. NKX3.1 functions as a suppressor regulating the proliferation and differentiation of prostatic luminal epithelium. NKX3.1 is strongly expressed in the nuclei of normal prostatic secretory epithelium but negative in prostatic stromal cells. NKX3.1 is a specific marker for primary acinar prostatic carcinoma and ductal adenocarcinoma, whereas the intensity of the nuclear expression correlates with the differentiation grade of the carcinoma and can be very weak in poorly differentiated carcinomas (Figs. 13.1 and 13.2) [9].

Diagnostic Pitfalls NKX3.1 is also expressed in testicular germ cells and seminoma in situ (GCNIS) but lost in invasive seminoma and embryonal carcinoma. Low to moderate NKX3.1 expression intensity is also found in a subset of estrogen- and/or androgen-positive breast carcinomas, i.e., invasive lobular carcinoma (Fig. 13.4) [10, 11]. Mucinous units of salivary glands and bronchial glands also reveal a nuclear NKX3.1 expression, which is to consider in the interpretation of small biopsies (Fig. 13.3). Furthermore, the TAL-1 genetic aberration associated with a subset of T-ALL causes the activation of NKX3.1 expression in neoplastic lymphocytes [12]. NKX3.1 is also a characteristic marker for the mesenchymal chondrosarcoma and Ewing-like sarcoma harboring the EWSR1-NFATC2 translocation [13].

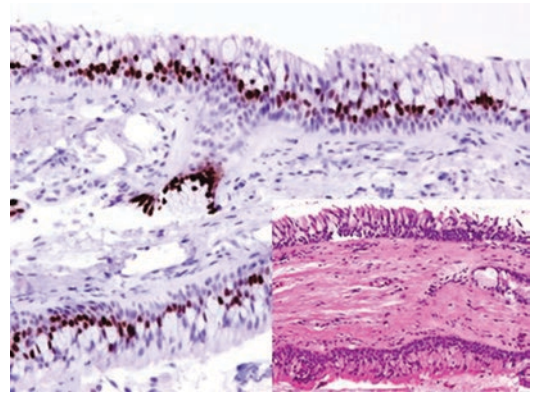


Fig. 13.3 Nuclear NKX3.1 expression in mucinous cells of bronchial mucosa

13.1.2.7 Alpha-methylacyl-CoA Racemase

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 – Papillary renal cell carcinoma	Gastrointestinal adenocarcinoma, hepatocellular carcinoma, carcinoma of breast and ovaries, endometrial clear cell carcinoma, urothelial carcinoma, extramammary Paget's disease, mucinous tubular and spindle cell carcinoma, mesothelioma, lymphoma, pancreatic islet tumor, dysplastic nevi	Periurethral glands, liver, salivary glands, sebaceous glands, renal tubular epithelium, pancreas epithelium, mesothelial cells
Positive control: prostatic carcinoma		

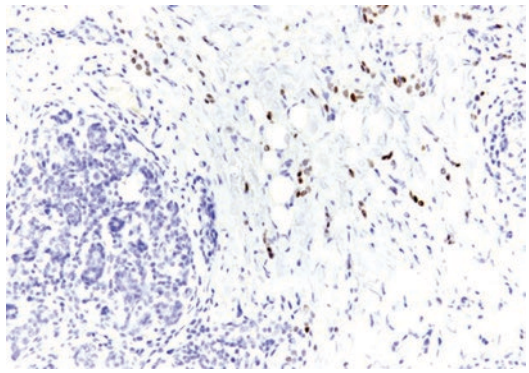


Fig. 13.4 NKX3.1 highlighting a subset of the tumor cells of invasive lobular carcinoma

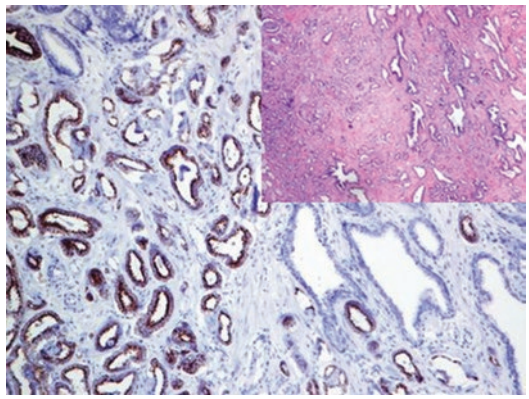


Fig. 13.5 Cytoplasmic AMACR expression in the neoplastic luminal cells of prostatic adenocarcinoma

Diagnostic Approach Alpha-methylacyl-CoA racemase (also known as p504S) is a member of the isomerase 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. 13.5) [14–16]. 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 marker for basal/myoepithelial cells exhibiting a strong nuclear stain listed in detail in previous chapters with the epithelial and renal tumor markers (see Chap. Sects. 2.5 and 12.1) [17].

The dual immunohistochemical stain with the PIN cocktail can show one of the following three expression patterns

– 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, a pattern characteristic for normal prostatic glands.

High molecular cytokeratins such as CK5/6/14 can be used as alternatives to p63 in a separate reaction (Fig. 13.6).

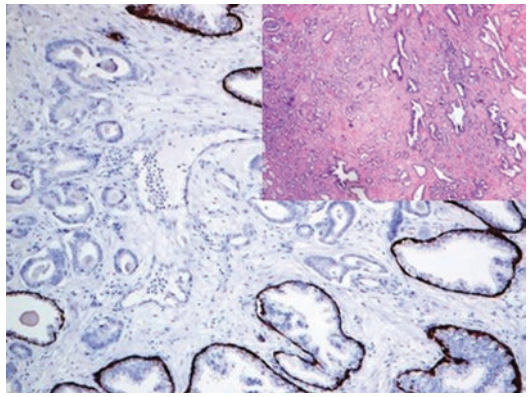


Fig. 13.6 Neoplastic glands of prostatic adenocarcinoma lacking the myoepithelial cells positive for high molecular weight cytokeratin (CK5/14)

The AMACR expression is characteristic for papillary renal cell carcinoma, mucinous tubular and spindle cell carcinoma, and Xp11 translocation renal cell carcinoma and may be also expressed in a very small subset of clear cell carcinoma but is constantly absent in chromophobe renal cell carcinoma [18, 19].

Diagnostic Pitfalls The luminal epithelium of high-grade prostatic intraepithelial neoplasia is frequently positive for AMACR, and it is also to consider that the expression of the high molecular cytokeratins may be partially lost in such lesions. Low AMACR expression levels may be also seen in benign histologic mimics of prostatic adenocarcinoma, including atrophic prostatic glands and post-atrophic hyperplasia, adenosis, seminal vesicle, and periurethral glands. The expression of AMACR is also characteristic for nephrogenic adenoma but later is positive for PAX-8 and GATA-3 and negative for NKX3.1.

In general, the expression of AMACR is found in many benign and malignant tumor types and cannot be considered a specific lineage marker of prostatic carcinoma [20].

13.1.2.8 ERG

ERG		
Expression pattern: nuclear		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Prostatic adenocarcinoma, endothelial tumors – Angiosarcoma/ endothelial tumors	Acute myeloid leukemia, B and T lymphoblastic leukemia, meningioma, GIST, solitary fibrous tumor, chondrosarcoma, chondroblastic osteosarcoma, epithelioid sarcoma, synovial sarcoma, malignant rhabdoid tumor, phosphaturic mesenchymal tumor	Endothelial cells, myeloid precursors
Positive control: blood vessels		

Diagnostic Approach **E**26 transformation-specific **r**egulated **g**ene-1 (ERG) is a member of the ETS family of transcription factors, which also includes Fli-1 and EST-1 encoded by the gene located on chromosome 21q22.3. ERG plays a role in the regulation of angiogenesis and differentiation of hematopoietic stem cells. ERG is normally expressed in endothelial cells and cells with endothelial differentiation in addition to myeloid precursors and tumors derived from these cells (Fig. 13.7) [5].

The ERG gene is the fusion partner of the TMPRSS2 gene involved in the regulation of response to androgen. This genetic mutation is the most frequent genetic abnormality associated

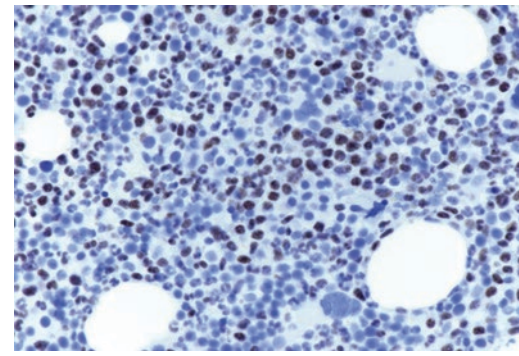


Fig. 13.7 Bone marrow with normal myeloid precursors showing nuclear ERG expression

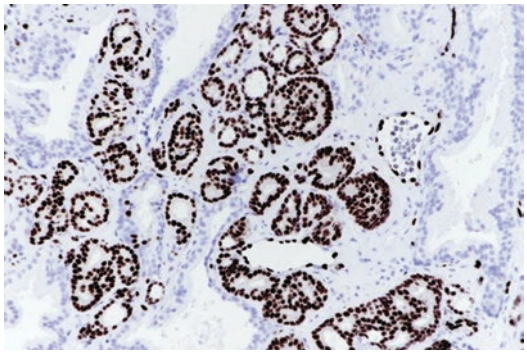


Fig. 13.8 Nuclear ERG expression in neoplastic cells of prostatic acinar adenocarcinoma; normal prostatic glands lack the ERG expression. Note that ERG also labels the endothelium of normal blood vessels

with prostatic carcinoma and is found in 40–80% of acinar adenocarcinoma and about 10% of ductal adenocarcinoma. This mutation generates the TMPRSS2-ERG gene fusion causing the overexpression of the ERG protein detected by immunohistochemistry (Fig. 13.8) in acinar prostatic carcinoma and frequently also in prostatic neuroendocrine carcinomas [21, 22].

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. Consequently, the gold standard remains the labeling of the myoepithelial basal cells [23]. Both antibodies to ERG and p63 can be used as a cocktail for the diagnosis of prostatic carcinoma but have less sensitivity than the above-described PIN cocktail [24].

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

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 a few other mesenchymal tumors, including chondrosarcoma, chondroblastic osteosarcoma, epithelioid sarcoma, synovial sarcoma, GIST, fibrous meningioma, and t(21;22)(q22;q12) associated Ewing's sarcoma [25]. ERG expression is also described in rare cases of invasive ductal carcinoma of the breast and papillary thyroid carcinoma. Moreover, EGR is expressed in a subset of acute myeloid leukemia and myeloid sarcoma in addition to B and T lymphoblastic leukemia/lymphoma.

13.1.2.9 Phosphatase and Tensin Homolog (PTEN)

PTEN is a tumor suppressor mentioned in a previous chapter (Chap. 11). The loss of PTEN is found in about 20% of primary carcinomas, with higher rates of PTEN loss in higher Gleason scores. PTEN loss is prognostically unfavorable. Diagnostically, the loss of PTEN expression may help to distinguish intraductal carcinoma (commonly lost) from high-grade PIN (often positive) if the morphological context supports this.

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 Cytokeratins (CK5/CK6/CK14, CK-34E12), p63, p40, LP34			
Acinar adenocarcinoma	Pan-CK, CK8/18, CK19, **PSA**, PAP, **NKX3.1**, **Prostein**, hK2, **p504S (racemase)** **Diagnostic is the loss of basal myoepithelial cell layer: negativity for high molecular Cytokeratins (CK5/6/14, CK-34E12), p63, p40**	**Androgen receptor, ERG**[a]	CK7	CK5/14, CK10, CK20, CDX-2, GATA-3, Uroplakin, CEA
Ductal adenocarcinoma	Pan-CK, CK8/18, CK19, NKX3.1, androgen receptor	PSA, Prostein, p504S (racemase), CK20		CK5/CK14, CK7, CDX-2

Intraductal carcinoma	Pan-CK, CK19, PSA, PAP, NKX3.1, p504S (racemase), **ERG** **Preserved basal myoepithelial cell layer**: positive for high molecular Cytokeratins (CK5/6/14) and p63, p40			PTEN
Adenoid cystic (basal cell) carcinoma of the prostate	CK8/18, **CK5/6/14**, **p63**, **p40**, bcl-2[c], CK7 only in luminal	**Myb**[b], HER-2	P504S (AMACR), androgen receptor	ERG, PSA, CK20
Neuroendocrine neoplasms – Adenocarcinoma with neuroendocrine differentiation – Well-differentiated neuroendocrine tumors (NET G1, G2, G3) – Neuroendocrine carcinoma of small and large cell type	Chromogranin, Synaptophysin, CD56 See neuroendocrine tumors (Chap. 14.7)		Androgen receptors, NKX3.1	
Prostatic stromal tumor of uncertain malignant potential	PgR, AR, vimentin	Desmin, actin, CD34	ER	CD117
Prostatic stromal sarcoma	Vimentin	CD34	ER, PgR	CD117, Desmin, actin
Adenocarcinoma of seminal vesicle	CK8, CK18, CK19, **PAX-8**, PAX-2, MUC-6, CA-125, CEA	CK7, androgen receptor		CK20, PAP, PSA, NKX3.1, GATA-3, Oct-4, WT-1, CDX-2
Squamous cell carcinoma of seminal vesicle	CK5/CK14, p63			CK7, CK20, PSA, CEA
Mixed epithelial and stromal tumor of seminal vesicle	*Stromal cells*: vimentin, CD34, ER, PgR, Desmin, h-Caldesmon *Epithelial cells*: CK7			Inhibin, CD117, PSA, NKX3.1, CK20

[a] Positive in tumors associated with the TMPRSS2-ERG gene fusion
[b] See adenoid cystic carcinoma of the salivary glands (Chap. 6.2)
[c] Negative in basal cell hyperplasia

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, Sox-17, NUT, CD117, D2 40, PLAP, AFP, CD30, CDX-2, GATA-3, β-hcG, and cytokeratin profile.

13.2.2 Sex Cord-Stromal Tumors

Diagnostic antibody panel for sex cord-stromal tumors.

Inhibin, Steroidogenic factor-1 (SF-1, Ad4BP), FOXL2, Calretinin, CD56, anti-Müllerian hormone, Melan A, CD99.

13.2.2.1 SALL-4

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 – Choriocarcinoma – Ovarian dysgerminoma – CNS germinoma	A subset of gastrointestinal and 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 a member of the **sp**al**l**-like 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, Sox-2, and NANOG [13–15]. 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. 13.9). SALL-4 is negative in sex cord tumors.

Diagnostic Pitfalls SALL-4 is strongly expressed in the neoplastic cells of intratubular germ cell neoplasms (GCNIS) but can also be expressed in adult normal testicular intratubular germ cells, specifically in undifferentiated spermatogonia; consequently, SALL-4 is not a suitable marker to highlight the cells of intratubular germ cell neoplasms. In routine immunohistochemistry, it is important to remember that the expression of SALL-4 is not restricted to germ cell tumors as it is expressed in various intensity in different non-germ cell epithelial and mesenchymal tumors, including serous ovarian carcinoma, pulmonary adenocarcinoma, gastric adenocarcinoma, cholangiocarcinoma and hepatocellular carcinoma, urothelial carcinoma, and small cell carcinoma in addition to embryonal rhabdomyosarcoma, renal rhabdoid tumor beside subsets of lymphoblastic lymphoma, and anaplastic large cell lymphomas. This aberrant expression must be carefully considered in the interpretation of this marker [28].

13.2.2.2 Oct-4

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, renal medullary carcinoma, diffuse large B cell lymphoma	Germ cells (pluripotent germ cells)
Positive control: seminoma		

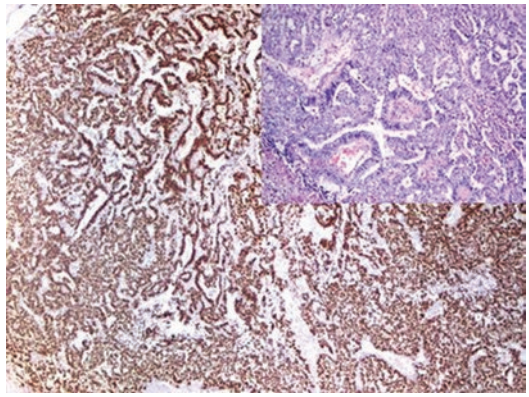


Fig. 13.9 SALL-4 labeling the nuclei of yolk sac tumor cells

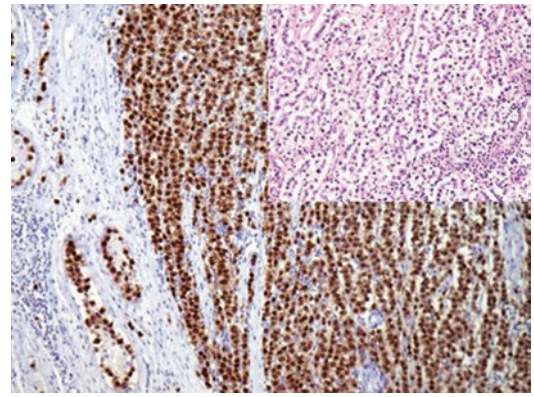


Fig. 13.10 Oct-4 staining the nuclei of seminoma cells and the cells of intratubular germ cell neoplasm (left)

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 an important role in the differentiation of pluripotent germ cells and downregulated when these cells started to differentiate. A high expression level of Oct-4 is characteristic for seminoma and embryonal carcinoma, whereas spermatocytic tumor (formerly spermatocytic seminoma) lacks the expression of Oct-4 (Fig. 13.10) [29]. Oct-4 labels the nuclei of the majority of the dysplastic cells of intratubular germ cell neoplasms but not the non-neoplastic testicular cells, making Oct-4 a helpful and specific maker for intratubular germ cell neoplasms (Fig. 13.11) [30]. Atypical cytoplasmic Oct-4 expression is also found in neuroendocrine tumors with different differentiation grades.

Diagnostic Pitfalls The expression of Oct-4 is found in a subset of pulmonary non-small cell carcinoma and breast carcinoma [31]. Oct-4 expression is also found in some cases of testicular and extra-testicular diffuse large B cell lymphoma, which is to consider in the differential diagnosis [32].

13.2.2.3 Placental Alkaline Phosphatase

Placental alkaline phosphatase (PLAP)		
Expression pattern: membranous		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Germ cell tumors: seminoma, embryonal carcinoma, yolk sac tumor, choriocarcinoma	Proximal GIT 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 of phosphoric acid monoesters. Placental alkaline phosphatase (PLAP) is a membrane-associated glycoprotein primarily expressed in placental syncytiotrophoblasts from the eighth week throughout pregnancy. PLAP is a marker for several germ cell tumors such as seminoma, dysgerminoma, and, to a lesser degree also, embryonal carcinoma, yolk sac tumor, and gonadoblastoma. Since PLAP is not specific for any germ cell tumor (but has a preference for seminoma and dysgerminoma), a panel of antibodies is required to differentiate between the PLAP-positive germ cell tumors (see below) [33–35]. PLAP is negative in spermatocytic tumors and immature teratoma.

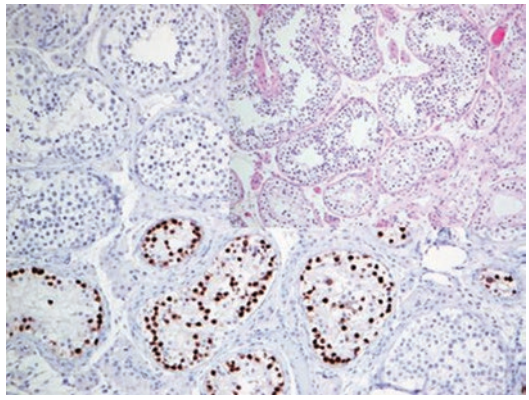


Fig. 13.11 Oct-4 highlighting the cells of intratubular germ cell neoplasm (IGCN)

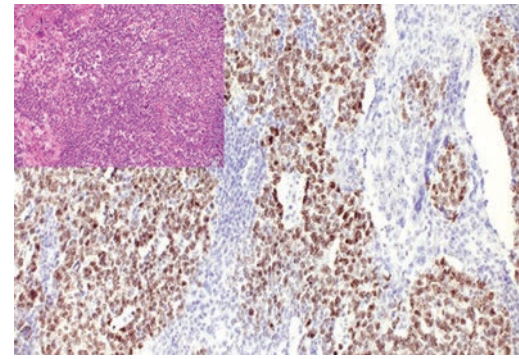


Fig. 13.12 Testicular mixed germ cell tumor with nuclear Sox-2 expression in the cells of embryonal carcinoma. Other tumor components lack the expression of Sox-2

Diagnostic Pitfalls Aberrant PLAP expression is rarely found in other non-germ cell tumor types such as breast and lung carcinoma. Additionally, it is essential to consider that a cytoplasmic PLAP stain is reported in tumors with myogenic differentiation, such as embryonal rhabdomyosarcoma and smooth muscle tumors [23].

13.2.2.4 Sox-17

Sox-17 (SRY-box transcription factor 17) is a member of the SOX family of transcription factors detailed in Chap. 11.6. In germ cell tumors, Sox-17 is positive in seminoma, dysgerminoma, and yolk sac tumor, whereas embryonal cell carcinoma and choriocarcinoma lack the expression of Sox-17 [36].

13.2.2.5 Sox-2

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 several genes involved in the 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 is negative in seminoma, yolk sac tumor, and choriocarcinoma (Fig. 13.12) [36, 37]. Sox-2 is also expressed in glial brain tumors and supratentorial PNET [38]. Ectopic Sox-2 expression is found in a subset of pulmonary squamous cell carcinomas and adenocarcinomas. Variable Sox-2 expression is also reported in some neuroendocrine carcinomas [26].

13.2.2.6 Podoplanin (D2–40)

Podoplanin (D2–40) is a type I transmembrane mucoprotein listed in detail with the markers of vascular tumors (Chap. 25). D2–40 is an excellent seminoma marker that also stains intratubular neoplastic germ cells but is negative in all other non-seminomatous 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 (Fig. 13.13).

13.2.2.7 Human Chorionic Gonadotropin

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, high-grade urothelial carcinoma	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 [39, 40]. Focal expression is also noted in high-grade urothelial carcinoma. Generally, the expression of β-HCG in nontrophoblastic tumors indicates aggressive behavior.

13.2.2.8 CD30

CD30 is a membrane-bound glycoprotein listed in detail in a later section as an essential marker for Hodgkin and anaplastic lymphomas (Chap. 16.6). Additionally, the expression of CD30 is characteristic for embryonal carcinoma (Fig. 13.14). In rare cases, CD30 may faintly stain yolk sac tumor, which is to consider in the differential diagnosis of combined germ cell tumors.

13.2.2.9 Inhibin A

Inhibin A		
Expression pattern: cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Sex cord-stromal tumors (granulosa cell tumor, Leydig cell tumor, Sertoli cell tumor, steroid cell tumor, thecoma and fibrothecoma) – Adrenocortical tumors	Choriocarcinoma and trophoblastic lesions and placental site nodule, hepatocellular carcinoma	Sertoli cells, granulosa cells, theca interna, intermediate trophoblasts, syncytiotrophoblasts, adrenal cortex, brain tissue
Positive control: granulosa cell tumor/adrenal gland		

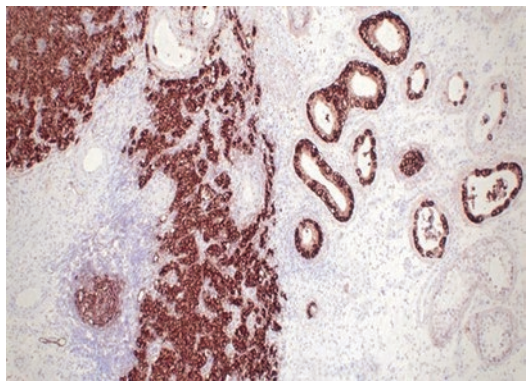


Fig. 13.13 Seminoma associated with intratubular neoplastic germ cells. Podoplanin stains both tumor components in addition to small lymphatic vessels

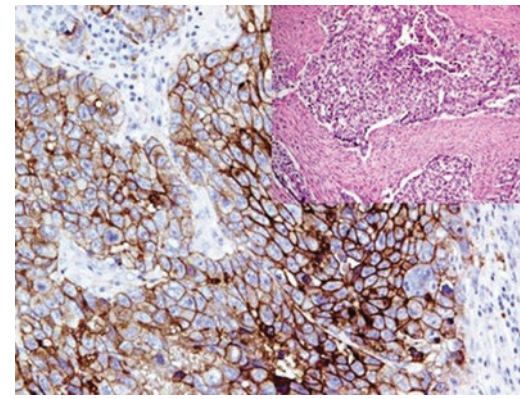


Fig. 13.14 Embryonal carcinoma, tumor cells with strong membranous CD30 expression

Diagnostic Approach Inhibin is a member of the transforming growth and differentiation factor family, a glycoprotein hormone composed of α- and β-subunits expressed in the ovarian granulosa cells, gonads, and adrenal gland, functioning as an inhibitor for the pituitary follicle-stimulating hormone (FSH) secretion and stimulating the synthesis of androgen in ovarian theca cells. Antibodies to Inhibin A, anti-Müllerian hormone, and Melan A are important diagnostic markers for sex cord tumors, including adult and juvenile granulosa cell tumor, Leydig cell tumor, Sertoli cell tumor, steroid cell tumors, thecoma, fibrothecoma, and hyperthecosis [40]. Inhibin and anti-Müllerian hormone are consistently negative in ovarian surface epithelial-stroma tumors, seminoma, and embryonal carcinoma.

Diagnostic Pitfalls Inhibin is also expressed in other tumors, mainly tumors of the adrenal cortex.

13.2.2.10 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. Anti-Müllerian hormone mediates male sexual differentiation by inhibiting the development of the Müllerian duct and preventing the transformation of the Müllerian duct into the uterus, fallopian tubes, and other Müllerian structures and plays a role in 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 [41]. Other sex cord-stromal tumors are usually negative for AMH.

13.2.2.11 Adrenal 4 Binding Protein (SF-1)

Steroidogenic factor 1 (SF-1) is listed in detail with the markers of adrenocortical tumors (Chap. 14.6). SF-1 is expressed in normal testicular Sertoli and Leydig cells in addition to granulosa cells. SF-1 is a sensitive marker for Sertoli cell tumors and granulosa cell tumors. Leydig cell tumor lacks the expression of SF-1.

13.2.2.12 Glypican-3

Glypican-3 was listed in detail in a previous chapter (Chap. 9.1). 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.

13.2.2.13 CDX-2

Caudal-related homeobox 2 (CDX-2) is an intestine-specific transcription factor protein regulating the differentiation and proliferation of intestinal epithelial cells and is popularly used as a marker for gastrointestinal adenocarcinomas (see Chap. 7.1). CDX-2 is also a sensitive and specific marker for yolk sac tumor (Fig. 13.15) [42].

13.2.2.14 GATA-3

GATA-3, also known as endothelial transcription factor 3, is one of the six members of the GATA family of transcription factors listed in detail with the markers of breast and urothelial in addition to salivary gland tumors (Chaps. 6.2, 10, and 12.2). GATA-3 is also a transcription factor important for the differentiation of trophoblasts and is strongly expressed in trophoblasts and trophoblastic tumors, including choriocarcinoma and gestational trophoblastic tumors. GATA-3 is also expressed in the neoplastic cells of yolk sac tumor (Fig. 13.16) [43].

13.2.2.15 CD56

CD56 (neural cell adhesion molecule) is listed in later chapters as a marker for NK lymphomas and neuroendocrine tumors (Chap. 16.5). CD56 is a sensitive marker for ovarian and testicular sex cord-stromal tumors but lacks specificity as it is expressed in a wide range of other tumors. The combination of CD56 with SF-1, Inhibin, and Melan A will make the diagnosis of sex cord tumors more precise.

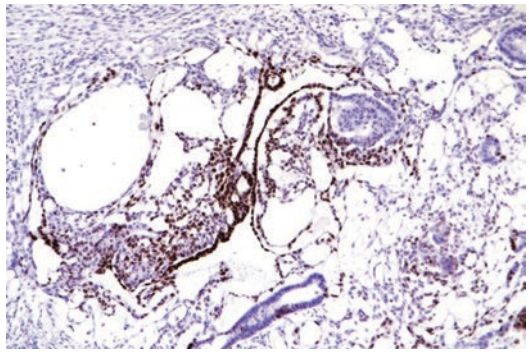


Fig. 13.15 Testicular mixed germ cell tumor with strong nuclear CDX-2 expression in yolk sac tumor component

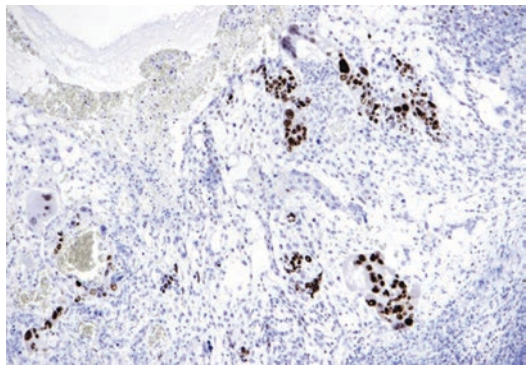


Fig. 13.16 Testicular mixed germ cell tumor with nuclear GATA-3 expression in the neoplastic trophoblasts and syncytiotrophoblasts besides few cells of yolk sac tumor. Cells of embryonal carcinoma lack the GATA-3 expression

13.2.2.16 Melan A and CD99

Melan A and CD99 are further markers that label the neoplastic cells of sex cord-stromal tumors. Both markers are listed in detail in later chapters: Melan A as a melanoma marker (Chap. 22) and CD99 as a marker for Ewing sarcoma (Chap. 29).

13.3 Paratesticular Tumors

Diagnostic antibody panel for paratesticular tumors.

Cytokeratin profile, PAX-8, PAX-2, Calretinin.

13.3.1 PAX-8 and PAX-2

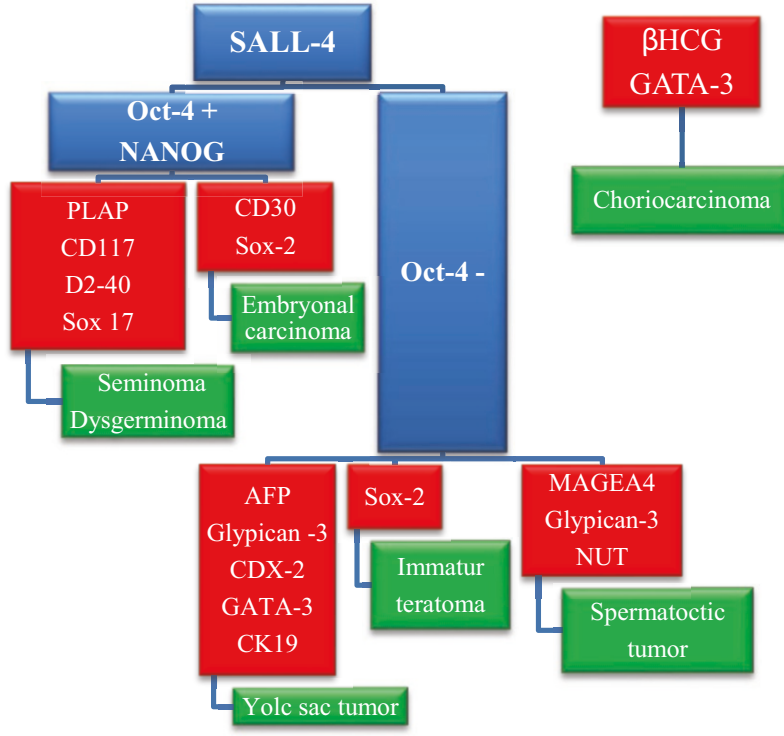
Both PAX-8 and PAX-2 are transcription factors expressed in the organs derived from Wolffian and Müllerian ducts and strongly stain the rete testis, epididymal and seminal vesicle epithelium, and carcinomas derived from these cells. They can be used to differentiate between prostatic carcinoma and carcinoma of seminal vesicles (see markers of renal cell tumors; Chap. 12.1) (Algorithm 13.1).

Immunoprofile of testicular and paratesticular tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
A. Germ cell tumors				
Intratubular germ cell neoplasms (GCNIS)	**Oct-4**, **PLAP**, **D2–40**, **TCL-1**, SALL-4[a], SOX-17, sCD143, LIN28 angiotensin-converting enzyme (ACE), NSE	Ferritin, CD117		AFP, βhcG, Glypican-3, inhibin, CD30
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-3, EMA, CK19, CK20, CEA, AFP, βhcG, inhibin
Spermatocytic tumor (formerly spermatocytic seminoma)	**SALL-4**, **NUT**, MAGEA4, Oct-2	CD117, vimentin, CK8/CK18, NSE		AFP, **Oct-4**, **PLAP**, ßhcG, CEA, EMA, CD30, CD143, **D2–40**

Embryonal carcinoma	**Oct-3/4**, **SALL-4**, **NANOG**, **Sox-2**, **PLAP**, **CD30**, LIN28, CK8, CK18	CK7, CK19, NSE	βhcG, AFP, vimentin	EMA, CK20, CEA, GATA-3, **Sox-17**, **CD117**, **Glypican-3**, D2–40
Yolk sac tumor	**AFP**, **SALL-4**, **Glypican-3**, **CDX-2**, **CK19**, Pan-CK, LIN28	PLAP, CD34, GATA-3, NUT[b]	CD117, HepPar1, NSE, GFAP	**NANOG**, **Sox-2**, **Oct-3/4**, **CK7**, EMA, βhcG, CD30, CEA, vimentin
Choriocarcinoma Syncytiotrophoblastic cells Cytotrophoblastic cells	**βHCG**, inhibin, GATA-3, CD10, Pan-CK, CK8/CK18, CK19, Glypican-3, EGFR CD10, Pan-CK, CK8/CK18, CK19, CEA	PLAP, human placental lactogen, EMA, CEA Glypican-3, PLAP	Vimentin	CD30, AFP, Oct-4, NANOG, Sox-2, Sox-17, D2–40 βhcG, 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**, **SF-1**, Calretinin, vimentin	CD99, FOXL-2	Pan-CK, S100, Synaptophysin, chromogranin	EMA, PLAP, AFP, WT-1, β-catenin(n), anti-Müllerian hormone, Oct-4, SALL-4
Sertoli cell tumor	**SF-1**, **inhibin**, **FOXL2**, **anti-Müllerian hormone**, CD56, **β-catenin (n)**, vimentin	AFP, CD99, pan-CK, chromogranin, Synaptophysin, Calretinin, SOX-9	NSE, S100	EMA, PLAP, Oct-4, SALL-4
Granulosa cell tumor	**Inhibin**, **FOXL2**, **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/CK6, CK7, WT-1, Thrombomodulin (CD141), vimentin			CD31, CD34, CEA
Adenocarcinoma of collecting ducts and rete testis	Pan-CK, CK7, EMA, vimentin	**PAX-8**, WT-1, CD10	CEA	Inhibin, AFP, PLAP, Oct4, Sall-4, GATA-3, NKX3.1
Ovarian type tumors of collecting ducts and rete testis	See ovarian tumors			
Adenocarcinoma of the epididymis	Pan-CK, CK7, PAX-2, PAX-8, CEA			Oct-4, WT-1, CDX-2, NKX3.1, PSA
Melanotic neuroectodermal tumor	*Large pigmented cells:* Pan-CK, EMA, NSE, HMB45, Synaptophysin *Small cells:* NSE, HMB45, CD57, Synaptophysin, CD56	S100	GFAP Pan-CK, GFAP, PAX-5, CD99, Calretinin	

[a] SALL-4 is not a suitable marker to highlight the cells of intratubular germ cell neoplasms
[b] Positive only in hepatoid variant of yolk sac tumor [42]

Algorithm 13.1
Immunoprofile of germ cell tumors



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14 Markers and Immunoprofile of Tumors of Endocrine Organs and Neuroendocrine Tumors

Contents

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14.1 Screening Markers of Neuroendocrine Differentiation

Chromogranin, Synaptophysin, Secretogranin, NSE, S100, PGP9.5, CD56, PAX-6, INSM-1, Islet-1, and Somatostatin receptor (SSTR).

Endocrine and neuroendocrine tumors are a heterogeneous group of tumors sharing a common phenotype arising from different cells of endocrine glands or from multipotent neuroendocrine stem cells that migrated from the neural crest to different organs [1, 2].

Neuroendocrine cells and tumors derived from these cells share different common antigens and transcription factors characteristic for neuroendocrine differentiation. The immunohistochemical markers listed in this chapter are used to screen for neuroendocrine differentiation in normal or neoplastic cells; however, none of these markers is a universal marker for neuroendocrine differentiation; consequently, screening for this immunophenotype must include two or more antibodies to neuroendocrine molecules or transcription factors. In routine immunohistochemistry, chromogranin and synaptophysin are the most commonly used markers, and a mixture of both markers gives better results and superior stain intensity. The new neuroendocrine transcription factors, such as INSM-1 and Islet-1, are very helpful to confirm the neuroendocrine differentiation [3].

Neuroendocrine tumors may have epithelial or neuroectodermal histogenesis; accordingly, the absence of cytokeratin expression in tumors does not exclude the diagnosis of neuroendocrine neoplasia.

14.1.1 Chromogranin A

Chromogranin A		
Expression pattern: cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Endocrine and 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 Chromogranins are glycosylated calcium-binding acidic proteins and members of the Chromogranin/Secretogranin family that includes Chromogranin A, Chromogranin B (known as Secretogranin I), and Chromogranin C (known as Secretogranin II), located in the neurosecretory granules of neuroendocrine cells and synaptic vesicular walls. Chromogranin A is the most commonly used marker in routine immunohistochemistry. Chromogranins are expressed in almost all neuroendocrine cells and neuroendocrine tumors. The intensity of the immunostaining depends on the quantity of neurosecretory granules present in the cytoplasm of targeted cells; an example is small cell carcinoma, which actively synthesizes Chromogranin, but because of the paucity of cytoplasm and scarcity of neurosecretory granules, small cell carcinoma usually shows very weak or negative Chromogranin stain.

Diagnostic Pitfall Poorly differentiated neuroendocrine carcinomas can lose the expression of neurosecretory granules and are frequently negative for Chromogranin and Synaptophysin. To exclude the neuroendocrine differentiation, it is recommended to use other neuroendocrine transcription factors such as INSM-1 and Islet-1.

14.1.2 Synaptophysin

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, choroid plexus epithelium, 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 found in all neurons. Synaptophysin is a broad-spectrum marker for neuroendocrine cells and tumors with endocrine and neuroendocrine differentiation. Strong expression is also noted in astrocytic and ependymal tumors in addition to central neurocytoma. 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.

14.1.3 Insulinoma-Associated Protein 1 (INSM-1)

Insulinoma-associated protein 1 (INSM-1)		
Expression pattern: nuclear		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Neuroendocrine tumors (NET and NEC) of different origin – Extraskeletal myxoid chondrosarcoma	Pheochromocytoma, paraganglioma and neuroblastoma, pituitary adenoma, medullary thyroid carcinoma, Merkel cell carcinoma	Pituitary gland, thyroid C cells, pancreatic islet cells, adrenal medulla, GIT enterochromaffin cells, pineal gland
Positive control: pancreatic tissue		

Diagnostic Approach Insulinoma-associated protein 1 (INSM-1) is a transcriptional factor involved in the regulation of proliferation (repressor) and differentiation of neuroendocrine cells. INSM-1 is expressed in all neuroendocrine cells and tumors derived from these cells, including low-grade and intermediate neuroendocrine tumors (NET G1/2), small cell and large cell neuroendocrine carcinomas, paraganglioma, medullary thyroid carcinoma, and pituitary tumors (Fig. 14.1) [4–7]. Compared to other neuroendocrine markers, including chromogranin, synaptophysin, CD56, and NSE, INSM-1 showed a higher sensitivity and speci-

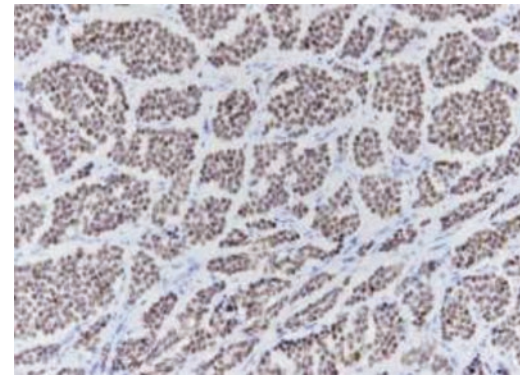


Fig. 14.1 Neuroendocrine tumor (NET G2) with strong nuclear INSM-1 expression

ficity in many studies. INSM-1 is also found to be a specific marker for extraskeletal myxoid chondrosarcoma. INSM-1 is usually negative in non-neuroendocrine epithelial tumors and in melanocytic tumors.

14.1.4 Islet-1

The human insulin gene enhancer binding protein (Islet-1/ISL-1) is a transcription factor involved in the differentiation of sympathetic neurons and neuroblasts and neuroendocrine pancreatic cells. Islet-1 is a marker for different neuroendocrine tumors in addition to thyroid medullary carcinoma, pheochromocytoma, and paraganglioma. This transcription factor is listed in detail with the markers of pancreatic tumors (Chap. 8).

14.1.5 CD56

CD56 (N-CAM) is a transmembrane neural adhesion molecule 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 a sensitive but less specific marker for neuroendocrine cells and neuroendocrine tumors, especially small cell carcinoma. CD56 is listed in detail.

14.1.6 Neuron-Specific Enolase

Neuron-specific enolase (NSE) γ-subunit		
Expression pattern: cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Neuroendocrine and neuroectodermal 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 phosphoenolpyruvate, playing a role in intracellular energy metabolism. Enolases are homo- or heterodimers composed of 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.

14.1.7 Somatostatin Receptor Type 2

Somatostatin receptor type 2 (SSTR2)		
Expression pattern: membranous		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Neuroendocrine tumors (NET G1–3) and neuroendocrine carcinoma (NEC) – Olfactory neuroblastoma – Meningioma – Follicular dendritic cell sarcoma	Pituitary adenoma, paraganglioma, pheochromocytoma, GIST, synovial sarcomas	CNS/cerebellum, neuroendocrine cells, kidney, follicular dendritic cells
Positive control: pancreas		

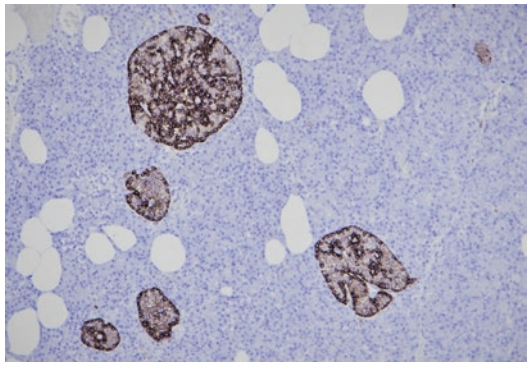


Fig. 14.2 Pancreatic tissue with exocrine and endocrine components. SSTR2 strongly stains the α- and β-endocrine cells in pancreatic islets

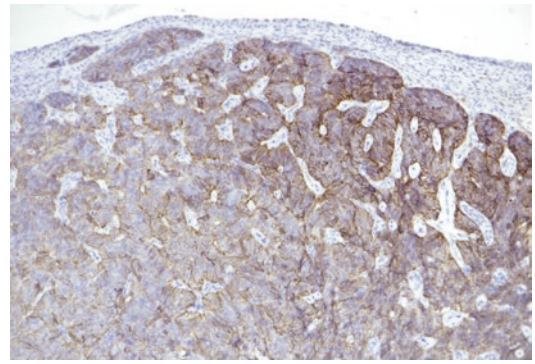


Fig. 14.4 NET G2 (atypical carcinoid) of the lung with strong membranous SSTR2 expression

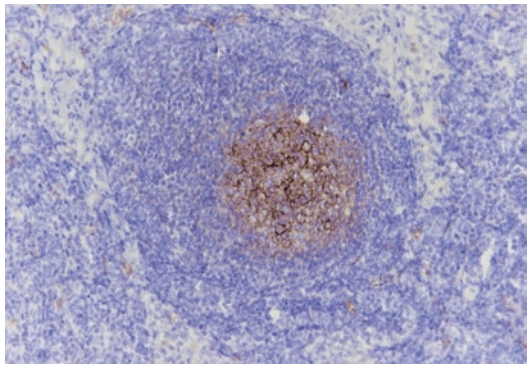


Fig. 14.3 SSTR2 highlighting the follicular dendritic cells in lymphoid germ centers

Diagnostic Approach Somatostatin receptor type 2 (SSTR2) is a transmembrane G protein-coupled receptor for somatostatin-14 and somatostatin-28. SSTR2 inhibits the secretion of several hormones and other secretory proteins in several organs with high expression levels in the cerebrum, and neuroendocrine tissue, including α- and β-cells in pancreatic islets and to a lesser degree in all neuroendocrine cells of the gastrointestinal tract (Fig.14.2) and pituitary gland in addition to kidney and follicular dendritic cells (Fig. 14.3). In routine histopathology, SSTR2 is a diagnostic marker for neuroendocrine and endocrine tumors, whereas the expression intensity decreases with the dedifferentiation of the neuroendocrine neoplasia as the highest expression levels are noted in NET G1–2 (Fig. 14.4) and frequently disappear in high-grade neuroendocrine carcinomas. Consequently, SSTR2 is a prognostic factor for related tumors and a therapeutic target of somatostatin analog-based treatment.

SSRT2 is also a diagnostic marker for other endocrine tumors, meningioma, and follicular dendritic cell sarcoma. SSTR2 is negative in schwannoma and neurofibroma.

Diagnostic Pitfalls The expression of SSTR2 is found in other tumors with similar morphology, such as synovial sarcomas and gastrointestinal stromal tumors, and must be used in a panel with more specific markers.

14.1.8 Serotonin

Serotonin (5-hydroxytryptamin, Enteramin) is a neurotransmitter molecule synthesized in the peripheral and central nervous system and enterochromaffin cells in the gastrointestinal tract. Serotonin is a neuroendocrine marker mainly used for pancreatic and gastrointestinal neuroendocrine tumors.

14.1.9 S100

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, neurilemoma, 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 The S100 proteins are a family comprising 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 specificity, and the final diagnosis must be confirmed by additional more specific markers.

Further markers for endocrine and neuroendocrine tumors are listed in detail in related chapters.

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, pituitary hormones (GH, PRL, TSH, ACTH, FSH, LH, α-SU), transcription factors (PIT-1, Tpit, GATA-2/GATA-3, and SF-1) [8].

The adenohypophysis is composed of six secretory cell types (α, β, δ, γ, ε cell), and all but one of them are able to produce only one of the anterior lobe hormones. The new classification of pituitary gland adenomas is 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): GH is a 191 amino acid single chain 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 the pituitary gland. Prolactin-producing cells may also be found in prostatic glands.
- Thyroid-stimulating hormone (TSH): TSH is a glycoprotein consisting of the β- and α-chain regulating the T4 production in the thyroid gland.
- Adrenocorticotropic hormone (ACTH): ACTH is a 39 amino acid polypeptide that acts on the cells of the adrenal cortex. Besides cells of the 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): FSH is a glycoprotein consisting of the β- and α-chain regulating folliculogenesis, spermatogenesis, and proliferation of Sertoli cells.
- Luteinizing hormone (LH): LH is a glycoprotein consisting of the β- and α-chain regulating folliculogenesis and testosterone production 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 most of the TSH-, FSH-, and LH-producing adenomas, whereas some pituitary gland adenomas exclusively express the α-SU.

14.2.3 Pituitary Transcription Factors

14.2.3.1 Pituitary Transcription Factor-1 (PIT-1, PUO1 F1)

PIT-1 is a member of the POU family of transcription factors regulating the differentiation of somatotroph, lactotroph, and thyrotroph cells of the pituitary gland and stimulating the synthesis of related hormones in these cells. Pit-1 is a marker for PIT1-lineage adenomas, including somatotroph, thyrotroph, lactotroph, and plurihormonal adenomas.

14.2.3.2 T-box Pituitary Transcription Factor (T-pit, T box 19)

T-pit is a transcription factor regulating the synthesis of pro-opiomelanocortin in corticotroph and melanotroph cells. T-pit is a marker for corticotroph adenoma and null cell adenoma (TPIT lineage adenomas).

14.2.3.3 Steroidogenic Factor 1 (SF-1)

This marker is listed in detail in a later chapter as a marker for adrenocortical tumors (see Chap. 14.6). SF-1 is strongly expressed in gonadotroph cells and is one of the markers for gonadotroph adenoma (SF-1 lineage adenoma).

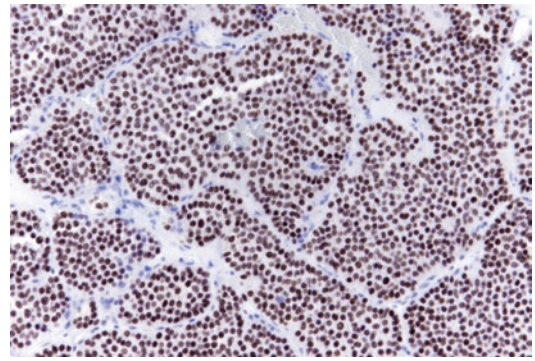


Fig. 14.5 Pituitary adenoma with strong nuclear GATA-3 expression

14.2.3.4 GATA-2

GATA-2 is a transcriptional activator involved in the regulation of endothelin-1 gene expression in endothelial cells besides the differentiation and proliferation of hematopoietic and endocrine cells. GATA-2 is homologous to GATA-3 and, in immunohistochemistry, can be detected using the same antibody.

SF-1, in association with GATA-2/GATA-3 and estrogen receptor-α, regulate the differentiation of gonadotroph cells and are characteristic markers for gonadotroph adenoma (Fig. 14.5). TSH-producing and plurihormonal adenomas can also be positive for GATA-2/GATA-3. Somatotroph, lactotroph, and null cell adenomas lack the expression of both markers [9].

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

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

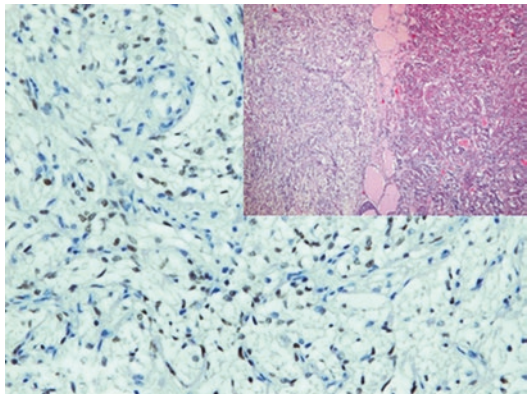


Fig. 14.6 TTF-1 staining the cells of the neurohypophysis

14.2.4.1 Thyroid Transcription Factor-1 (TTF-1)

TTF-1 was listed in detail 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. 14.6); consequently, TTF-1 is also a diagnostic marker for tumors derived from these cells, including pituicytoma and granular cell tumor of the sellar region [10, 11]. These tumors constantly lack the expression of Cytokeratins, which is important to consider in the differential diagnosis.

Immunoprofile of pituitary gland tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
A. Tumors of adenohypophysis				
Pituitary adenoma (pituitary neuroendocrine tumor) General markers	Synaptophysin, chromogranin, INSM-1, NSE Proliferation index (Ki-67): In pituitary adenoma, <3% In pituitary carcinoma, >12%	Pan-CK[a], EMA, GATA-3	CD99	Vimentin, CK5/6, CK7, CEA, Sox-10
– Somatotroph adenoma	**GH** **PIT-1**	Prolactin, TSH, FSH, LH, α-subunit, SSRT2, ER-α		
– Lactotroph adenoma	**Prolactin** **PIT-1**	α-Subunit, Galectin-3, ER-α		
– Corticotroph adenoma	**ACTH** **Tpit**	NeuroD1, α-subunit		
– Gonadotroph adenoma	**LH**, **FSH** **SF-1**, **GATA-2/GATA-3**	ER-α, α-subunit		
– Thyrotroph adenoma	**TSH** **PIT-1**	Prolactin, GATA-3, α-subunit		
– Plurihormonal adenoma		STH, TSH, LH, FSH, prolactin		
– Null cell adenoma	Nonfunctional: no endocrine hormone secretion; no expression of pituitary transcription factors			
B. Tumors of neurohypophysis				
Granular cell tumor of the sellar region (neurohypophysis)	**S100, TTF-1**	GFAP	TFE-3	Neurofilaments, **Pan-CK**, HMB45, Olig-2, Synaptophysin, chromogranin, pituitary hormones

Pituicytoma Grade 1	MAP2, S100, **TTF-1**, vimentin Proliferation index (Ki-67): 1–2%	GFAP	EMA	Synaptophysin, chromogranin, Neurofilaments, Pan-CK, pituitary hormones, Olig-2, CD34
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
Pituitary blastoma	*In small cells:* Pan-CK, EMA *In secretory cells:* Synaptophysin, chromogranin, Pan-CK Proliferation index (Ki-67): 1–60%			Oct-4
Rathke cleft cyst	Pan-CK, CK7, β-catenin			

[a]Characteristic perinuclear expression pattern in somatotroph adenoma

14.3 Tumors of the Thyroid Gland

14.3.1 Diagnostic Antibody Panel for Tumors of Follicular Cell Origin

Thyroglobulin, thyroperoxidase, TTF-1, PAX-8, IGF2BP-1, cytokeratin profile [12].

14.3.2 Markers for the Evaluation of Malignancy

CD56, cytokeratin 19, Galectin-3, HBME-1.

14.3.3 Therapy-Related and Diagnostic Markers

BRAF-$_{V600E}$, NRAS-$_{Q61R}$, Trop-2, RET, ALK [13].

14.3.4 Diagnostic Antibody Panel for Tumors of C Cell Origin

Calcitonin, TTF-1, INSM-1, Islet-1, CEA, and other markers of neuroendocrine differentiation.

14.3.4.1 Thyroglobulin

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 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. In diagnostic immunohistochemistry, it is recommended to use thyroglobulin in a panel with TTF-1 and PAX-8. Anaplastic thyroid carcinoma is usually negative for thyroglobulin. Thyroid parafollicular C cells and neoplasms originating from these cells 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.

14.3.4.2 Thyroid Transcription Factor-1 (TTF-1)

TTF-1 is mentioned in detail with the markers of pulmonary tumors (Chap. 3). In addition to pulmonary adenocarcinoma, 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 (Fig. 14.7). In tumors with unknown primary, TTF-1 is to use in a panel with PAX-8 to discriminate between primary pulmonary adenocarcinoma and thyroid carcinomas.

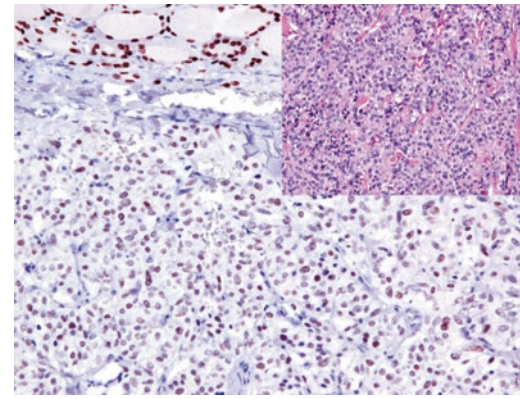


Fig. 14.7 Medullary thyroid carcinoma. TTF-1 staining nuclei of the tumor cells. Note intensive nuclear staining in normal follicular cells

14.3.4.3 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 [14]. Pulmonary parenchyma, gastrointestinal and hepatopancreatic epithelium, and corresponding tumors are constantly negative for TTF-2.

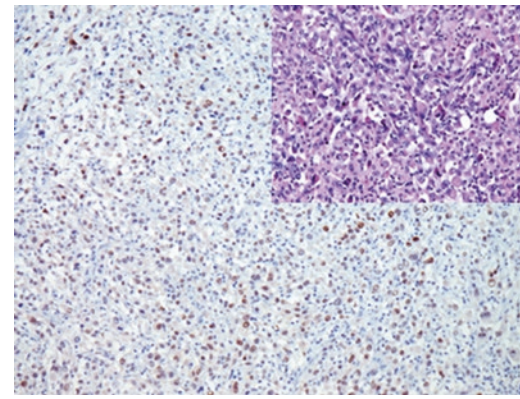


Fig. 14.8 Nuclear PAX-8 staining of anaplastic thyroid carcinoma cells

14.3.4.4 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 normal thyroid follicular cells and more than 90% of papillary and follicular thyroid carcinomas, including Hürthle cell carcinoma in addition to thyroid squamous cell carcinoma and in the majority of poorly differentiated thyroid carcinoma and more than 50% of anaplastic thyroid carcinomas (Fig. 14.8). Medullary thyroid carcinoma is usually negative for PAX-8 or shows patchy weak expression. Pulmonary adenocarcinomas and breast carcinoma are constantly negative for PAX-8. It is important to consider that parathyroid tissue and parathyroid tumors, in addition to thymoma and other different neuroendocrine tumors in the head and neck region, may be positive for PAX-8. It is important to consider that PAX-2 shows in normal and neoplastic thyroid tissue a different expression behavior than PAX-8 and cannot be used as equivalent markers. PAX-8 is listed in detail with the markers of genitourinary tumors in a previous chapter.

14.3.4.5 Insulin-Like Growth Factor 2 mRNA Binding Protein 1 (IGF2BP-1)

IGF2BP-1 is an oncofetal protein that regulates the transcription and splicing of different genes by binding to the mRNAs, including insulin-like growth factor 2 [15]. IGF2BP1 is found to be a marker for anaplastic thyroid carcinoma. Other carcinomas of thyroid follicular cells, including papil-

lary thyroid carcinoma, follicular thyroid carcinoma, and high-grade follicular cell-derived non-anaplastic thyroid carcinoma (poorly differentiated thyroid carcinoma), lack the expression of IGF2BP-1 [16].

14.3.4.6 Galectin-3

Galectin-3 is 1 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 the malignant transformation, which makes it a helpful marker for the diagnosis of different carcinoma types. Galectin-3 is positive in most papillary, follicular, and undifferentiated thyroid carcinomas, as well as in parathyroid carcinoma, head and neck squamous cell carcinoma, and colorectal and hepatocellular carcinoma.

14.3.4.7 HBME-1

HBME-1 (Hector Battifora mesothelial cell 1) was initially recognized as a marker expressed on the microvilli of normal and neoplastic mesothelial cells. HBME-1 is also expressed on the membrane of different normal epithelial and carcinoma cells, including pulmonary, breast, pancreatic, and ovarian adenocarcinomas. Furthermore, HBME-1 is strongly expressed in papillary and follicular thyroid carcinomas but negative or weakly positive in hyperplastic thyroid tissue and benign thyroid lesions. Similar to CK19, CD56, and Galectin-3, HBME-1 may be helpful in differentiating between malignant and benign encapsulated thyroid lesions.

14.3.4.8 Trophoblastic Cell Surface Antigen 2

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, lung, uterus, uterine cervix and ovaries, bladder, and prostate	Epithelium of salivary glands, pancreas, bile ducts, breast, uterus, prostate, squamous epithelium
Positive control: prostatic tissue		

Diagnostic Approach Trophoblast cell surface antigen 2 (Trop-2), also known as tumor-associated calcium signal transducer 2, is a type I transmembrane glycoprotein functioning as a calcium signal transducer. Low baseline Top-2 expression is found in different normal tissue types such as the breast, pancreas, ovaries, lung, and kidney. During malignant transformation, the expression of Trop-2 is upregulated, and overexpression of Trop-2 is noticed in different carcinoma types, including gastrointestinal, pulmonary, genitourinary, and breast carcinomas [17]. In the majority of tumors, the overexpression of Trop-2 correlates with aggressive behavior and poor prognosis.

In routine histopathology, Trop-2 is a helpful marker for the diagnosis of different histological types of papillary thyroid carcinomas. More than 90% of papillary thyroid carcinomas express Trop-2, while benign thyroid nodules, follicular adenomas, and follicular carcinomas usually lack the expression of this protein [18]. Trop-2 can be used in combination with CD56 and CK19.

As a cell surface protein, Trop-2 is an interesting target for specific humanized therapeutic antibodies and specific inhibitors in different carcinoma types exhibiting Trop-2 overexpression, such as triple-negative breast carcinoma (Fig. 14.9).

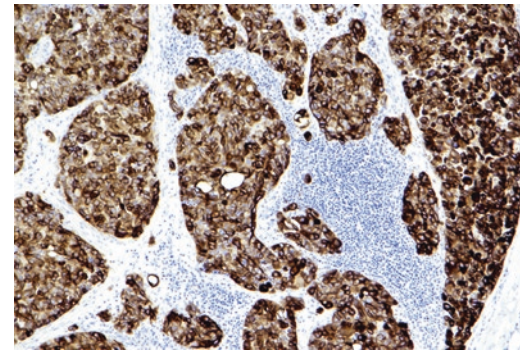


Fig. 14.9 Metastatic triple-negative breast carcinoma exhibiting Trop-2 overexpression

14.3.4.9 CD44v6

CD44v6 is an isoform of CD44 (heparan sulfate proteoglycan), a surface glycoprotein functioning as a cell-to-cell and cell-to-matrix mediator. CD44 is expressed in different carcinoma types, including breast, colonic, hepatocellular, and renal cell carcinomas, in addition to papillary and follicular thyroid carcinoma. In combination with other markers, CD44v6 can be a helpful marker to differentiate between papillary carcinoma and other benign thyroid lesions mimicking this carcinoma type. CD44 is also helpful in differentiating between reactive urothelium and carcinoma in situ (see respective section).

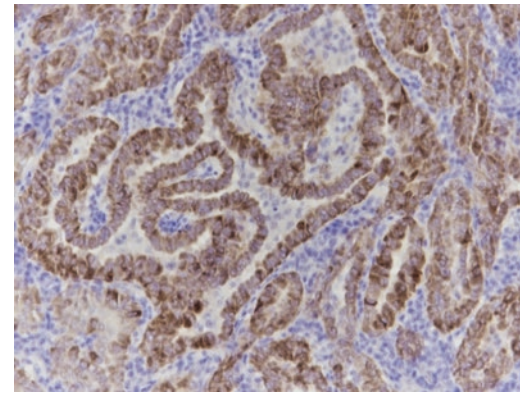


Fig. 14.10 Papillary thyroid carcinoma exhibiting strong cytoplasmic staining with the antibody to BRAF-V600E

14.3.4.10 BRAF

The RAF (**r**apidly **a**ccelerated **f**ibrosarcoma) kinase family includes three isoforms, ARAF, BRAF, and CRAF, which are cytoplasmic serine–threonine kinases that play an important role in the RAS–RAF–ERK kinase signaling pathway as the mitogen-activated protein kinase (MAPK) cascade. Among the three RAF kinases, mutations occur mainly in the BRAF gene located on chromosome 3, causing the activation of the MAPK signaling pathway and uncontrolled kinase activity affecting cell proliferation and differentiation [19]. BRAF mutations are among the most common mutations in human malignancies, found in ~50% of malignant melanoma, ~70% of papillary thyroid carcinoma, up to 10% of colorectal adenocarcinomas, >95% of hairy cell leukemia, ~65% of Langerhans cell histiocytosis, and >90% of papillary craniopharyngioma. About 60 variant mutations are described within the BRAF gene, whereas the most common mutation occurs at position 1799 in the nucleotide sequence, causing the substitution of thymine to adenine resulting in a valine to glutamic acid replacement in the encoded amino acid sequence at residue 600 (V600E). The BRAF-V600E variant makes ~99% of all BRAF mutations associated with papillary thyroid carcinoma and is found in about 50% of adult papillary thyroid carcinoma and ~95% of tall cell papillary thyroid carcinoma. BRAF-V600E negative papillary thyroid carcinoma is frequently associated with other mutations within the genes encoding further molecules of the MAPK signaling pathway, including RET, NTRK, and ALK. Different BRAF mutation variants are also found in poorly differentiated and anaplastic thyroid carcinomas but are rarely associated with radiation-induced thyroid carcinomas and are not characteristic for follicular thyroid carcinoma. The BRAF-V600E mutation is absent in follicular and Hürthle cell carcinoma as well as in medullary thyroid carcinoma.

In routine immunohistochemistry, the mutated V600E amino acid sequence can be detected using different specific antibodies and considered as a diagnostic marker and therapeutic target (Fig. 14.10).

Diagnostic Pitfalls The available antibodies can only detect a specific mutated amino acid sequence, mainly the BRAF-V600E variant. To detect other possible variants, the molecular sequencing of the complete BRAF gene is required.

14.3.4.11 RAS

The Ras proteins (KRAS, HRAS, and NRAS) are a group of closely related proteins with high sequence homology expressed in all mammalian cells and encoded by different genes discussed in Chap. 35. In thyroid tumors, RAS mutations (mostly NRAS) are described as the second most common genetic mutations and play an important role in thyroid oncogenesis. The NRAS mutations are mainly found in association with thy-

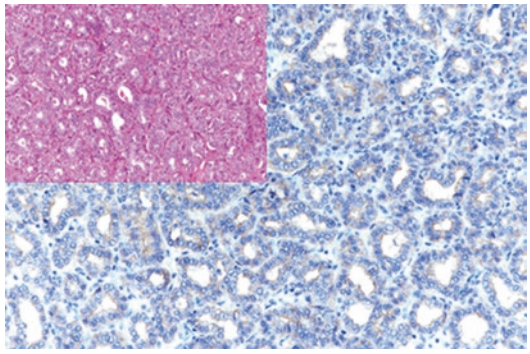


Fig. 14.11 Papillary thyroid carcinoma exhibiting moderate cytoplasmic staining with the antibody to NRAS-Q61R

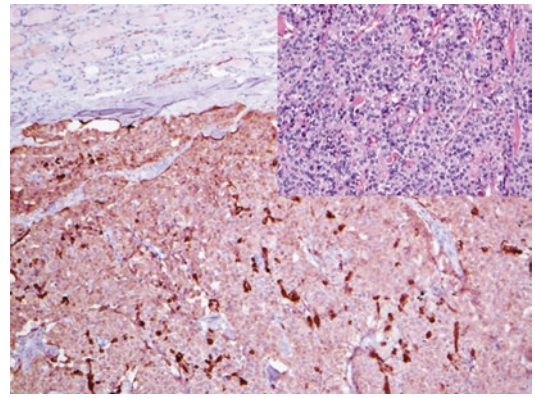


Fig. 14.12 Medullary thyroid carcinoma exhibiting cytoplasmic expression of calcitonin in the tumor cells

roid tumors with follicular morphology, including follicular adenoma, 40–50% of follicular carcinoma, and 10–20% of follicular variant of papillary thyroid carcinoma. The NRAS-Q61R mutation is the most common variant found in up to 65% of NRAS mutated cases. The mutated NRAS-Q61R protein can be effectively detected by immunohistochemistry using specific antibodies (Fig. 14.11) [20].

14.3.4.12 Calcitonin

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 phosphorus 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 (Fig. 14.12). Tumors originating from the thyroid follicular cells are constantly negative for calcitonin but also positive for TTF-1 and PAX-8. Best stain results are obtained using monoclonal antibodies.

Diagnostic Pitfalls Rare cases of neuroendocrine tumors such as neuroendocrine carcinoma of the larynx and pheochromocytoma are reported to be positive for calcitonin, whereas the latter is 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, $BRAF\text{-}_{V600E}$
Follicular thyroid carcinoma/oncocytic carcinoma (Hürthle cell carcinoma)	**Thyroglobulin**, thyroid peroxidase, **TTF-1**, **PAX-8**, CK7, CK8, CK18, CD44V6, S100	Galectin-3, HBME1, E-cadherin, bcl-2, vimentin	CK19, $NRAS\text{-}_{Q61R}$	Calcitonin, CK5/6, CK20, Islet-1, INSM-1, Trop-2, CEA, PAX-2, $BRAF\text{-}_{V600E}$

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, **BRAF-$_{V600E}$**, CD44[a], vimentin	CD15, CK17, CD34, ER, PgR	CK20, CEA, calcitonin, Islet-1, INSM-1, CD56[a], Synaptophysin, chromogranin, PAX-2
Follicular-derived carcinoma, high grade (poorly differentiated thyroid carcinoma)	**Thyroglobulin**, thyroid peroxidase, **TTF-1**, **PAX-8**, Pan-CK, Galectin-3, CD44V6	Vimentin, bcl-2	p53	CK5/6, CK19, CK20, calcitonin, INSM-1
Anaplastic thyroid carcinoma	Pan-CK, CK8/18, **IGF2BP-1**	CK19, **PAX-8**, CEA, p53, cyclin D1, CD10, vimentin	TTF-1, EMA, Galectin-3, bcl-2	Thyroglobulin, calcitonin, INSM-1
Medullary thyroid carcinoma	**Calcitonin**, **INSM-1**, **Islet-1**, 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, BRAF-$_{V600E}$
Cribriform morular thyroid carcinoma	*Cribriform component:* TTF-1, β-catenin *Morulae:* CK5, CD5, CD10, CDX-2		PAX-8, ER, PgR	Thyroglobulin, CK20, calcitonin, BRAF TTF-1, PAX-8, p40, p63
Carcinoma showing thymus-like differentiation (CASTLE)/ intrathyroid thymic carcinoma	Pan-CK CK5/14, p63, p40, CD5, CD117			TTF-1, calcitonin
Spindle epithelial tumor with thymus-like differentiation (SETTLE)	CK7	CD117		TTF-1, calcitonin, CD5, S100
Primary squamous cell carcinoma of the thyroid	CK5/6/14, p63, p40, **PAX-8**		TTF-1	
Hyalinizing trabecular tumor	**Thyroglobulin**, **TTF-1**, Ki-67 (MIB-1 clone)[b]	CK 7, Galectin-3		

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

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, except for chronic lymphocytic thyroiditis (Fig. 14.13)
- **Galectin-3:** positive in PTC and follicular carcinoma but negative in benign thyroid tissue
- **CD56:** negative in PTC but positive in benign thyroid tissue, BPH, and FN (Fig. 14.14) [21]
- **p63:** focal expression in PTC; constantly negative in non-PTC lesions
- **Trop-2:** positive in >90 PTC but negative in follicular adenoma/carcinoma
- **HBME-**1: positive in PTC and follicular carcinoma but negative or weakly positive in benign thyroid tissue
- **BRAF-V600E:** positive in ~50% PTC but negative in FN and BPH (Fig. 14.10)
- **CD44:** positive in PTC and follicular carcinoma but negative or weakly positive in BPH and benign thyroid tissue

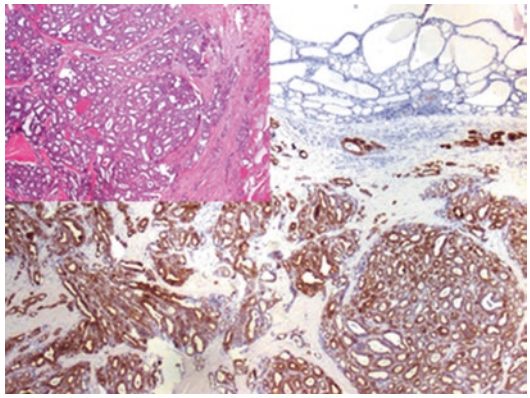


Fig. 14.13 CK19 highlighting the cells of papillary thyroid carcinoma. Normal thyroid tissue lacks CK19 expression

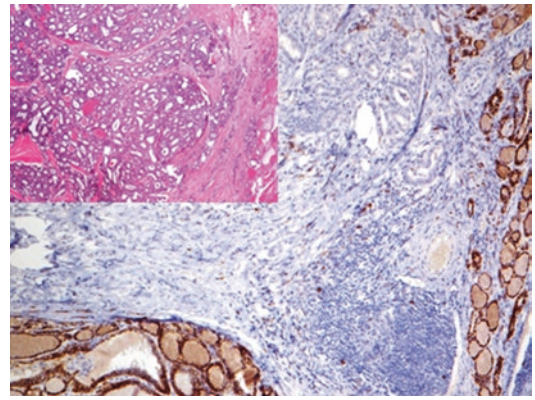


Fig. 14.14 CD56 staining normal thyroid tissue, whereas areas infiltrated by papillary thyroid carcinoma lack CD56 expression

14.4 Tumors of the Parathyroid Gland

14.4.1 Diagnostic Antibody Panel for Parathyroid Neoplasms

Parathyroid hormone, PAX-8, GATA-3, CD4, Thyroglobulin, TTF-1 [22].

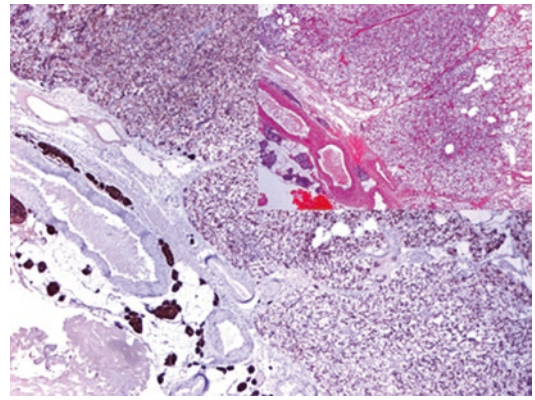


Fig. 14.15 Parathyroid hormone labeling parathyroid tissue and cells of parathyroid adenoma

14.4.1.1 Parathyroid Hormone

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 serum. Antibodies to PTH and related peptides are specific markers for the diagnosis of parathyroid neoplasms. PTH is helpful in recognizing ectopic parathyroid tissue and tumors, which may be situated in the mediastinum or intrathymic (Fig. 14.15).

Diagnostic Pitfalls Parathyroid chief cells usually rapidly discharge PHT after the synthesis, which may cause false negative immunohistochemical results. More challenging are nonsecretory clear cell parathyroid carcinomas, which may resemble metastatic renal cell carcinoma or any

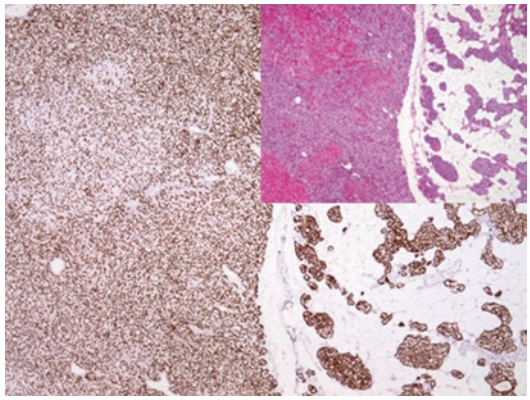


Fig. 14.16 GATA-3 staining cells of the suppressed parathyroid gland (right) and neighboring parathyroid adenoma

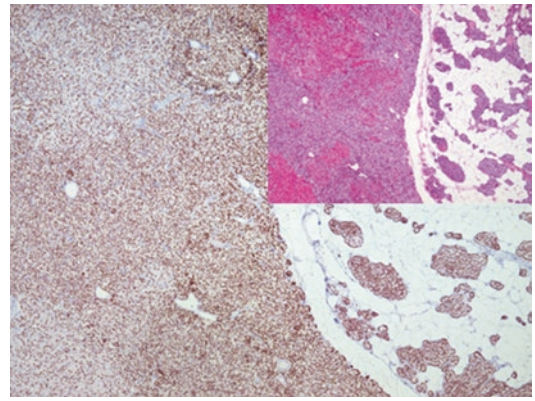


Fig. 14.17 PAX-8 staining cells of the suppressed parathyroid gland (right) and neighboring parathyroid adenoma

other clear cell carcinoma. The diagnostic panel for thyroid/parathyroid tumors must include thyroid and parathyroid hormones in addition to other differentiation markers such as PAX-8 and GATA-3.

14.4.1.2 Parathyroid Hormone-Related Peptide

This polypeptide (PtHrP) is a member of the parathyroid hormone family, also involved in calcium metabolism, and regulates the endochondral bone development. Antibodies to PtHrP stain parathyroid cells and parathyroid tumors in addition to several other malignant tumors such as breast carcinoma, cholangiocarcinoma, and transitional cell carcinoma, especially poorly differentiated types. PtHrP can also be used as a marker to discriminate between cholangiocarcinoma and metastatic colorectal adenocarcinoma [23, 24].

14.4.1.3 PAX-8, GATA-3, and CD4

Both transcription factors were listed in detail in previous chapters as markers for breast, renal, and urinary tract tumors (Chaps. 10 and 12.1). PAX-8 and GATA-3 also label 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. 14.16 and 14.17) [25]. It is important to remember that PAX-8 labels also thyroid follicular cells and tumors.

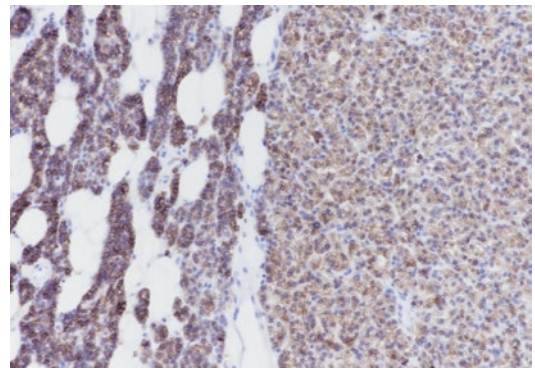


Fig. 14.18 Parathyroid gland (left) and parathyroid adenoma (right) with strong membranous CD4 expression in both normal and neoplastic cells

CD4 is a marker for T lymphocytes listed. CD4 labels also the chief cells of the parathyroid gland. Parathyroid adenomas and carcinomas are also positive for CD4, and the expression intensity correlates with the differentiation grade of the neoplasia (Fig. 14.18).

Immunoprofile of parathyroid tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Parathyroid adenoma/ parathyroid lipoadenoma	**PTH**, Synaptophysin, chromogranin, Neurofilaments, Pan-CK, CK8, CK18, CK14[a]. PAX-8, GATA-3, CD4 Proliferation index (Ki-67): <5%	CK19, RCC (gp200), vimentin	Cyclin D1, calcitonin, CK7, CK20	**TTF-1**, thyroglobulin, INSM-1, CD56, CK5/6/14

Atypical parathyroid tumor and parathyroid carcinoma	Synaptophysin, chromogranin, Neurofilaments, PGP9.5, Pan-CK Proliferation index (Ki-67): >6%	**PTH**, CK19, PAX-8, GATA-3, cyclin D1[b], CD4, vimentin	Calcitonin, Galectin-3, p53, CK7	Thyroglobulin, CK5/6/14, CK20, TTF-1, CD56[c], INSM-1

[a]Negative in parathyroid carcinoma
[b]See Fig. 14.19
[c]May be positive in oxyphil parathyroid adenoma

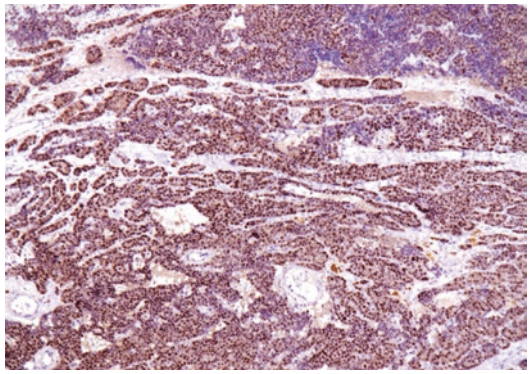


Fig. 14.19 Parathyroid carcinoma with nuclear Cyclin D1 expression

14.5 Pancreatic Endocrine Tumors

14.5.1 Diagnostic Antibody Panel for Pancreatic Endocrine Tumors

Islet-1, PDX-1, insulin, gastrin, glucagon, somatostatin receptor, vasoactive intestinal polypeptide (VIP) and human pancreatic polypeptide (hPP), PAX-6, PAX-8, progesterone receptors, and general screening neuroendocrine markers.

The immunophenotype of pancreatic endocrine tumors and the description of related immunohistochemical markers are listed in the chapter on pancreatic tumors (see Chap. 8).

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 [26].

14.6.1.1 Adrenal 4 Binding Protein/Steroidogenic Factor-1

Adrenal 4 binding protein/steroidogenic factor-1 (SF-1, Ad4BP)		
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), gonadotroph pituitary adenoma	Adrenal cortex, ovarian stromal cells, Sertoli cells, red pulp sinus cells of the spleen, gonadotrophic cells in the anterior pituitary gland
Positive control: adrenal gland		

Diagnostic Approach Adrenal 4 binding protein (Ad4BP), also known as steroidogenic factor 1 (SF-1), is a member of the orphan nuclear receptor family and is a transcriptional factor regulating steroidogenesis.

Initially, SF1 is expressed in the developing urogenital ridge; in adult tissue, it is constantly expressed in the pituitary gland, neurons of the ventromedial nucleus of the hypothalamus, adrenal cortex, testicular Sertoli and Leydig cells, granulosa cells, and different tumors derived from these tissue and cell types (Fig. 14.20). 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

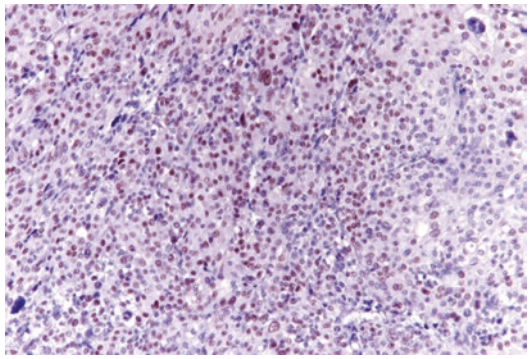


Fig. 14.20 Nuclear SF-1 expression in the cells of adrenocortical adenoma

Calretinin and the co-expression of Vimentin support the adrenocortical origin of the tumor [27–29]. SF-1 is also helpful for the classification of pituitary adenomas as it is selectively expressed in gonadotroph adenomas.

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

14.6.1.2 DAX-1

DAX-1 is a nuclear receptor protein and a member of the orphan nuclear receptor family encoded by the NR0B1 gene (**n**uclear **r**eceptor subfamily **0** group **B** member **1**) located on chromosome Xp21 acting as a suppressor for the steroid hormone production in the adrenal cortex by inhibiting the effect of the steroidogenic factor 1 (SF-1) [30, 31]. Furthermore, DAX-1 plays an active role in the development of the 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 adrenocortical tumors and 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 [32, 33].

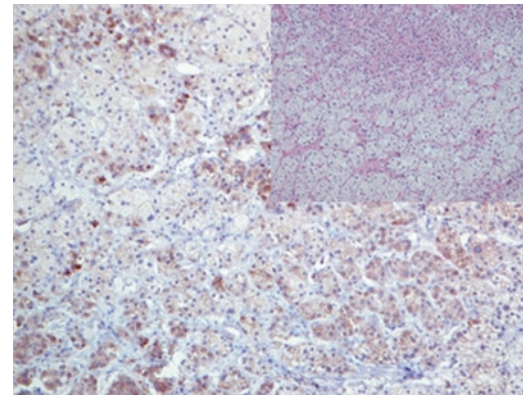


Fig. 14.21 Adrenocortical adenoma exhibiting cytoplasmic expression of inhibin

14.6.1.3 Inhibin

Inhibin is a glycoprotein hormone listed 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 fasciculate and reticulares of the cortex. The adrenal medulla lacks the expression of Inhibin.

Besides testicular and ovarian sex cord tumors, Inhibin is an important marker for benign and malignant adrenocortical tumors (Fig. 14.21) [34].

14.6.1.4 Melan A

Melan A is listed in the chapter on melanoma markers (Chap. 21). Melan A is also a marker for adrenal cortex cells and adrenocortical tumors. Characteristic for adrenal cortical cells and adrenocortical tumors is the strong granular cytoplasmic expression pattern of Melan A.

14.6.1.5 CYP11B2

CYP11B2 is an enzyme that catalyzes the biosynthesis of aldosterone, normally expressed in the zona glomerulosa of the adrenal cortex. The immunohistochemical stain of the adrenal gland with the CYP11B2-specific antibodies labels the functional areas of aldosterone production in the adrenal cortex. CYP11B2 is an immunohistochemical marker for aldosterone-producing adrenal cortical adenoma.

CYP11B1 is a further enzyme of the adrenal cortex that catalyzes the biosynthesis of cortisol, physiologically expressed in the zona fasciculata/reticularis and related pathological lesions.

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

14.6.2.1 Diagnostic Antibody Panel for Pheochromocytoma and Tumors of Extra-Adrenal Paraganglia

Chromogranin, Synaptophysin, INSM-1, Islet-1, CD56, GATA-3, NSE, S100.

These antibodies were listed in detail in other chapters (Figs. 14.22, 14.23 and 14.24).

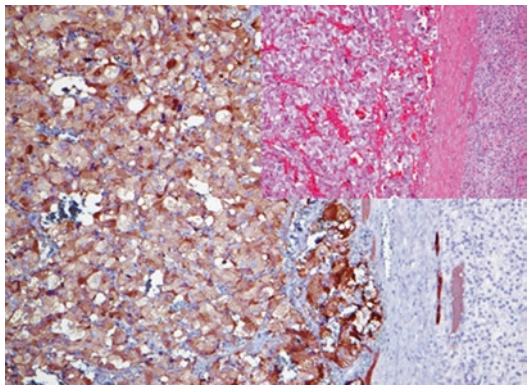


Fig. 14.22 Pheochromocytoma with strong CD56 expression

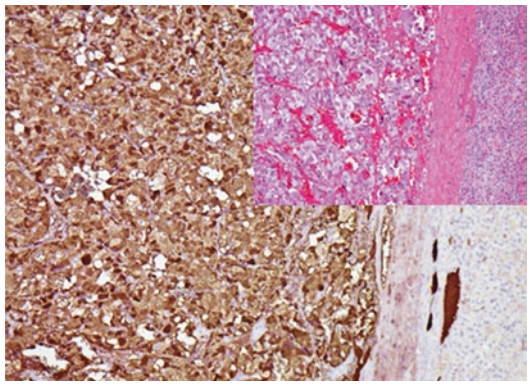


Fig. 14.23 Pheochromocytoma exhibiting strong Synaptophysin expression

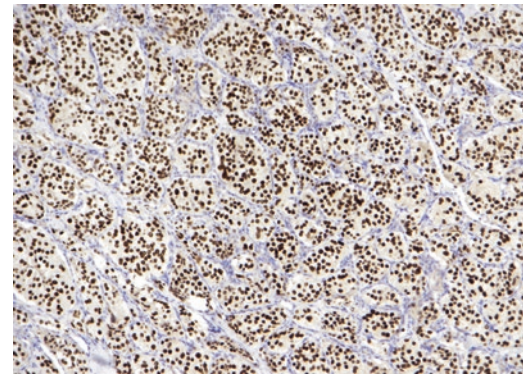


Fig. 14.24 Extra-adrenal paraganglioma with strong nuclear INSM-1 expression in the tumor cells

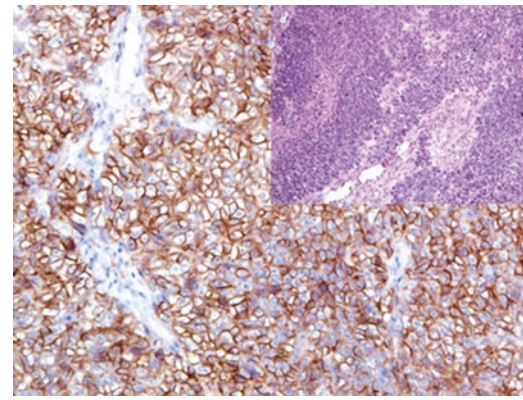


Fig. 14.25 CD56 staining the membrane of olfactory neuroblastoma cells

14.6.2.2 Diagnostic Antibody Panel for Neuroblastoma

Chromogranin, Synaptophysin, INSM-1, Islet-1, CD56, NSE, NB84, PGP9.5, PHOX2B, GATA-3, CD117, S100, and neurofilaments (Fig. 14.25) [35].

The tumors mentioned above are of neuroectodermal origin and usually lack the expression of cytokeratins, and the general neuroendocrine markers are characteristic for these tumors (Figs. 14.22, 14.23 and 14.24).

Neural Cell Adhesion Molecule (CD56)

CD56 is a member of the immunoglobulin superfamily clustered as CD56 functioning as a media-

tor of cell-to-cell adhesion and cell-to-matrix interaction, involved in the regulation of cell adhesion, synaptic plasticity, migration, proliferation, differentiation, and apoptosis. CD56 is an important molecule for developing and differentiating 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. 14.24). CD56 is a helpful wide-spectrum marker for neural and neuroendocrine tumors. Furthermore, CD56 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.

NB84

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 human neuroblastoma cells. It stains about 100% of differentiated and about 90% of undifferentiated neuroblastomas. NB84 is more sensitive but less specific than Synaptophysin [36]. For an appropriate diagnosis of adrenal or extra-adrenal tumors, a panel of three to four of the antibodies mentioned above 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 that includes CD99 and different cytokeratins is required. It is essential to consider that about 5% of undifferentiated neuroblastomas lack the expression of NB84.

Paired Mesoderm Homeobox Protein 2B (PHOX2B)

PHOX2B is a transcription factor encoded by the PHOX2B gene on chromosome 4p13, essential for the differentiation and maturation of sympathetic neurons and chromaffin cells. The expression of PHOX2B is limited to the cells of the autonomic nervous system, mainly to the cells originating from neural crest precursors. The expression of PHOX2B is demonstrated in all neuroblastoma, ganglioneuroblastoma, and ganglioneuroma cases as well as in about 40% of paragangliomas (Fig. 14.26).

Other small round blue cell tumors such as rhabdomyosarcoma, Ewing sarcoma, and different lymphoma types, in addition to epithelial neuroendocrine neoplasms, are negative for PHOX2B. Unlike GATA-3, epithelial tumors such as squamous cell carcinoma, different types

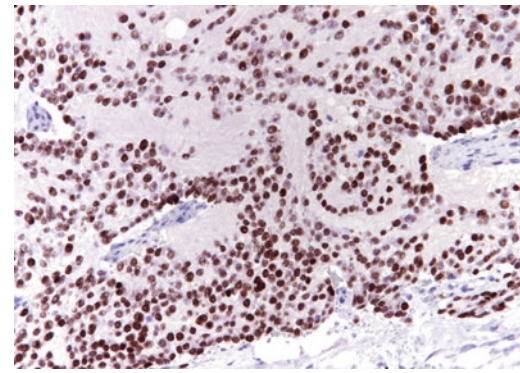


Fig. 14.26 Nuclear PHOX2B expression in the cells of neuroblastoma

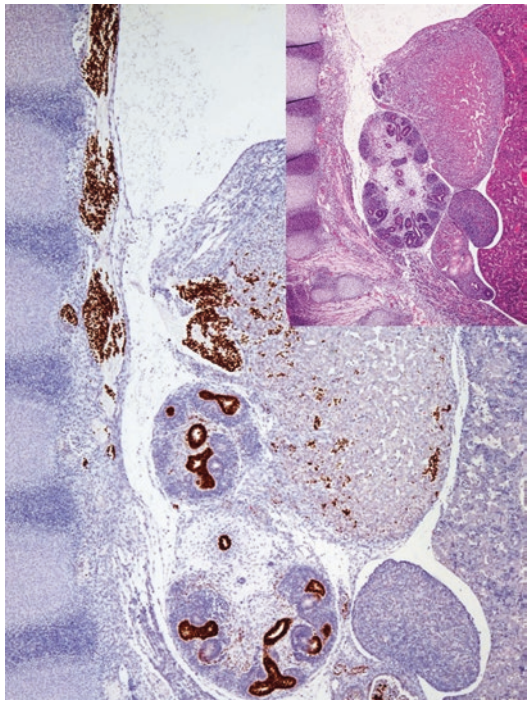


Fig. 14.27 Section through a 12-week embryo showing paravertebral sympathicoblasts of neural crest labeled by GATA-3. These sympathicoblasts migrate into the dorsomedial part of the primordial adrenal gland to form the adrenal medulla. GATA-3 is also highlighting the urothelium of the collecting system of the kidney

of adenocarcinomas, and transitional cell carcinoma are negative for PHOX2B [37, 38].

GATA-3

This transcription factor was listed in previous chapters as a marker for breast, salivary gland, parathyroid, and urothelial tumors (Chaps. 10). GATA-3 strongly labels the fetal sympathicoblasts and the chromaffin cells of the adrenal medulla and sympathetic paraganglia derived from sympathicoblasts (Fig. 14.27). Consequently, GATA-3 is a marker for tumors of the adrenal medulla and extra-adrenal paraganglia, including pheochromocytoma and neuroblastoma (Figs. 14.28, 14.29 and 14.30). Very low GATA-3 expression is also found in the adrenal cortex and adrenocortical tumors.

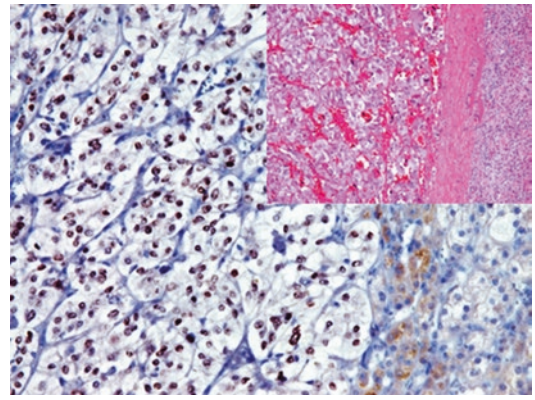


Fig. 14.28 GATA-3 staining the nuclei of pheochromocytoma cells

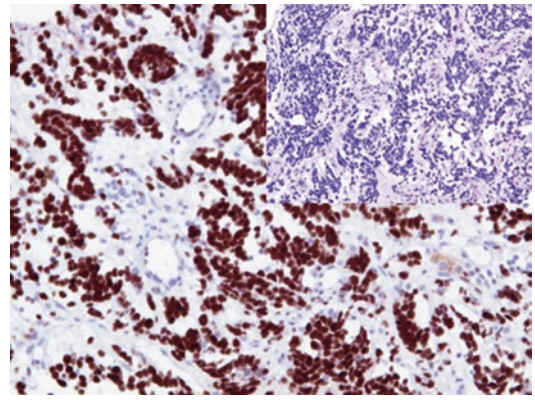


Fig. 14.29 GATA-3 highlighting the nuclei of neuroblastoma cells in an adrenal gland biopsy

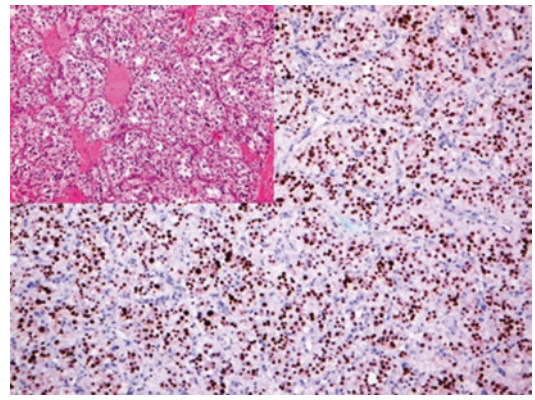


Fig. 14.30 Paraganglioma. Tumor cells exhibiting nuclear GATA-3 expression

Immunoprofile of adrenal gland tumors and tumors of extra-adrenal paraganglia				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Adrenocortical adenoma/carcinoma	**Adrenal 4 binding protein (SF-1)**, **inhibin**, Melan-A Proliferation index (Ki-67): in adrenocortical adenoma, <2.5% In adrenocortical carcinoma, >4%	Synaptophysin, NSE, Calretinin, CD56, CYP11B2[a], vimentin	Pan-CK, CK5, bcl-2	CK7, CK19, CK20, EMA, CEA, CD10, INSM-1, chromogranin, RCC, PAX-8
Pheochromocytoma and tumors of extra-adrenal paraganglia (sympathetic and parasympathetic paraganglioma):	**INSM-1**, Islet-1, CD56, **chromogranin**, Synaptophysin, NSE Proliferation index (Ki-67): in benign pheochromocytoma, <2% In malignant pheochromocytoma[c], >3%	**S100**[b], SSTR2, GATA-3, bcl-2	GFAP, vimentin, pan-CK, calcitonin	CK5/CK6, CK7, CK19, CK20, EMA, D11, PAX-8, CA IX, Melan-A
Neuroblastoma	**INSM-1**, **CD56**, NSE, Neurofilaments, PGP9.5, NB84, **GATA-3**, **PHOX2B**, vimentin	**S100**, ALK, Synaptophysin, chromogranin, CD117, Calretinin	Pan-CK, WT-1	CK5/CK6, CK7, CK20, CD99
Olfactory neuroblastoma (esthesioneuroblastoma)	**CD56**, CD57, NSE, Fli-1, PGP9.5, Neurofilaments, NB84, **S100**	SSTR2, Bombesin, Synaptophysin, chromogranin	Pan-CK	GATA-3, PHOX2B, EMA, WT-1, CD99, PAX-7

[a]Labels aldosterone-producing adrenal cortical tumors
[b]Strong nuclear and cytoplasmic S100 stain in sustentacular cells (Fig. 14.31)
[c]This criterion cannot be used exclusively to define malignancy

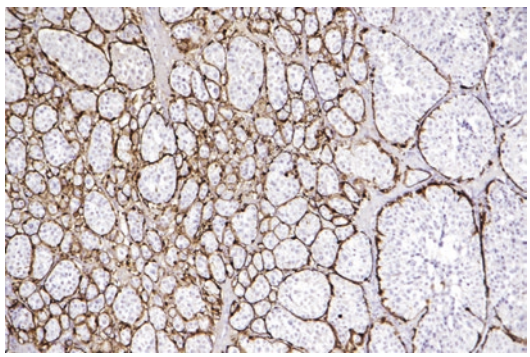


Fig. 14.31 Extra-adrenal paraganglioma showing strong S100 expression in sustentacular cells

14.7 Diagnostic Antibody Panel for the Classification of Neuroendocrine Neoplasms: Neuroendocrine Tumors (NET G1, G2, G3) and Neuroendocrine Carcinomas (NEC) (Small and Large Cell Types)

The general neuroendocrine markers, including INSM-1, Chromogranin, Synaptophysin, NSE, S100, CD56, and Secretogranin and Somatostatin receptor (SSTR), are characteristic markers for

neuroendocrine neoplasms [27, 39, 40]. The mitotic proliferation and index estimated by PHH3 and Ki-67 are essential for tumor grading. The tissue-specific transcriptional factors such as CDX-2, SATB-2, PDX-1, PAX-6, Istet-1, TTF-1, OTP, and NKX3.1 in addition to the cytokeratin profile are helpful markers to ascertain the site of the primary tumor (see the chapter below).

14.8 Approach to the Diagnosis of Neuroendocrine Neoplasms (NET, NEC)

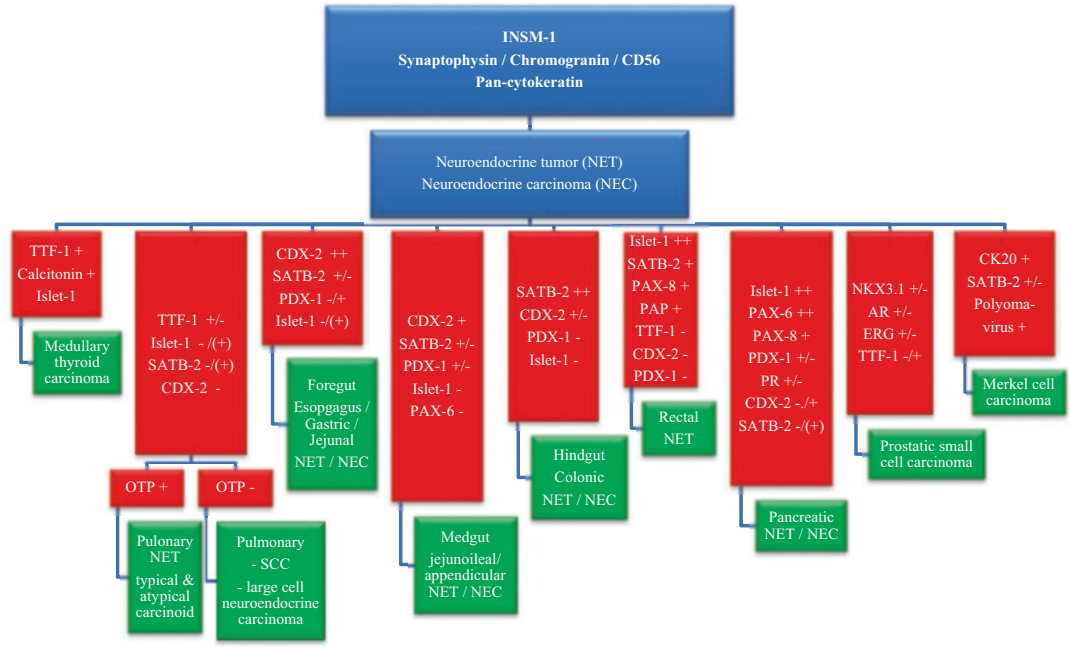
Neuroendocrine neoplasms are tumors that arise from the neuroendocrine cells that migrated from the neural crest and include the cells of the adenohypophysis, thyroid C cells, bronchial K cells, gastroenteropancreatic neuroendocrine cells, paraganglionic cells and cells of the adrenal medulla, urogenital neuroendocrine cells, and cutaneous Merkel cells. Neuroendocrine neoplasms are divided into two main groups with distinct morphological, genetic, biological, and clinical features: [41]

- Well-differentiated neuroendocrine tumors (NET) include three differentiation categories G1, G2, and G3.
- Neuroendocrine carcinomas (NEC) include both small and large cell types and always are G3.

For the optimal diagnosis of this heterogeneous group and to determine the most probable site of origin, it is recommended to consider the following points

- The morphological features of the tumor: neuroendocrine tumors (NET G1, G2, or G3) show the characteristic neuroendocrine growth pattern that includes insular, trabecular, acinar, or glandular growth patterns. Neuroendocrine carcinoma (NEC) shows an undifferentiated anaplastic morphology.
  - The epithelial nature of the tumor can be confirmed by the expression of one of the pan-cytokeratin markers or another cytokeratin like CK7/19/20, whereas the neuroendocrine tumors usually exhibit a characteristic dot-like or paranuclear cytoplasmic expression pattern. It is important to remember that neuroendocrine neoplasms of neuronal/neuroectodermal origin, such as paraganglioma, pheochromocytoma, and neuroblastoma, usually lack the expression of cytokeratins.
  - The neuroendocrine differentiation must be confirmed using one or more neuroendocrine markers (INSM-1, synaptophysin, chromogranin, CD56).
  - A panel of tissue-specific markers can be used to define the most probable site of origin (see Algorithm 14.1) [42–45].
  - Several markers may be useful to differentiate between NET G3 and NEC (see endocrine tumors of the pancreas; Chap. 8).

Classification and immunophenotype of neuroendocrine neoplasms				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Neuroendocrine tumors NET G1 (carcinoid) – NETG2 (atypical carcinoid) – NET G3	**INSM-1**, **Islet-1**, chromogranin, synaptophysin, somatostatin, CD56, Secretogranin, NSE, PGP9.5, Leu7	Pan-CK, CK8, CK18, CK19, S100, SSTR2		CK5/CK6, CK7, CK20
Neuroendocrine carcinoma – NEC of small cell type – NEC of large cell type	Proliferation/mitotic index (Ki-67 and PHH3) NET G1 (carcinoid)—proliferation index (Ki-67): <3%; mitotic rate: <2/2 mm^2 NET G2 (atypical carcinoid)—proliferation index (Ki-67): 3–20%; mitotic rate: 2–20/mm^2 NET G3 and NEC—proliferation index (Ki-67): >20%; mitotic rate:>20/2 mm^2			



Algorithm 14.1 Differential diagnosis of neuroendocrine neoplasms

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15 Markers and Immunoprofile of Mesothelioma and Tumors of the Peritoneum

Contents

15.1 Diagnostic Antibody Panel for Mesothelial Tumors

Markers of mesothelial cells: Calretinin, Thrombomodulin (CD141), Mesothelin, Podoplanin (D2–40), WT-1, CK5/CK6/CK7/CK14.

Markers for the differentiation between benign mesothelial cells and malignant mesothelioma: BAP-1, Glut-1, IMP-3, h-Caldesmon, E-Cadherin, Osteonectin, CD56, 5-hmC, L1CAM (CD171), CD146 [1–4].

15.2 Diagnostic Antibody Panel for Epithelial Tumors of Müllerian Type

Cytokeratin profile, CEA, CA125, PAX-8, WT-1, p53, p16. The expression of p16 is regulated by the activity of the retinoblastoma gene (Rb), 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 (see Sect. 11.6) [5, 6].

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15.3 Diagnostic Antibody Panel for Smooth Muscle Tumors

Actin, h-Caldesmon, Calponin, Smoothelin, and cytokeratin profile.

15.4 Diagnostic Antibody Panel for Miscellaneous Primary Peritoneal Tumors

CD34, CD99, DOG-1, Actin, h-Caldesmon, Desmin, ALK, and cytokeratin profile.

15.4.1 Calretinin

Calretinin		
Expression pattern: cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Mesothelioma/adenomatoid tumor – Adrenocortical tumors – Sex cord-stromal tumors	Squamous cell carcinoma, ameloblastoma, thymic tumors, transitional cell carcinoma, colonic carcinoma, granular cell tumor, fibrosarcoma, mesonephric adenocarcinoma, PEComa, myxoid chondrosarcoma, synovial sarcoma, desmoplastic small round cell tumor, cardiac/atrial myxoma, lipogenic tumors, mast cell lesions, neurocytoma, neuroblastoma	Central and peripheral neuronal 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, and apocrine 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, central, and peripheral neurogenic tissue types. Calretinin is strongly expressed in normal and neoplastic mesothelial cells and is considered an important sensitive marker for mesothelial tumors (Figs. 15.1 and 15.2). Calretinin is a marker for 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. Calretinin also labels normal and neoplastic mast cells. About one-third of squamous cell carcinomas also show 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 [7, 8]. Moreover, Calretinin is an optimal marker to highlight the ganglion cells in colonic biopsies for the diagnosis of Hirschsprung disease.

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. In pleural biopsies, it is important to consider that submesothelial fibroblasts are usually positive for pan-Cytokeratin and other keratins that may be a source of misinterpretation.

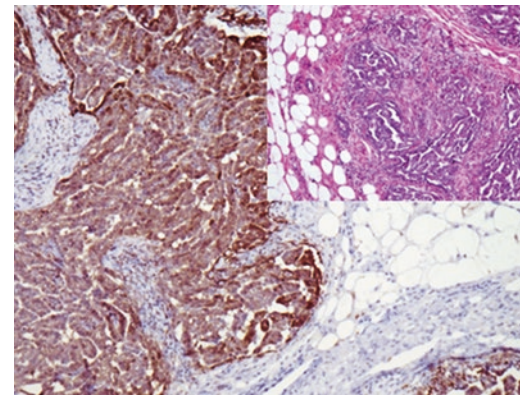


Fig. 15.1 Calretinin highlighting mesothelioma cells infiltrating the chest wall

Diagnostic Pitfalls It is important to consider that ~50% of sarcomatoid mesothelioma is negative for Calretinin. Furthermore, Calretinin has a broad expression spectrum, and the Calretinin positivity alone is not enough to confirm the diagnosis of mesothelioma.

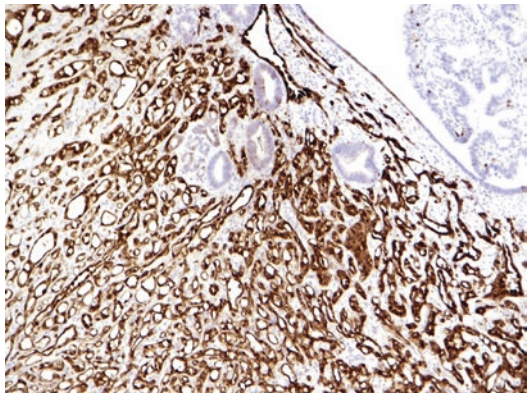


Fig. 15.2 Adenomatoid tumor of the fallopian tube. Tumor cells stained by calretinin

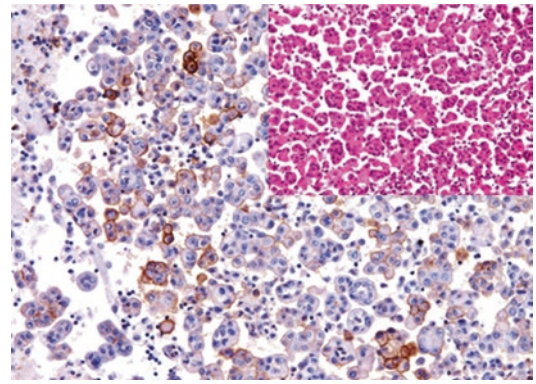


Fig. 15.3 Thrombomodulin labeling mesothelioma cells in malignant pleural effusion

15.4.2 Thrombomodulin

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, thymoma	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		

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 also found in various cell and tissue types, including mesothelial cells, squamous epithelial cells, and transitional epithelium of the urinary tract. Thrombomodulin is a helpful screening antibody for mesothelioma, transitional cell carcinoma, and squamous cell carcinoma in addition to vascular tumors (Fig. 15.3). In routine immunohistochemistry, Thrombomodulin stains 50–90% of mesotheliomas. Sarcomatoid mesothelioma usually lacks the expression of Thrombomodulin. Thrombomodulin is constantly negative in renal cell carcinoma, prostatic carcinoma, gastrointestinal adenocarcinoma, and endometrioid carcinoma.

Diagnostic Pitfalls A subset of pulmonary non-small cell carcinoma may be positive for Thrombomodulin; consequently, it is important to use other more specific markers to differentiate between mesothelioma and primary lung carcinoma. Generally, it is important to use other more specific tissue-specific markers to discriminate between Thrombomodulin-positive tumors.

15.4.3 Mesothelin

Mesothelin		
Expression pattern: membranous		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Mesothelioma – Non-mucinous ovarian surface carcinomas – Pancreatic ductal adenocarcinoma	Adenocarcinoma of different origins, acinar cell carcinoma and squamous cell carcinoma, thymic 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 to some other types of epithelial cells. Antibodies to Mesothelin can be included in an immunohistochemical panel for diagnosis of mesothelioma as they strongly label epithelioid mesothelioma. Sarcomatoid mesothelioma is negative for Mesothelin.

Diagnostic Pitfalls In addition to epithelioid mesothelioma, Mesothelin variously labels other carcinoma types, including non-mucinous ovarian carcinoma and endometrium, pulmonary, gastrointestinal, pancreatic, and cholangiocellular adenocarcinomas. In small pancreatic and duodenal biopsies, Mesothelin is an informative marker to discriminate between nonneoplastic glands negative for mesothelin and neoplastic glands positive for this marker (Fig. 15.4). Generally, Mesothelin is best regarded as a screening antibody as it cannot be considered as a specific mesothelioma marker.

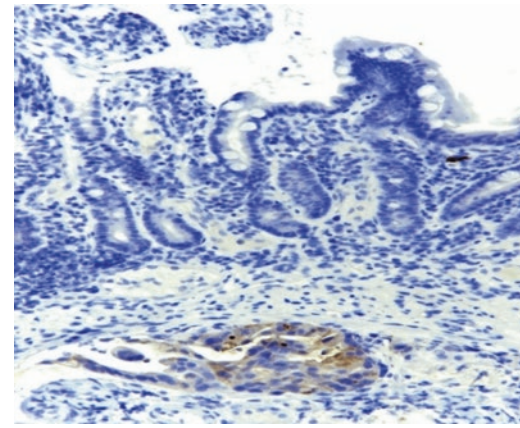


Fig. 15.4 Mesothelin highlighting the neoplastic glands of pancreatic ductal adenocarcinoma infiltrating the submucosa of the duodenal wall. Normal duodenal mucosa negative for Mesothelin

15.4.4 WT-1

WT-1 protein encoded by the Wilms tumor gene is one of the important mesothelial markers discussed in detail in a previous chapter (Chap. 11.6). In benign and malignant mesothelial cells, WT-1 shows a nuclear expression pattern and can be used as the double stain in combination with other markers exhibiting membranous stain (Fig. 15.5). More than 50% of sarcomatoid mesothelioma is negative for WT-1.

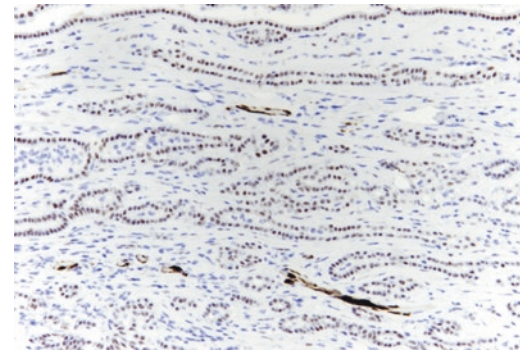


Fig. 15.5 Nuclear WT-1 expression in mesothelioma cells. Note also strong WT-1 expression in the endothelial cells

15.4.5 Podoplanin

Podoplanin (also known as D2–40) is a mucoprotein expressed on the membrane of lymphatic endothelium discussed in the chapter on vascular tumors (Chap. 25). Podoplanin is not specific for lymphatic endothelium but is also expressed in many other cell and tumor types, such as meningeal cells, germ cells, and germ cell tumors, in addition to many other mesenchymal tumors. Podoplanin is strongly expressed in mesothelial cells and both epithelioid and sarcomatoid mesotheliomas (Fig. 15.6) [9, 10].

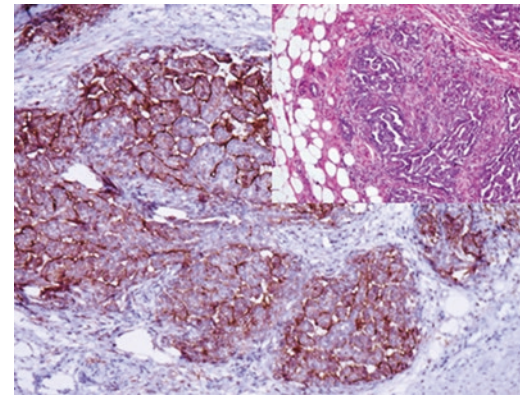


Fig. 15.6 Mesothelioma infiltrating the pleura. Neoplastic mesothelial cells with strong Podoplanin (D2–40) expression

15.4.6 Glut-1

Glut-1		
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 – Erythroid leukemia	Perineurioma, hemangioma, chordoma, epithelioid sarcoma, a wide range of carcinomas of different origins	Red blood cells and erythroid precursors, testicular germinal cells, renal tubules, placental trophoblasts, brain capillaries, perineural cells
Positive control: mesothelioma		

Diagnostic Approach Glucose transporter 1 (Glut-1) is a member of the sodium-independent glucose transporter family and a membrane-associated erythrocyte glucose transport protein maintaining the basal glucose transport in most cell types. Glut-1 is not a tissue-specific marker but is expressed in a wide range of epithelial and non-epithelial tumors. In diagnostic histopathology, Glut-1 is a potential marker for malignant transformation as it is overexpressed in many types of malignant epithelial, mesenchymal, and neuronal malignant tumors. Generally, Glut-1 is useful for differentiating between reactive and malignant proliferations. It is a helpful marker to discriminate between malignant mesothelioma and reactive proliferation of mesothelial cells, between atypical endometrial hyperplasia and functional or reactive endometrial hyperplasia, and between invasive and noninvasive implants of serous ovarian tumors. Glut-1 is also a helpful marker to distinguish between hemangioma, usually positive for Glut-1 and vascular malformation, pyogenic granuloma, and granulation tissue lacking the expression of Glut-1 (Fig. 25.9).

Diagnostic Pitfalls Glut-1 is a hypoxia-inducible factor (HIF) target gene, which is also induced by the hypoxia-inducible factor 1α (HIF-1α) [11]. Consequently, hypoxic tissue areas will also show the overexpression of Glut-1.

15.4.7 Insulin Like Growth Factor II mRNA-Binding Protein 3

IMP3		
Expression pattern: cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Mesothelioma – Hodgkin cells – Malignant melanocytic lesions	Many adenocarcinoma types, including lung, gastrointestinal, and pancreatic carcinoma, and uterine and ovarian carcinoma. Germ cell tumors, medullary and anaplastic thyroid carcinoma, neuroendocrine tumors, and Merkel cell carcinoma	Placenta, lymphoblasts, mucinous cells (bronchial glands, colorectal mucosa, endocervix), ciliated cells, neuroendocrine cells
Positive control:		

Diagnostic Approach 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, fibroblasts, and brain. In routine immunohistochemistry, IMP3 is used to discriminate between malignant and reactive proliferative lesions. Similar to GLUT-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 (Fig. 15.7) [12]. IMP3 is also positive in malignant pancreatic glands and negative in normal pancreatic tissue. Furthermore, IMP3 is a helpful marker for discriminating between serous endometrial carci-

noma positive for IMP3 and endometrioid carcinoma negative for IMP3 (Fig. 15.8) [13].

IMP3 is also a marker for classical Hodgkin cells; however, it can also be found in some extrafollicular blasts or cells of B cell lymphoma.

Diagnostic Pitfalls IMP3 is not a specific marker as it is expressed in a wide range of malignant tumors with different histogenesis.

15.4.8 BRCA1 Associated Protein 1 (BAP-1)

BAP-1 is a nuclear ubiquitin hydrolase involved in chromatin remodeling and functions as a transcriptional regulator and tumor suppressor. BAP-1 is encoded by a gene located on chromosome 3p12.124, a genomic region that is found to be deleted in different fractions of several human malignancies, including mesotheliomas, uveal and cutaneous melanomas, clear cell renal cell carcinoma, pulmonary adenocarcinoma, intrahepatic cholangiocarcinoma, and meningioma [14, 15].

For related tumor types, the loss of BAP-1 expression is associated with higher metastatic potential and aggressive behavior.

In routine immunohistochemistry, BAP-1 is a helpful marker to discriminate between malignant mesothelioma that usually lacks the nuclear BAP-1 expression and reactive mesothelial proliferation exhibiting the nuclear BAP-1 expression (Fig. 15.9). The sensitivity of BAP-1 to differentiate between benign and malignant mesothelial lesion is reported to be up to 90% in epithelioid mesothelioma and up to 50% in biphasic mesothelioma but insufficient in sarcomatoid mesothelioma. The diagnosis can be supported by p16 FISH analysis [16, 17]. The loss of BAP-1 expression is also reported in clear cell renal cell carcinomas and some melanocytic tumors.

The loss of BAP-1 expression is also an important criterion for the diagnosis of BAP-1 inactivated melanocytic tumors, usually exhibiting a spitzoid morphology.

5-Hydroxymethylcytosine (5-hmC) is a further immunohistochemical marker reported to be helpful for the differentiation between malignant mesothelioma and benign mesothelial proliferation. Similar to BAP-1, up to 90% of malignant mesotheliomas show the loss of the nuclear 5-hmC expression [18].

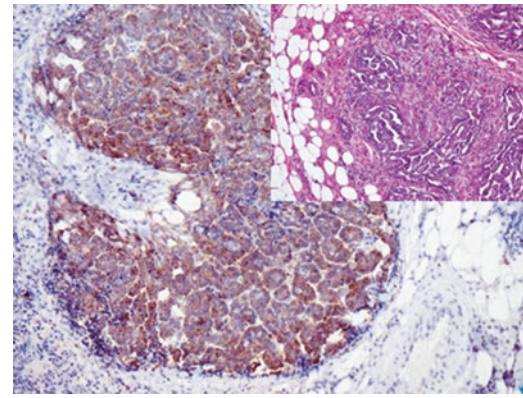


Fig. 15.7 Malignant mesothelioma with strong cytoplasmic IMP3 expression in tumor cells

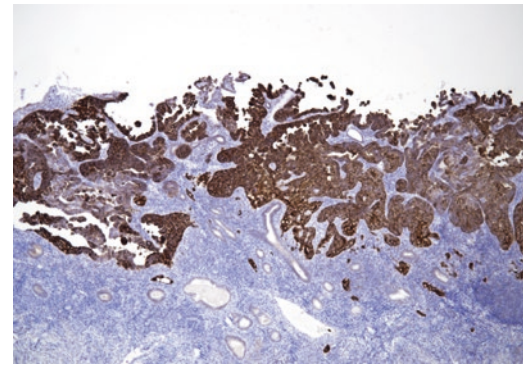


Fig. 15.8 Serous endometrium carcinoma with strong IMP3 expression

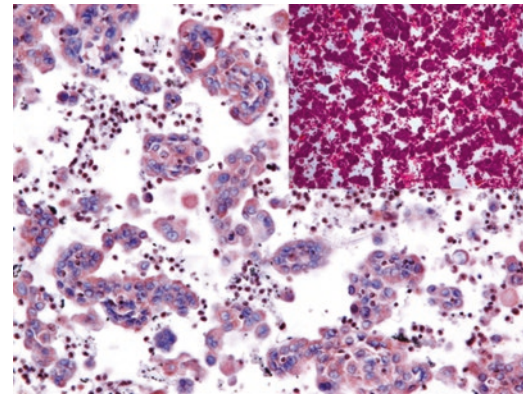


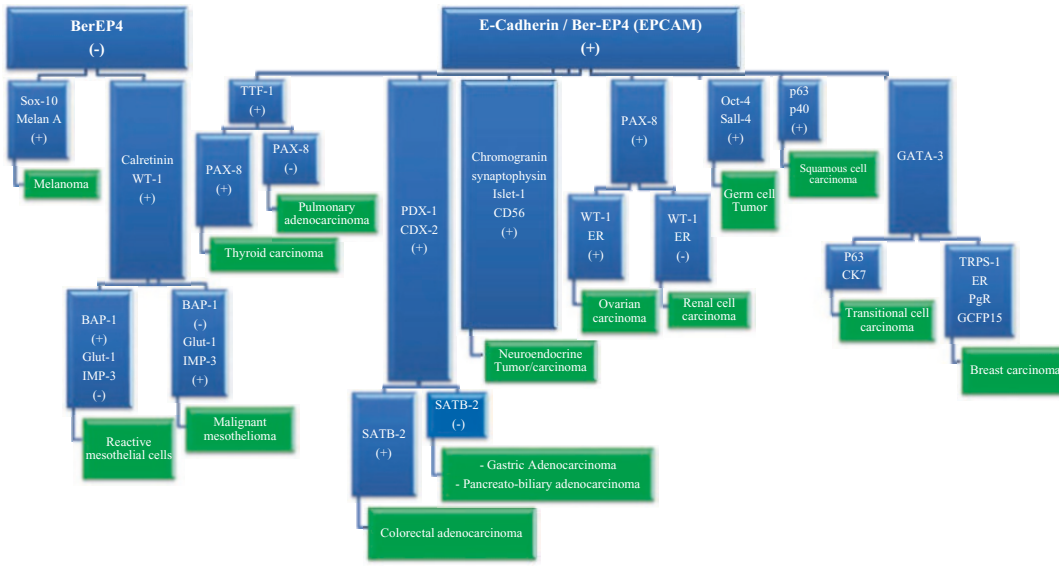
Fig. 15.9 Malignant pleural effusion with mesothelioma cells lacking the nuclear expression of BAP1. Lymphocytes show regular nuclear BAP1 expression

Diagnostic Criteria for the Diagnosis of 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, p63, and CD15, which are consistently negative in mesothelioma. Generally, it is advisable to confirm the diagnosis of mesothelioma by three to four mesothelial markers [1]. Other markers such as Glut-1, BAP-1, and CD146 help confirm the neoplastic nature of the mesothelial proliferation.

Markers Constantly Negative in Reactive and Malignant Mesothelial Cells, Positive in Malignant Cells of Pleural and Peritoneal Carcinosis Different epithelial and tissue-specific markers are used to discriminate between mesothelial proliferations and metastatic epithelial cells. The epithelial-specific antigen (clone BerEp4), MOC-31, p63, Claudin-4, CEA, and CD15 are epithelial markers usually negative in mesothelial cells. TTF-1, Napsin A, CDX-2, SATB-2, GATA-3, PDX-1, PAX-8, NKX3.1, and Arginase are makers and transcriptional factors negative in mesothelial cells and specific for different tissue and cell types (see Algorithm 15.1).

Markers discriminating between malignant mesothelioma (MM) and benign/reactive mesothelial proliferation (BMP)	
BAP-1	− in MM, + in BMP
Glut-1	+ in MM, − in BMP
5-hmC	−/+ in MM, + in BMP
Desmin	− in MM, +/− in BMP
CD146	+ in MM, − in BMP
E-cadherin	+ 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
PAX-8	− in MM, + in well-differentiated papillary mesothelial tumor
L1CAM (CD171)	− in MM, + in well-differentiated papillary mesothelial tumor

+ expression in >90%; +/− in 50–90%; −/+ in 10–50%; − in <10%



Algorithm 15.1 Immunoprofile of tumor cells in effusion cytology

Immunoprofile of peritoneal tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
A. Immunoprofile of mesothelioma				
Adenomatoid tumor	Pan-CK, CK5/CK6, CK7, WT-1, **Calretinin**, **BAP1**, Mesothelin, Podoplanin (D2–40)	Thrombomodulin (CD141), HBME-1		p63, CD15, EPCAM, Claudin-4, CK20, CEA, TTF-1, CDX-2, PAX-8, Smoothelin
Well-differentiated papillary mesothelial tumor	Pan-CK, CK5/CK6, CK7, WT-1, **Calretinin**, **BAP1**, **L1CAM (CD171)**, Mesothelin, Podoplanin (D2–40)	**PAX-8**,	EMA	p63, CD15, EPCAM, ER, PgR, CK20
Epithelioid mesothelioma	Pan-CK, CK5/CK6, CK7, CK8, CK14, CK18, CK19, **WT-1**, **Calretinin**, Podoplanin (D2–40), Mesothelin, **h-Caldesmon**, CD44s	**Thrombomodulin** (CD141), **IMP3**, **Glut-1**, HBME-1, vimentin	N-cadherin, E-cadherin, GATA-3, CD30, actin, EMA, bcl-2	**BAP1**, p63, CD15, **L1CAM (CD171)**, EPCAM (BerEp4), Claudin-4, CK20, CEA, TTF-1, CDX-2, Napsin, PAX-8. Myoglobin, Myogenin
Sarcomatoid mesothelioma	Pan-CK, Podoplanin (D2–40)	**Calretinin**, CK7	Mesothelin, WT-1	CK5/CK6, EMA, EPCAM Claudin-4, CK20, CEA, TTF-1, CDX-2, Napsin, PAX-8. Myoglobin, Myogenin
B. Epithelial tumors of Müllerian origin				
Serous/endometrioid/ clear cell and transitional cell tumors	See epithelial tumors of the ovary			
C. Smooth muscle tumors				
Leiomyomatosis peritonealis disseminata	Actin, Smoothelin, h-Caldesmon			CK5/CK14
D. Miscellaneous tumors				
Pseudomyxoma peritonei	CK20, CDX-2, SATB-2, PDX-1, CEA	MUC-2		CK7
Desmoid fibromatosis	β-Catenin (nuclear), vimentin		Desmin	
Calcifying fibrous tumor	CD34, FXIIIa			
Histiocytic nodule	CD4, CD64, CD68, CD163			Calretinin, Pan-CK
Gliomatosis:	GFAP	S100		Pan-CK
Extra gastrointestinal stromal tumor	See gastrointestinal GIST			
Endometrioid stromal sarcoma of the peritoneum	See endometrial stromal sarcoma			
Desmoplastic small round cell tumor	See miscellaneous soft tissue tumors			

Differential diagnosis of epithelioid mesothelioma versus metastatic carcinoma																	
	BER-EP4	CK5/CK14	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)	+	−	+	−	−	−		−	−	−	+	−	−	+	−	−	−

+ expression in >90%; +/− in 50–90%; −/+ in 10–50%; − in <10%

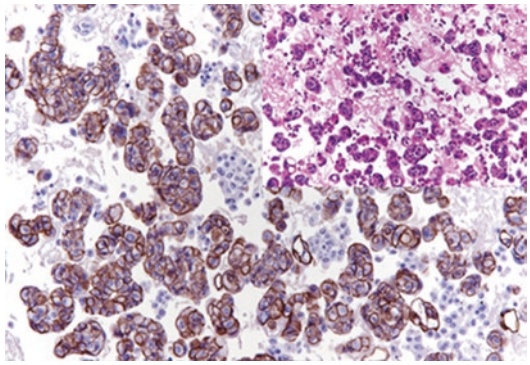


Fig. 15.10 Pleural effusion embedded in gelatin block, E-cadherin highlighting carcinoma cells exhibiting strong membranous stain

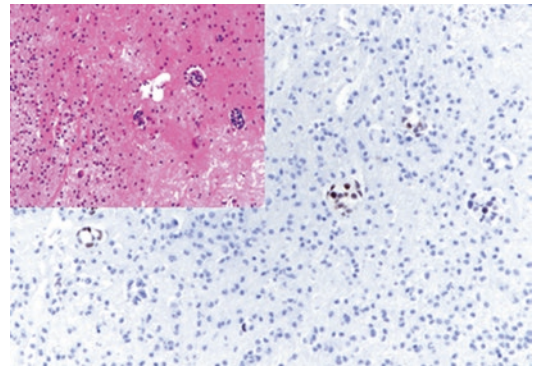


Fig. 15.11 Ascitic fluid embedded in gelatin block; few primary gastric adenocarcinoma tumor cells exhibiting nuclear CDX-2 expression

15.5 Management of Effusion Cytology

For reproducible and reliable interpretation of effusion and FNA cytology, it is recommended to perform the immunostaining on sections prepared from cell blocks after sedimentation and embedding in a suitable matrix (gelatin, agarose, thrombin, or albumin). To screen for tumor cells with epithelial differentiation in effusion cytology, E-cadherin is found to be one of the most informative markers to highlight the epithelial cells taking into consideration those epithelial tumors associated with the loss of E-cadherin expression such as lobular breast carcinoma and poorly cohesive gastric adenocarcinoma (Fig. 15.10). It is also important to consider that E-cadherin is also expressed in malignant mesothelioma and occasionally weakly expressed in reactive mesothelial cells. A good alternative for E-cadherin is the Ber-EP4 clone of EPCAM but in our experience less sensitive than E-cadherin (see Chap. 2). Both markers can also be combined in an antibody mixture. In the case of known primary neoplasia, the use of tissue- or tumor-specific markers or transcription factors such as TTF-1, PAX-8, PDX-1, CDX-2, SATB2–2, p63, Islet-1, Insm-1, Arginase, steroid hormones, or DOG-1 will precise the diagnosis (Fig. 15.11; see the table above and Algorithm 15.1).

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Markers and Immunoprofile of Lymphoid Neoplasms

16

Contents

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The 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, granulocytes, stromal cells, and capillaries. All of these components must be considered for the interpretation of lymphoproliferative neoplasms. For the initial diagnosis, screening markers can be helpful. Additional markers for specific lymphoma types must be used to precise the diagnosis. Markers listed in different parts of this chapter are essentially used for orientation. The final diagnosis must be made taking into consideration the clinical data, histomorphology, and immunophenotype, including immunohistochemistry and flow cytometry, in addition to molecular genetic analysis if necessary. The fifth revision of the World Health Organization classification of hematolymphoid neoplasms was considered in this chapter [1].

16.1 Screening Markers for Lymphoid Neoplasms

CD45 (LCA), B-cell markers, T-cell markers, TdT, CD34, and Ki-67 [2–4].

16.1.1 CD45

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	Mature and immature hematopoietic cells (including B- and T-lymphocytes, monocytes, macrophages, plasma cells, and mast cells), dendritic cells, osteoclasts, 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 mature and immature 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. Most representative examples of CD45 negative lymphomas are ALK-positive large B-cell lymphoma, anaplastic large-cell lymphoma, and plasmablastic lymphoma. In suspicious cases, the use of other lymphoid markers is required. Membranous CD45 expression is reported in sporadic cases of undifferentiated, neuroendocrine, and small-cell carcinomas. Necrotic carcinomas can also imitate a membranous LCA positivity, which also holds true for other immunohistochemical markers, as, in general, necrosis may display a false positivity.

16.1.2 Terminal Deoxynucleotidyl Transferase

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

Diagnostic Approach Terminal deoxynucleotidyl transferase (TdT) is a DNA nuclear polymerase encoded on chromosome 10, 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, including prothymocytes and thymocytes. The expression of TdT is specific for precursor cell lymphomas of T- and B-cell origin, namely, acute lymphoblastic leukemia. In the normal bone marrow, 1–2% of nucleated cells are positive for TdT, and most are B-cell precursors. In the normal thymus, different percentages of cortical T-cells are TDT positive depending on their maturation stage.

Diagnostic Pitfalls It is essential to consider that TdT may be positive in some types of acute myeloid leukemia, especially minimally differentiated AML (M0) and AML with t(6;9) in addition to blast crisis of chronic myeloid leukemia (CML) and myeloid sarcoma. The expression of TdT is also characteristic for the immature T-lymphocytes associated with the thymoma types A, B, and AB but not thymic carcinoma.

The expression of TdT is also reported in a large percentage of Merkel cell carcinoma, which may also be positive for PAX-5 [5, 6].

CD5 and CD10 are further markers important 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, mainly epithelial tumors.

16.1.3 CD10

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 – Clear cell renal cell carcinoma – Hepatocellular carcinoma	Follicular lymphoma; plasma cell neoplasms; transitional cell carcinoma; various adenocarcinomas including pulmonary, colorectal, and prostatic adenocarcinomas; melanoma; placental site trophoblastic tumor; choriocarcinoma; myofibroblastoma; mesothelioma; rhabdomyosarcoma; osteosarcoma; leiomyosarcoma; Ewing's sarcoma; solitary fibrous tumor; atypical fibroxanthoma	Pro-B- and pre-B-cells, germinal center B-cells, prothymocytes, subcapsular thymocytes and follicular T-helper cells, granulocytes, adrenal cortex, endometrial stroma cells, hepatocytes and bile duct canaliculi, glomerular epithelial cells and cells of proximal renal tubules, epithelium of seminal vesicles, 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 (B- and T-cells), myelogenous, epithelial/myoepithelial, and mesenchymal origin mentioned in the above table [7, 8]. CD10 is a maturation marker of granulocytes and, together with CD15, labels the blasts of low-risk MDS.

In diagnostic immunohistochemistry, CD10 must be used in a panel with other tissue- and cell-specific markers [2]. The expression pattern of CD10 (membranous or cytoplasmic) is highly variable, depending not only on the tumor type but also on differentiation grade, as the cytoplasmic stain is usually seen in poorly differentiated carcinomas.

16.1.4 CD5

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 origins, atypical thymoma and thymic carcinoma	T-cells, a subset of B-cells of the mantle zone of the spleen and lymph nodes
Positive control: appendix/tonsil		

Diagnostic Approach CD5 (lymphocyte antigen T1, Leu-1) is a glycoprotein receptor encoded on chromosome 11. The expression of CD5 begins at the prothymocyte stage and persists in the majority of T-lymphocytes. CD5 labels the majority of T-cell lymphomas, including 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 is also found in a small subset of adult B-lymphocytes, including mantle zone lymphocytes, in addition to more than 50% of fetal B-lymphocytes and lymphomas of B-cell origin, mainly mantle cell lymphoma and B-CLL (Figs. 16.1 and 16.2).

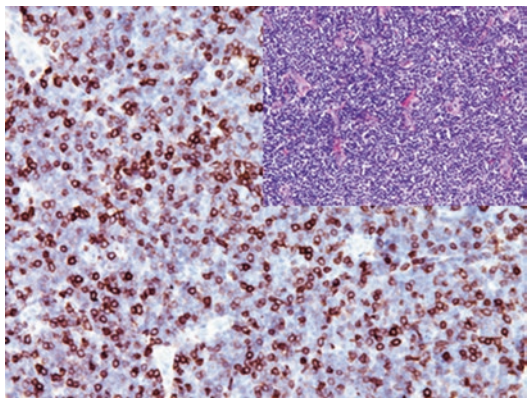


Fig. 16.1 Weak to moderate CD5 expression in the cells of B-CLL. T-lymphocytes with strong membranous CD5 expression

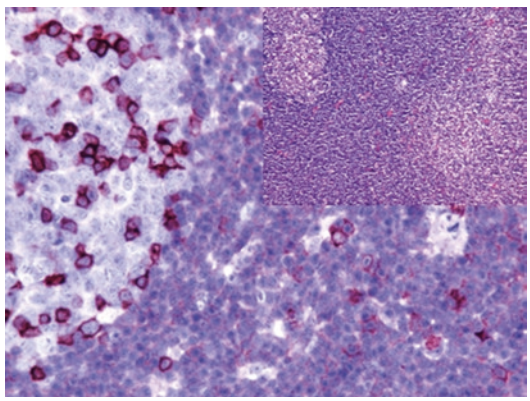


Fig. 16.2 Cells of mantle cell lymphoma showing moderate membranous CD5 expression. Associated T-lymphocytes with strong CD5 expression

Diagnostic Pitfalls The expression of CD5 is not limited to lymphoid tissue but is found in adenocarcinomas of different origins, renal cell carcinoma, and adrenocortical carcinoma in addition to squamous cell carcinoma. Furthermore, CD5 is a diagnostic marker for atypical thymoma and thymic carcinoma (see Chap. 4). A focal weak CD5 expression can also be found in mesothelioma, transitional carcinoma, squamous cell carcinoma, and adenocarcinomas of different origins [9].

16.1.5 CD34

CD34 is a cell surface adhesion glycoprotein and a marker for endothelial and stem cells listed with the markers of vascular tumors (Chap. 25). CD34 is an important marker for the diagnosis of lymphoid and hematopoietic neoplasia. Besides CD117, CD34 is also expressed on the precursors of B- and T-lymphocytes, myeloblasts, and mast cell progenitors. CD34 is expressed on different percentages of B-ALL, T-ALL, and AML blasts and is helpful for the diagnosis of MDS.

16.1.6 Ki-67

Ki-67 is a nonhistone nuclear protein in humans encoded by the MKI67 gene on chromosome 10q26.2, involved in the early steps of polymerase I-dependent ribosomal RNA synthesis and DNA replication and expressed in the active cell cycle. The expression of Ki-67 begins in the G_1 phase and persists during the active phases of the 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.

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 pro-

liferation. 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. 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 lymphomas (Fig. 16.3). 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 considerable interlaboratory variability.

Noteworthy is the aberrant membranous expression of Ki-67 characteristic for sclerosing pneumocytoma and hyalinizing trabecular tumor of the thyroid.

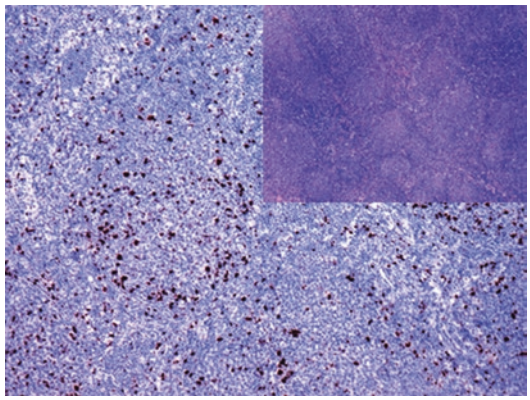


Fig. 16.3 Characteristic low proliferation index (Ki-67) in neoplastic follicles of follicular lymphoma grade 1–2

16.2 Markers and Immunoprofile of B-Cell Neoplasms

16.2.1 B-Lineage-Specific Markers

CD10, CD19, CD20, CD79a, PAX-5.

16.2.2 Markers for Specific Lymphoma Types

CD5, CD23, CD34, LEF-1, bcl-2, Bcl-6, LMO2, HGAL, cyclin D1, SOX11, ARTA1, TRAP, HHV-8, and TdT [2–4, 10].

16.2.3 Therapy-Related Markers

CD19, CD20, CD30, p53, Ki-67.

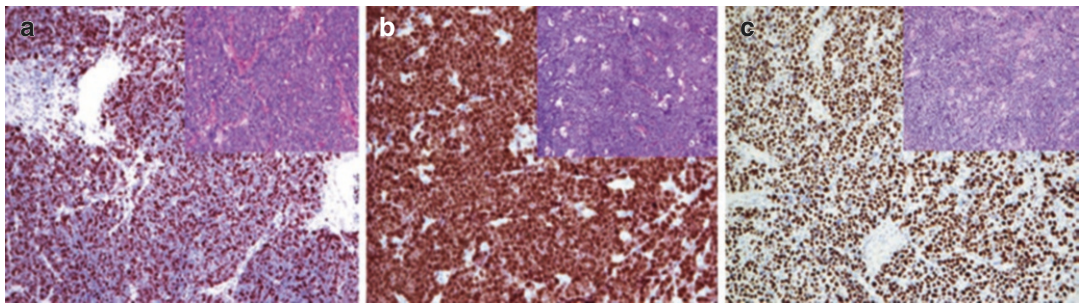


Fig. 16.4 Three tumor types with high Ki-67 index (~100%). (**a**) Small-cell carcinoma, (**b**) Burkett's lymphoma, (**c**) plasmablastic lymphoma

16.2.4 CD19

CD19 (B4)		
Expression pattern: membranous		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– B-cell lymphoma/leukemia	Myeloblasts in (M0) AML with t(8;21), blast phase of CML	B-cells, follicular dendritic cells
Positive control: appendix/tonsil		

Diagnostic Approach CD19 (β-integrin) is a single-chain glycoprotein and a member of the immunoglobulin family encoded on chromosome 16. CD19 is an early naïve B-lymphocyte antigen, which remains through the B-lymphocyte differentiation stages and disappears in the plasma cell stage. The CD19 expression is also found 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]. CD19 is negative in ALK + large B-cell lymphoma, primary effusion lymphoma, and plasma cell neoplasia.

16.2.5 CD20

CD20 (B1 antigen)		
Expression pattern: membranous		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– B-cell lymphoma/leukemia	Epithelial cells of thymomas type A and AB	B-cells, follicular dendritic cells
Positive control: appendix/tonsil		

Diagnostic Approach CD20 is a transmembrane non-glycosylated phosphoprotein encoded by the MS4A1 gene on chromosome 11, acting as a receptor during B-cell activation and differentiation. CD20 appears on the B-cells after CD19 and CD10 in the naïve B-lymphocytes and remains until the late stages of B-lymphocyte differentiation but disappears in the plasma cell stage. Characteristic for CD20 is the membranous expression pattern, whereas the cytoplasmic or nucleolar expression patterns are nonspecific.

Diagnostic Pitfalls CD20 is a pan B-lymphocyte marker, but some types of B-cell lymphomas may be CD20 negative or show a very weak expression level; consequently, in doubtful cases, it is important to use two B-cell markers to ensure or exclude the B-cell origin of the neoplasm. Optimal combinations are CD20/CD19 and CD20/PAX-5 or CD20/CD79. Few B-cell lymphoma types are negative for CD20, such as plasmablastic lymphoma, ALK + large B-cell lymphoma, and primary effusion lymphoma. Hodgkin and Reed–Sternberg cells in classical Hodgkin lymphoma are usually negative for CD20 but often express the nuclear B-cell marker PAX5 (see below). Generally, the expression of CD20 is restricted to B-lymphocytes; nevertheless, CD20 expression is reported in rare cases of peripheral T-cell lymphoma.

CD20 expression is also characteristic for rare epithelial tumors, found on the neoplastic epithelial cells of thymomas type A and AB, whereas thymomas type B1, B2, B3, and C and in normal thymic epithelium lack the expression of CD20. The CD20-positive thymic cells are negative for all other B-cell markers (Fig. 16.5). Aberrant CD20 expression is also reported in a small subset of thyroid carcinoma, mainly papillary thyroid carcinoma [12].

A diagnostic pitfall is the interpretation of CD20 stain in tissue or bone marrow samples after targeted anti-CD20 immunotherapy (rituximab) exhibiting the loss of CD20-positive

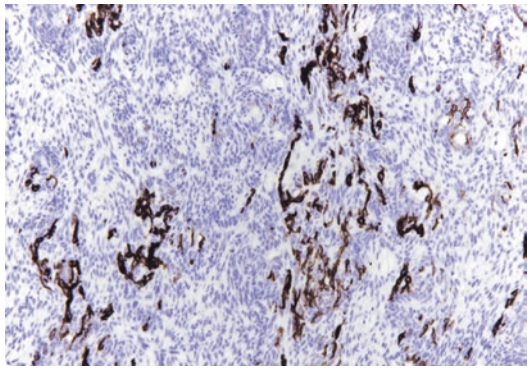


Fig. 16.5 CD20 expression on the epithelial cells of type A thymoma

B-lymphocytes, which also may be associated with the loss of CD19. In such biopsies, the absence of CD20-positive lymphocytes does not exclude the presence of lymphoma cells and other B-cell markers, such as PAX5 and CD79a, can be helpful in detecting lymphoma cells.

16.2.6 CD22

CD22 (sialic acid binding Ig-like lectin 2, Siglec-2) is a type I transmembrane glycoprotein composed of two α- and β-chains that acts as a mediator in B-cell–B-cell interaction. CD22 is expressed in the cytoplasm of early B-lymphocytes after CD19, followed by the membranous expression on mature B-lymphocytes, and disappears in plasma cells. CD22 is also expressed on basophils and mast cells. CD22 is a marker for B-cell lymphomas.

16.2.7 CD23

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, Reed–Sternberg cells	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 has two forms, a and b, with different amino acid sequences. Type a is involved in the differentiation of B-cells and expressed on mature B-cells, and type b plays a role in the regulation of allergic reactions and is expressed on B- and T-cells, activated macrophages, and eosinophils. CD23 is also a good marker for follicular dendritic cells. It is important to mention that the expression of CD23 is activated by EBV infection. CD23 is an important marker used to discriminate B-CLL (strongly positive) from other lymphoma types with similar morphology (Fig. 16.6), while it is negative in t(14;19) associated B-CLL. CD23 also labels mediastinal large B-cell lymphoma, lymphoplasmacytic lymphoma, and a small subset of multiple myeloma in addition to Reed–Sternberg cells in Hodgkin lymphoma. It is also an important marker for follicular dendritic cell tumors (see Chap. 19).

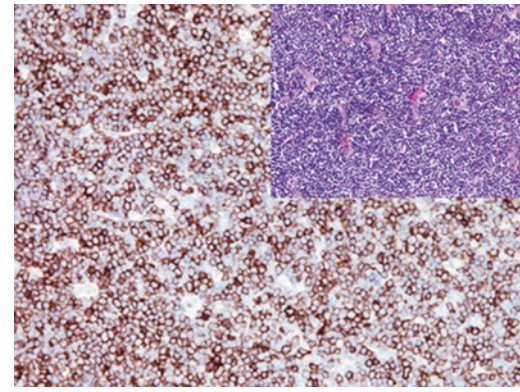


Fig. 16.6 B-CLL with strong membranous CD23 expression on neoplastic cells

16.2.8 CD79a

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, T-ALL	B-cells, a small population of CD3+ T-cells, a subset of megakaryocytes and endothelial cells
Positive control: appendix/tonsil		

Diagnostic Approach CD79a is a disulfide-linked heterodimer associated with the membrane-bound immunoglobulin receptor complex. CD79a appears in the pre-B-lymphocyte stage before the IgH chain rearrangement and persists until the plasma cell development, rendering the majority of normal and neoplastic plasma cells positive for CD79a. CD79a exhibits a membranous expression pattern, but plasma cells may also show a cytoplasmic stain 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.

16.2.9 PAX-5

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 lymphoma	AML with t(8;21), Merkel cell carcinoma, small-cell carcinoma, alveolar rhabdomyosarcoma, Wilms tumor, glioblastoma and neuroblastoma, mesonephric and Müllerian tumors	Pre-B- to mature B-cells
Positive control: appendix/tonsil		

Diagnostic Approach PAX-5 (also known as B-cell activator protein, BSAP) is a PAX (paired box) family member that includes nine transcription factors involved in tissue and organ differentiation. PAX-5 is a B-cell-specific transcription factor encoded by the gene located at chromosome 9p13 and expressed in the early pro-B, pre-B, and naïve stages of B-cell development until the mature B-cells [13]. Plasma cells, T-lymphocytes, and macrophages constantly lack PAX-5 expression. PAX-5 is one of the best markers of B-cell lymphomas (Fig. 16.7). It is also expressed in the L&H cells of nodular lymphocyte predominance Hodgkin lymphoma and in the majority of Hodgkin cells in classic Hodgkin lymphoma.

The PAX-5 gene is a partner of the t(9;14)(p13;q32) translocation associated with the plasmacytoid subtype of small lymphocytic lymphoma.

Diagnostic Pitfalls PAX-5 can be positive in some tumors resembling lymphoma, such as Merkel cell carcinoma, small-cell carcinoma,

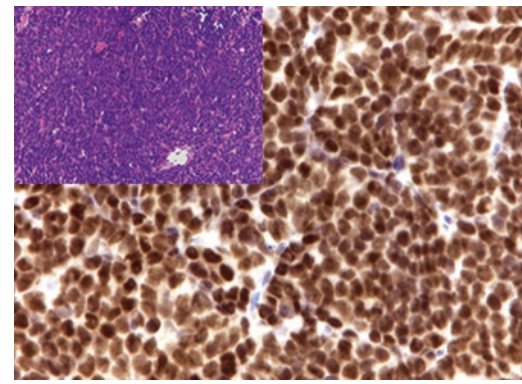


Fig. 16.7 Strong nuclear PAX-5 expression in the cells of diffuse large B-cell lymphoma

atypical carcinoid, and also rarely in acute lymphoblastic lymphoma of T-cell origin [14, 15]. 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 [16, 17].

16.2.10 Cyclin D1

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, solid pseudopapillary neoplasm of the pancreas	Cells in the G_1 phase of the cell cycle, histiocytes, endothelial cells
Positive control: mantle cell lymphoma		

Diagnostic Approach Cyclin D1 (also known as bcl-1) is a cell cycle protein encoded on chromosome 11q13 and 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 is 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. 16.8). In routine immunohistochemistry, cyclin D1 is usually used in combination with CD5, Sox-11, and other B-cell markers [2, 18].

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

Diagnostic Pitfalls Other lymphoma types exhibiting similar morphology, such as hairy cell leukemia and B-CLL, may also be positive for cyclin D1; however, the staining intensity is much less than mantle cell lymphoma [19]. A small subset of mantle cell lymphoma lacks the expression of cyclin D1; this subset is usually positive for Sox-11, which is to consider in the differential diagnosis. Cyclin D1 is also expressed in some carcinoma types, such as adenocarcinomas of the breast and prostate, besides some mesenchymal tumors, such as inflammatory myofibroblastic tumor.

16.2.11 Sox-11

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, solid pseudopapillary neoplasm of the pancreas	Immature neurons
Positive control: mantle cell lymphoma		

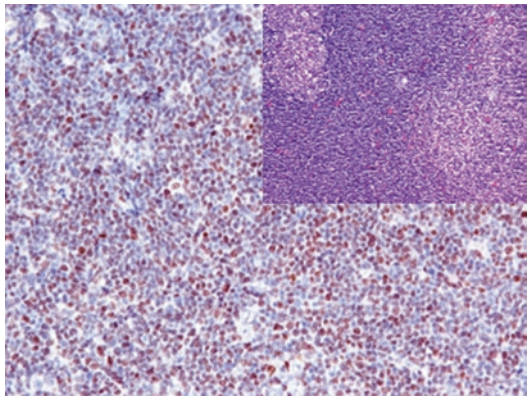


Fig. 16.8 Mantle cell lymphoma showing strong nuclear cyclin D1 expression in neoplastic lymphocytes

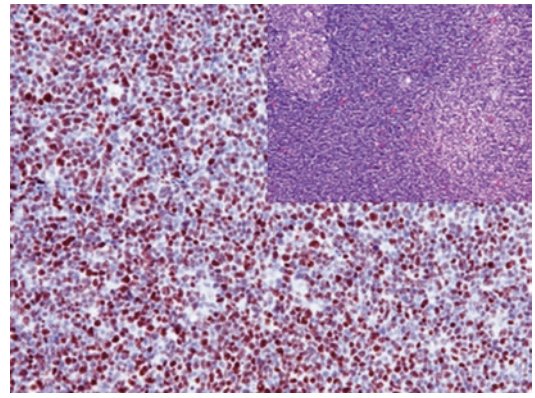


Fig. 16.9 Mantle cell lymphoma with strong nuclear Sox-11 expression in neoplastic lymphocytes

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 also takes part in the regulation of PAX-5 transcription.

Sox-11 strongly stains both cyclin D1 positive and negative mantle cell lymphomas (Fig. 16.9) in addition to other lymphoma types, including hairy cell leukemia, Burkitt lymphoma, and B- and T-ALL [20–22]. Sox-11 is constantly negative in B-CLL, follicular lymphoma splenic marginal zone lymphoma, diffuse large B-cell lymphoma, and multiple myeloma.

In epithelial tumors, the expression of Sox-11 is found in pulmonary neuroendocrine carcinomas (Fig. 16.10) and in a subset of ovarian carcinomas; the latter later are generally associated with a good prognosis [23].

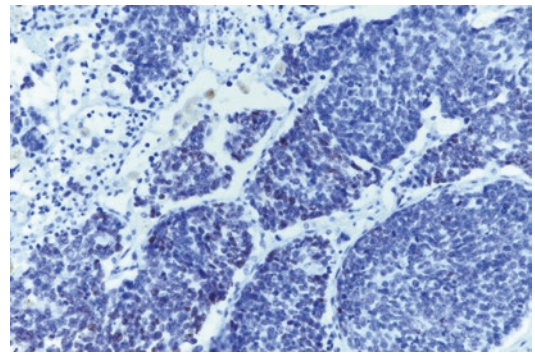


Fig. 16.10 Pulmonary neuroendocrine carcinoma with focal nuclear Sox-11 expression

16.2.12 bcl-2

bcl-2		
Expression pattern: cytoplasmic (mitochondrial membrane)		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Follicular lymphoma – Lymphoid tumors: the majority of B-cell lymphomas, a subset of T-cell lymphoma	– Epithelial tumors: carcinoma of the breast, nasopharyngeal carcinoma, basal cell carcinoma – Neuroendocrine tumors: adrenocortical tumors, thyroid carcinoma – Mesenchymal tumors: solitary fibrous tumor, synovial sarcoma, hemangiosarcoma, neurofibroma, schwannoma, dermatofibrosarcoma protuberans, spindle cell lipoma, rhabdomyosarcoma	Small B-lymphocytes in primary follicles and in the mantle and marginal zones, a subset of T-lymphocytes, medullary cells in the thymus and 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 an 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, translated into two homologous integral cell and mitochondrial membrane proteins.

The t(14;18)(q32;q21) translocation characteristic for 90% follicular lymphoma juxtaposes 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 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 associated with high proliferative activity and grade 1 follicular lymphoma with bcl-2 positive neoplastic follicular B-cells and usually with low proliferative activity (Fig. 16.11) [2]. Generally, all grade 1 follicular lymphomas are positive for bcl-2, and about 85% of grade 2 and up to 75% of grade 3 are positive for bcl-2. To consider are the bcl-2 negative follicular lymphoma types such as pediatric type follicular lymphoma.

The expression of bcl-2 is not specific for follicular lymphoma but found in the majority of B-cell lymphomas and in a subset of T-cell lymphomas.

The expression of bcl-2 is also found in a large number of epithelial, neuroendocrine, and mesenchymal tumors [2].

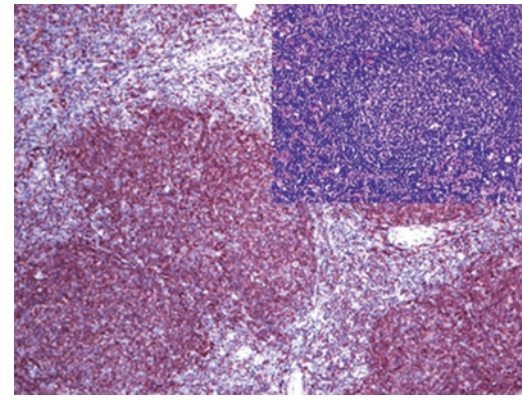


Fig. 16.11 Follicular lymphoma with strong diffuse bcl-2 expression in neoplastic follicles

Diagnostic Pitfalls Ten to 15% of grade 1–2 follicular lymphomas lack the expression of bcl-2 detected by immunohistochemistry. This phenomenon is also found in up to 70% of grade 3a and 3b follicular lymphomas. It can be either due to mutations within the bcl-2 gene producing mutated bcl-2 proteins not recognized by the standard antibodies or due to other equivalent mutations causing the upregulation of the bcl-2 expression.

In lymph nodes, the expression of bcl-2 is found in the B-cells of primary follicles, which may be misdiagnosed as the manifestation of grade 1 follicular lymphoma. Finally, different antibody clones to the bcl-2 molecules may show different stain results. In doubtful cases, it is recommended to repeat the immunohistochemical stain using another antibody clone. Finally, the molecular detection of the t(14;18) translocation or other equivalent genetic anomalies is also helpful for further characterization of the lymphoma types.

16.2.13 bcl-6

bcl-6		
Expression pattern: nuclear		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Follicular lymphoma (inter- and intrafollicular cells) – Anaplastic CD30+ large-cell lymphoma (ALK+/−)	Burkitt lymphoma, diffuse large B-cell lymphoma, mediastinal large B-cell lymphoma, L&H cells in nodular lymphocyte predominance Hodgkin lymphoma, angioimmunoblastic lymphoma, T-ALL	Germinal centers of lymph nodes, a subset of intrafollicular CD4+ T-lymphocytes (TFH)
Positive control: appendix/tonsil		

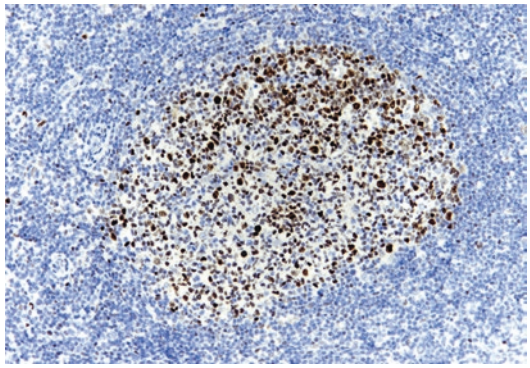


Fig. 16.12 bcl-6 expression in intrafollicular neoplastic cells of follicular lymphoma

Diagnostic Approach bcl-6 (**B-c**ell **l**ymphoma **6** protein) is a sequence-specific transcriptional repressor protein involved in the regulation of B-cell differentiation. Bcl-6 is normally expressed in nonneoplastic germinal center B-lymphocytes with a high proliferation rate and active somatic mutations. Furthermore, bcl-6 is a master transcription factor essential for the transformation of naïve CD4+ T helper cells into follicular helper cells (TFH cells).

bcl-6 is a marker for lymphomas of germinal center origin such as follicular lymphoma (intra- and interfollicular cells) (Fig. 16.12), Burkett's lymphoma, mediastinal large B-cell lymphoma, majority of Hodgkin cells, and nodular lymphocyte predominance Hodgkin lymphoma [2]. The bcl-6 gene is found to be translocated or hypermutated in ~40% of diffuse large B-cell lymphoma and ~15% of follicular lymphoma, causing the overexpression of the bcl-6 protein [24]. It is to consider that the immunohistochemical expression of bcl-6 is not a surrogate marker for mutations or rearrangements within the bcl-6 gene.

The expression of bcl-6 is also characteristic for some NK-cell/T-cell lymphoma types, such as angioimmunoblastic lymphoma and T-ALL. Mantle cell lymphoma, marginal zone lymphoma, and ALL are constantly bcl-6 negative.

16.2.14 bcl-10

bcl-10 (also known as **B-c**ell **l**ymphoma/leukemia **10**) is an apoptotic regulatory nuclear protein encoded on chromosome 1, involved in antigen-receptor-mediated lymphocyte activation through the NF-Kappa B pathway. Bcl-10 is expressed in the germinal center and marginal zone B-lymphocytes and is also weakly expressed in mantle zone B-lymphocytes beside a subset of T-lymphocytes. Bcl-10 labels different B-cell lymphoma types, including follicular lymphoma and extranodal marginal zone lymphoma of MALT type and weakly also mantle cell lymphoma. MALT lymphomas bearing the t(1;14)(p22;q32) translocation show a strong bcl-10 expression due to truncation of the bcl-10 gene and loss of the apoptotic activity of the encoded protein, while MALT lymphomas lacking this translocation and associated with other translocations show less expression intensity [25].

In the exocrine pancreas, bcl-10 is a marker for acinar cell differentiation and acinic cell carcinomas.

16.2.15 CD11c

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-cell lymphoma	Myeloid hematopoietic cells, granulocytes, macrophages, NK-cells, dendritic cells, a subset of activated T-lymphocytes, histiocytes
Positive control: appendix/tonsil		

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 natural killer cell lymphoma (Fig. 16.13). 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 cells of B-CLL is usually associated with a good prognosis.

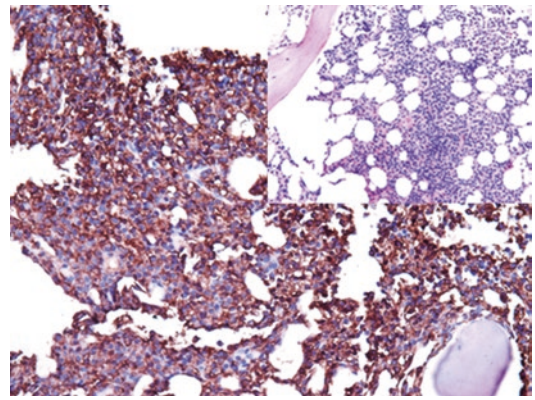


Fig. 16.13 Bone marrow infiltrated by hairy cell leukemia, neoplastic lymphocytes with strong CD11c expression

16.2.16 Tartrate-Resistant Acid Phosphatase (TRAP)

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)	Mantle 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; also called acid phosphatase 5) is a glycosylated monomeric iron-binding metalloprotein enzyme with high activity toward phosphoproteins, ATP, and 4-nitrophenyl phosphate, normally found in different tissue types, and is highly expressed in osteoclasts and macrophages.

TRAP is a specific marker for hairy cell leukemia but should be combined with other markers such as CD11c and DBA 44 (Fig. 16.14).

Diagnostic Pitfalls Another lymphoma type, such as marginal zone B-cell lymphoma, may reveal weak TRAP positivity. TRAP is also expressed in bone marrow macrophages [26].

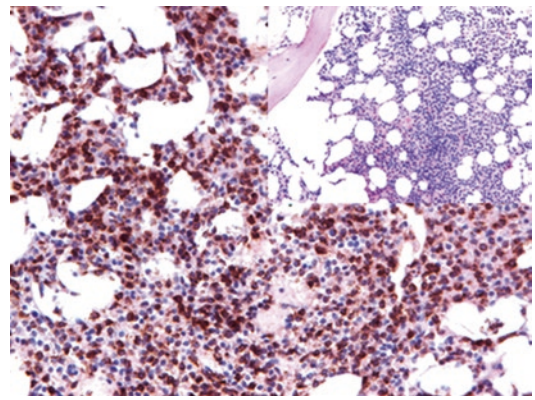


Fig. 16.14 Bone marrow trephine biopsy infiltrated by cells of hairy cell leukemia exhibiting strong cytoplasmic TRAP expression in neoplastic lymphocytes

16.2.17 Immunoglobulin Superfamily Receptor Translocation-1

IRTA-1 (CD307d, also called FCRL4) is the fourth member of the immune receptor translocation-associated protein family (IRTA-1-5) clustered as CD307. IRTA-1 is a cell surface receptor involved in the lymphogenesis of B-lymphocytes in addition to intercellular communication. IRTA-1 is positive in the B-cells of the marginal zone. IRTA-2 is also positive in the B-cells of the marginal zone and centrocytes. IRTA-3 is positive in the germinal centers. IRTA-4 and IRTA-5 are expressed in the mantle zone.

IRTA-1 is a helpful marker to discriminate between marginal zone lymphoma and other lymphoma types as it is expressed in more than 90% of extranodal marginal zone lymphoma, including MALT lymphoma, and in about 75% of nodal marginal zone lymphoma but negative in splenic marginal zone lymphoma (Fig. 16.15). B-CLL and mantle cell lymphoma may be also positive for IRTA-1 but can be distinguished from marginal cell lymphoma by other specific markers for both lymphoma types, including LEF-1 and cyclin D1 and SOX-11. Other lymphoma types, including follicular lymphoma, Burkitt lymphoma, hairy cell leukemia, and plasma cell neoplasms, also lack the expression of IRTA-1 [27, 28]. IRTA-1 cannot distinguish between reactive and neoplastic marginal zone lymphocytes.

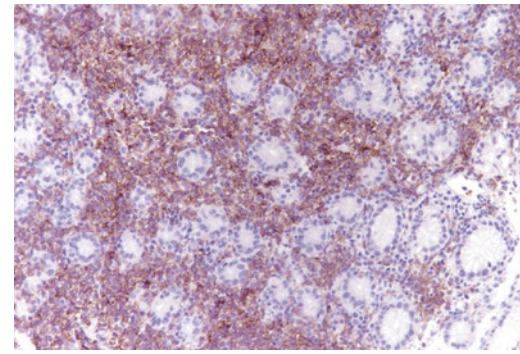


Fig. 16.15 IRTA-1 labels neoplastic lymphocytes of extranodal marginal zone lymphoma (MALT lymphoma)

16.2.18 LIM Only Transcription Factor 2

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	Subset of B- and T-ALL and AML, 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 the secretory phase
Positive control: tonsil/lymph node		

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 pro- and pre-B-lymphocytes in addition to germinal center B-lymphocytes. 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, mantle 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 lymphoma but not in classical Hodgkin lymphoma. Furthermore, LMO2 labels the myeloid blasts of acute myeloid leukemia [29, 30]. In addition to lymphoid and hematopoietic neoplasms, LMO2 labels normal blood and lymph vessel endothelium and the majority of benign and malignant endothelial tumors [31].

16.2.19 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 especially 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 and 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 mantle cell lymphoma and B-CLL are completely negative for HGAL [32, 33].

16.2.20 Lymphoid Enhancer Binding Factor

Lymphoid enhancer binding factor (LEF-1)		
Expression pattern: nuclear		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– B-CLL – Sinonasal glomangiopericytoma – Basal cell adenoma/ adenocarcinoma of salivary gland	Diffuse large B-cell lymphoma, ALK-negative anaplastic large-cell lymphoma, solid pseudopapillary neoplasm of the pancreas, pancreatoblastoma, invasive micropapillary breast carcinoma, papillary thyroid carcinoma (cribriform morular variant), cutaneous basal cell carcinoma	T-lymphocytes, hair follicles
Positive control: tonsil		

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 and differentiation of respiratory submucosal glands. LEF-1 is normally expressed in pre-B- and T-lymphocytes but not in mature B-cells. LEF-1 labels different types of T-cell lymphomas. In B-cell lymphomas, LEF-1 labels the neoplastic small lymphocytes of chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (Fig. 16.16), whereas other low-grade B-cell lymphomas, including mantle cell lymphoma, marginal zone lymphoma, and follicular lymphoma, lack the expression of LEF-1 [34]. The LEF-1 expression is found in

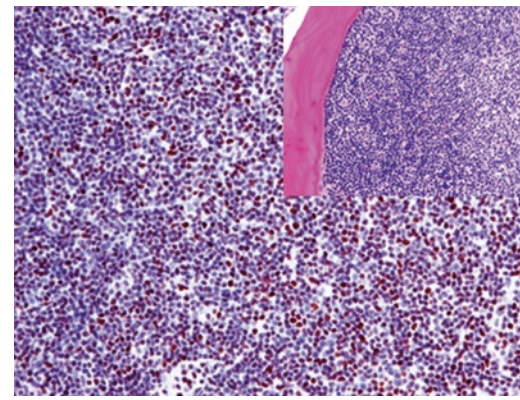


Fig. 16.16 Bone marrow, nuclear LEF-1 expression in CLL neoplastic cells

about one-third of diffuse large B-cell lymphoma and specific for ALK-negative anaplastic large-cell lymphoma with the DUSP22 rearrangement. LEF-1 is not a specific lymphoma marker as it is also expressed in different carcinoma types, such as colorectal adenocarcinoma [35]. Furthermore, the nuclear expression of LEF-1 is also characteristic for sinonasal glomangiopericytoma (see Chap. 3, Fig. 3.10) and solid pseudopapillary neoplasm of the pancreas in addition to invasive micropapillary carcinoma of the breast [36, 37].

In salivary gland tumors, LEF-1 is positive in most basal cell adenomas of the salivary glands, whereas adenoid cystic carcinoma and acinic cell carcinoma usually lack the expression of LEF-1.

16.2.21 Annexin A1

Annexin A1 (Lipocortin) is a member of calcium-dependent phospholipid binding proteins located on the cell membrane and in the cytoplasm, and involved in the regulation of inflammatory reaction and phagocytosis. Annexin A1 is highly upregulated in hairy cell leukemia and used as a specific marker for this lymphoma type. In nonlymphoid neoplasia, annexin A1 is highly expressed in cholangiocarcinoma. In renal tumors, the expression of Annexin A1 is an indicator of the response to TKI.

16.2.22 c-myc

c-myc is a member of the myc family composed of three related transcription factors c-myc, l-myc, and n-myc encoded on chromosomes 8, 1, and 2, respectively. The product of the c-myc gene is a nuclear phosphoprotein and a transcription factor involved in the regulation of different stages of the cell cycle, including growth, proliferation, differentiation, and apoptosis. The c-myc gene is one of the most common mutated genes in human malignancies. In routine immunohistochemistry, the overexpression of c-myc in more than 40% of tumor cells correlates with the presence of an activating mutation. In ~90% of Burkitt lymphoma, the expression of c-myc is activated by one of the specific translocations: t(8;14)(q24;q32) or t(8;22)(q24;q11) (Fig. 16.17). Eight to 14% of diffuse large B-cell lymphoma is also associated with a c-myc activating translocation. High-grade B-cell lymphomas associated with c-myc, bcl-2, and/or bcl-6 gene rearrangements, so-called double or triple hit lymphomas, usually have a poor prognosis.

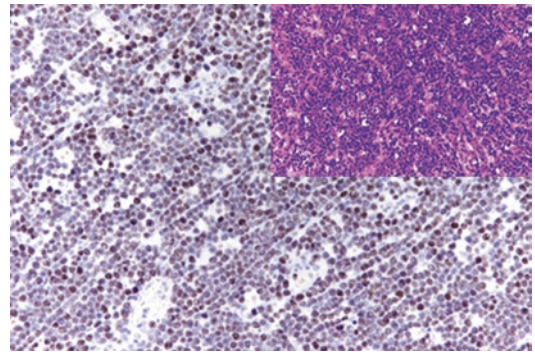


Fig. 16.17 Burkitt lymphoma with nuclear c-myc expression in neoplastic lymphocytes

16.2.23 FOXP1

FOXP1 (Forkhead box protein 1) is a member of the forkhead box family of transcription factors. FOXP1 is expressed in nonneoplastic activated B-lymphocytes and overexpressed in the nongerminal center (ABC) type diffuse large B-cell lymphomas (DLBCL).

Evolution of immunoprofile of nonneoplastic B-lymphocytes	
Cell type	Immunoprofile
Pluripotent stem cell	CD117, CDw123, CD243, CDw338, HLA-DR (CD74)
Lymphoid stem cell	CD10, CD34, CD38, CD117, CD124, CD127, CD228, TdT, HLA-DR (CD74)
Pro-B-cell	CD19, cCD22, CD24, CD34, CD38, CD72, cCD79a, CD79b, CD124, CD127, PAX-5, TdT, HLA-DR
Early B-cell	CD10, CD19, CD20, CD21, CD22, CD24, CD34, CD72, cCD79a, CD79b, CD124, PAX-5, TdT, HLA-DR
Pre-B-cell	CD9, CD19, CD20, CD21, CD22, CD24, CD38, CD40, CD72, CD74, cCD79, CD124, PAX-5, TdT, HLA-DR
Naïve B-cell	CD19, CD20, CD21, CD22, CD24, CD23, CD35, CD40, sCD79, CD124, PAX-5, s-IgM, s-IgD
Follicle center B-cell	CD10, CD19, CD20, CD21, CD22, CD38, CD79a, PAX-5, HLA-DR (CD74), sIgM, sIgG, s-IgA, bcl-6
Immunoblast	CD10, CD19, CD20, CD21, CD22, CD23, CD24, CD37, CD40, CD72, CD 74, CD79a, CD139, CD275, CD316, CD317, HLA-DR, s-IgM, s-IgG, s-IgA, bcl-6
Marginal zone B-cell	CD1c, CD19, CD20, CD21, CD27
Lymphoplasmacytoid cell	CD19, CD20, CD38, CD79a, CD79b, CD275, CD316, CD317
Plasmablast	CD27, CD38
Plasma cell	CD38, CD79a, CD126, CD138, CD269, CD275, CD316, CD317, CD317, cIg, IRF4/MUM-1

Immunoprofile of B-cell neoplasms				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
B-lymphoblastic leukemia/lymphoma	**CD19**, CD79a, **TdT**, **PAX-5**, HLA-DR (CD74), ERG, cIgM Proliferation index (Ki-67): 50–90%	**CD10**[a], CD22, CD34, CD24[a], CD45, CD99, **CD34**[j], FLI-1, LMO2	CD20, CD13	
	Flow cytometry			
	CD19, cCD22, CD38, cCD79a, HLA-DR	CD9[i], CD10[a], sCD22, CD24[a], CD34[j], TdT	CD13, CD20, CD45	MPO
Monoclonal B-cell lymphocytosis	CLL type: see B-CLL immunoprofile (B-cell accounts in peripheral blood <5 × 10^9/L with B-CLL phenotype with no signs of lymph node involvement) Non-CLL phenotype: CD5 and CD23 (−/+)			
	Flow cytometry			
	– CD5 and CD23 on >20% of CD19+ lymphocytes – Kappa-lambda ratio of >25% of mature B-lymphocytes			
B-cell chronic lymphocytic lymphoma (B-CLL)/small lymphocytic lymphoma (B-SLL)	**CD5**[h], CD19, **CD20**, CD22, **CD23**[h], CD74, CD79a, **CD160**, **CD200**, **LEF-1**, ROR-1, PAX-5, p27, bcl-2, sIgM Proliferation index (Ki-67): ~5%	CD22, CD43, MUM-1, sIgD	CD11c, CD38[b] ZAP-70[b], DBA44	CD10, SOX-11, bcl-6
	Flow cytometry			
	CD5[h], CD11cweak, CD19, CD23, CD25, CD43, CD200, sIgweak, ROR-1 κ-/λ-light-chain restriction	CD11c, CD20weak, CD22weak, CD81	CD38, CD49d[b], CD79a	CD3, CD10, CD25, CD79b, CD81, CD103, FMC7

B-cell prolymphocytic leukemia	**CD19**, **CD20**, CD22, CD25, CD27, CD74, CD79a, PAX-5, bcl-2	sIgM, sIgD	CD23	CD5, CD10, CD23, CD43, CD138, cyclin D1
	Flow cytometry			
	CD19, CD20, CD22, CD25, CD79a, FMC7		CD5, CD23, CD38	CD10
Lymphoplasmacytic lymphoma	Small B-lymphocytes: **CD19**, **CD20**, CD22, CD43, CD74, CD79a, **CD200**, PAX-5, IgM Plasmacytoid cells and plasma cells: CD19, CD38, CD79a, CD138, MUM-1 Proliferation index (Ki-67): ~5–10%	Bcl-2, MYD88	CD5, CD23,	Lymphocytes: CD3, CD10, CD103, cyclin D1 Plasma cells: CD56
	Flow cytometry			
	In B-cells: CD19, CD20, CD200 In plasma cells: CD19, CD38, CD138, cIgM	CD11c, CD22, CD43, CD25, FMC7	CD5, CD23,	CD3, CD10, CD56, CD103
Mantle cell lymphoma/in situ mantle cell neoplasm	**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	MUM-1	CD10, CD11c, CD23, CD138, bcl-6
	Flow cytometry			
	CD5, CD19, CD22, CD79a, CD79b, FCM-7, bcl-2	CD11c, CD43, SOX-11		CD3, CD10, CD11c, CD23, CD103, CD138, CD200
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 (see Fig. 19.3) **Proliferation index (Ki-67) in bcl-2 positive neoplastic follicles (low grade): <15%** **Proliferation index (Ki-67) in bcl-2 negative reactive follicles: >60%**	CD10, **bcl-6**, **LMO2**, **HGAL** κ/λ light-chain restriction		CD5, CD23, CD43, SOX-11, cyclin D1
	Flow cytometry			
	CD19, CD20, CD22, CD79a, CD10, sIg			CD3, CD5, CD11c, CD43, CD103, CD200
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]

Primary cutaneous follicle center lymphoma	CD20, PAX-5, **bcl-6**		CD10, CD30, CD23, bcl-2	CD3, CD5, CD43, MUM-1, bcl-2, cyclin D1
Nodal marginal zone B-cell lymphoma	CD19, **CD20**, CD21, CD22, CD35, CD74, CD79a, **IRTA-1**, MNDA, PAX-5, sIgM	sIgA, sIgG, CD43, CD11c, bcl-2	CD38, MUM-1, TRAP	CD3, CD5, CD10, CD21, CD 23, CD138, bcl-6, SOX-11, sIgD, cyclin D1
	Flow cytometry			
	CD1d, CD19, CD22, CD74, CD79a, sIg	CD43		CD5, CD10, CD21, CD23, Cd43, CD103, CD200, bcl-6
Extranodal marginal zone B-cell lymphoma of MALT type	CD19, **CD20**, CD21, CD22, CD35, CD74, CD79a, PAX-5, sIgM, bcl-2, **IRTA-1**	CD11c, CD43, MUM-1, bcl-10, sIgD, sIgA, sIgG	CD43	CD3, CD5, CD10, CD23, SOX-11, cyclin D1, bcl-6
	Flow cytometry			
	CD19, CD21, CD35, FMC7, IgM	CD11c	CD23, IgA, IgG	CD3, CD5, CD10, CD25, CD103, IgD
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, CD11c, DBA44	CD23, CD25, CD43, CD103, sIgG	CD3, CD5, CD10, CD43, CD103, bcl-6, **cyclin D1**, annexin A1, IRTA-1
	Flow cytometry			
	CD19, CD11c, CD20, CD22, CD200, FCM-7, IgM, IgD		CD11c, CD23, CD25, CD103	CD3, CD5, CD10, CD38, CD43, CD103, CD123
Splenic diffuse red pulp small B-cell lymphoma	CD20, CD19, CD79a, DBA44, PAX-5	Cyclin D3	CD103	CD3, CD5, CD10, CD21, CD23, CD25, CD38, CD43, cyclin D1, annexin A1
	Flow cytometry			
	CD11c, CD1d, CD19, CD20, CD22, CD25, CD103, CD123, CD180, CD200, FCM-7, sIg		CD103	CD3, CD5, CD10, CD21, CD23, CD25
Hairy cell leukemia	**CD11c**, CD19, **CD20**, CD22, **CD25**, CD74, CD79a, **CD103**, CD123, **annexin A1**, **TRAP**, **DBA.44 (CD76)**, **BRAF-v600E**, PAX-5, bcl-2, sIgM Proliferation index (Ki-67): <5%	CD23, CD68 (cytoplasmic dots), PCA-1, HC1, HC2, cyclin D1	CD5	**CD10**, **CD23**, **CD43**, bcl-6
	Flow cytometry			
	CD11c, CD1d, CD19, CD20, CD22, CD25, CD103, CD123, CD200, FCM-7, sIg		CD23weak	CD3, CD5, CD10, CD27, CD43, CD180
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, bcl-2 Proliferation index (Ki-67): >40%	Bcl-6, FOXP1[l]	CD5, CD10, CD30, Fascin, p63, MUM-1[e]. Islet-1[k]	CD3, CD15, CD200

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 microenvironment cells (>80% of cell population): lymphocytes positive for CD3, CD8, and cytotoxic molecules and histiocytes positive for CD68 and CD163		CD30, bcl-2, EMA	CD3, CD5, CD10, CD15, bcl-2, PU 1
Mediastinal (thymic) large B-cell lymphoma	CD19, **CD20**, CD45, CD74, CD79a, CD200, PAX-5, Oct-2, STAT-6	**CD23**, MUM-1, **CD30**, **HGAL**, **LMO2**, bcl-2, bcl-6, PD-L1, p16, p63	CD10	CD3, CD5, CD15, CD21
	Flow cytometry			
	CD19, CD22, CD79a, CD200	CD23, CD30, HLA-DR		CD3, CD5, sIg
ALK-positive large B-cell lymphoma	**ALK**, EMA, CD38, CD138, VS38c, MUM-1 κ-/λ-light-chain restriction Proliferation index (Ki-67): >90%		CD4, CD10, CD38, CD45, CD43, CD79a, EMA, Pan-CK	CD3, CD19, CD20, CD22, CD30, PAX-5
Large B-cell lymphoma with IRF-4 rearrangement	CD19, CD20, CD22, **MUM-1**, **bcl-6**	CD10, bcl-2	CD5	
Fibrin-associated large B-cell lymphoma	CD19, CD22, CD79a, PAX-5 Proliferation index (Ki-67): >90%	CD30, bcl-2, bcl-6		CD10
Primary cutaneous diffuse large B-cell lymphoma, leg type	CD19, CD20, CD79a, PAX-5, bcl-2, MUM-1 Proliferation index (Ki-67): >40%	Bcl-6, P63		CD10
KSHV-/HHV8-positive diffuse large B-cell lymphoma	**HHV-8**, CD19	CD30	CD20, CD38	CD79a, CD138
Intravascular large B-cell lymphoma	CD19, CD20, CD79a, PAX-5, MUM-1	Prostatic acid phosphatase, Bcl-2	CD5, CD10, bcl-6	CD3, CD23, cyclin D1
Primary effusion lymphoma	CD45, CD30, **CD79a**, CD38, CD138, VS38c, **HHV-8**, MUM-1	**PAX-5**, EMA, EBV	CD4, CD7	CD10, CD19, CD20, CD43, PAX-5, bcl-6
	Flow cytometry			
	CD138, CD71		CD20, CD23	CD10, CD19, CD22, FMC7
Burkitt lymphoma	**CD10**, CD19, **CD20**, CD22, CD38, CD74, CD79a, PAX-5, sIgM, **c-myc**, **HGAL**, CD43, Oct-2, p53 Proliferation index (Ki-67): >95%	Bcl-6, EBV, LMO2, SOX11, CD43, **adipophilin**[g]	MUM-1	CD5, CD23, TdT, bcl-2, cyclin D1
	Flow cytometry			
	CD10, CD19, CD20, CD22, CD38, CD71, CD77, sIgM, HLA-DR, FMC7			CD5, CD23, CD34, CD44, TdT
High-grade B-cell lymphoma with 11q aberrations (Burkitt-like lymphoma with 11q aberration)	CD19, CD20, CD22, CD38, CD74, CD79a, PAX-5, MUM-1 Proliferation index (Ki-67): >95%	CD10, CD43, bcl-6, LMO-2, sIgG, IgM	CD56	**c-myc**, bcl-2

EBV-positive DLBCL	**EBV**[f], CD19, **CD30**, MUM-1, PAX-5	CD20, CD15, bcl-2		CD10
Fibrin-associated large B-cell lymphoma	CD19, CD20, CD79a, PAX-5, MUM-1 Proliferation index (Ki-67): >90%	CD30, bcl-2, bcl-6	CD3, CD4, CD43	CD10
Fluid overload-associated large B-cell lymphoma	CD19, CD79a, PAX-5,	CD20	CD30, CD138	LMO-2
Primary large B-cell lymphoma of immune-privileged sites	CD19, CD20, CD79a, PAX-5, bcl-2, bcl-6, MUM-1 Proliferation index (Ki-67): >80%			
EBV-positive mucocutaneous ulcer	Large B-cells: EBV(f), CD19, MUM-1, PAX-5 T-cells: CD3, CD8	CD30		
Lymphomatoid granulomatosis	EBV, CD19, **CD20**, CD79a, PAX-5	CD30		CD15

[a]Negative in ALL with 11q23 translocation
[b]The expression of CD38, CD49d, or ZAP70 in B-CLL correlates with a worse prognosis
[c]Pediatric type follicular lymphoma lacks the t(14;18) translocation
[d]See the modified Hans Algorithm 16.1 and table below [38]
[e]Positive in ABC (activated B-cell-like) subtype of DLCBL
[f]EBV antigens: EBER, LMP1, EBNA2
[g]Due to the presence of intracytoplasmic lipid vacuoles in cells of Burkitt lymphoma (see Fig. 16.18)
[h]Atypical CLL may be negative for CD5/CD23 and strong CD20/FMC7 expression
[i]CD9 negative in precursor B-cell ALL with t(12;21)
[j]CD34 negative in precursor B-cell ALL with t(0;22)
[k]See Fig. 16.19
[l]Expressed only in non-GCB (ABC) type

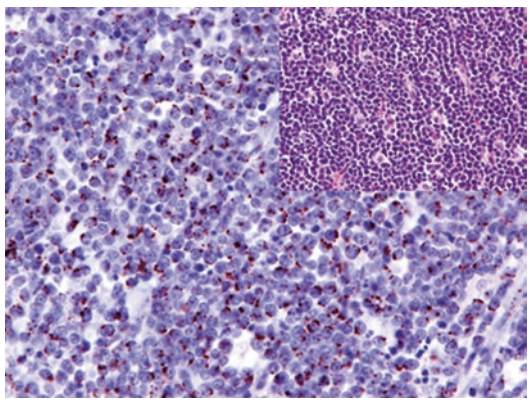


Fig. 16.18 Intracytoplasmic adipophilin expression in the cells of Burkitt lymphoma

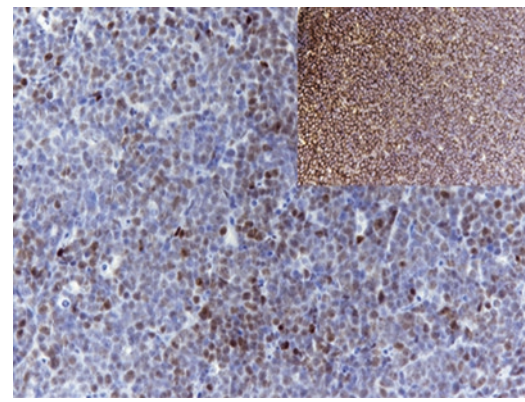


Fig. 16.19 Diffuse large B-cell lymphoma with strong membranous CD20 expression (upper left picture). Lymphoma cells also exhibit a marked nuclear Islet-1 expression

16.3 Markers and Immunoprofile of Plasma Cell Neoplasms

16.3.1 Immunohistochemical Markers for Plasma Cell Neoplasms

CD20, CD38, CD56, CD138, VS38c, CD79a, MUM-1, κ and λ light chains.

16.3.2 CD38

CD38		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Plasma cell neoplasms – Plasmablastic lymphoma	Pre-T-ALL, B-ALL, primary effusion lymphoma, subtypes of B-cell lymphoma, AML	Plasma cells, erythroid and myeloid precursors, early B- and T-cells, NK-cells, pancreatic islets, normal epithelium of prostate, astrocytes and pyramidal neurons
Positive control: appendix		

Diagnostic Approach CD38 (also known as ADP-ribosyl cyclase) is a transmembrane glycoprotein involved in signal transmission and regulation of intracytoplasmic calcium concentration. CD38 is expressed in most CD34 positive pluripotent stem cells and in different maturation stages of B- and T-lymphocytes, plasma cells, and myeloid cells [18]. In B-cells, the expression is found in germinal center B-cells and memory B-cells in the marginal zone. CD38 is commonly used in diagnostic panels for multiple myeloma. CD38 may also be expressed on a subset of B-CLL cells and is considered an adverse prognostic factor. CD38 is a target for specific therapeutic antibodies used for the treatment of multiple myeloma.

Diagnostic Pitfalls CD38 has a broad 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.

16.3.3 CD138

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, different carcinoma types including thyroid, breast, lung, head and neck, urothelium, prostate, 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. CD138 is expressed in different maturation stages of B-lymphocytes but lost at the pre-B stage. CD138 is strongly expressed in plasma cells in addition to different types of epithelial and mesenchymal cells and binds to various growth factors and extracellular matrix proteins regulating cell differentiation and cell adhesion.

Diagnostic Pitfalls CD138 is widely used as a marker for plasma cells and plasma cell neoplasms (Fig. 16.20); 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 [39]. A particular pitfall is the plasmacytoid urothelial carcinoma, which is often strongly positive for CD138 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 marker but not EMA, as EMA may also be positive in plasma cell disorders as well [9]. The cytoplasmic expression of κ or λ light chains in the plasma cells is also essential 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 [40].

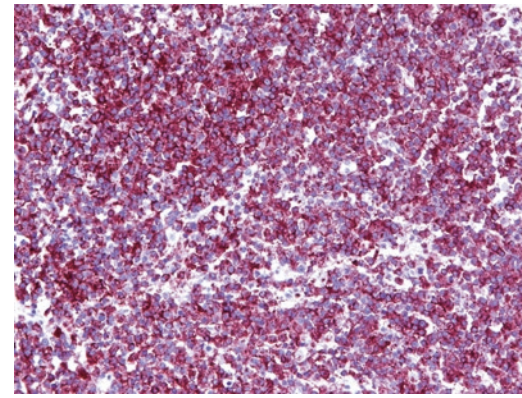
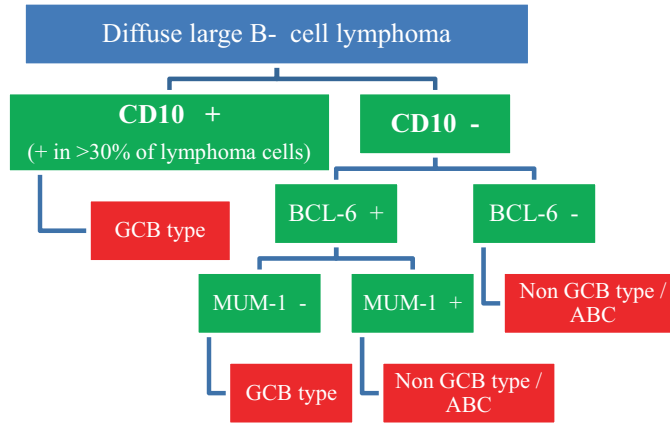


Fig. 16.20 Multiple myeloma with strong membranous CD138 expression

16.3.4 Multiple Myeloma Oncogene 1/IRF4

Multiple myeloma oncogene 1/IRF4 (MUM-1)		
Expression pattern: nuclear/cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Plasma cell neoplasms – Plasmablastic lymphoma – Diffuse large B-cell lymphoma ABC type – Anaplastic CD30+ large-cell lymphoma ALK +/− – Large B-cell lymphoma with IRF-4 rearrangement – Hodgkin and Reed–Sternberg cells in classical Hodgkin lymphoma	CLL, marginal zone lymphoma, intravascular large B-cell lymphoma, primary mediastinal large-cell lymphoma, angioimmunoblastic T-/NK-cell lymphoma, CNS lymphoma, malignant melanoma	B-cells (centrocytes), plasma cells, T-follicular helper (TFH) cells
Positive control: appendix		

Diagnostic Approach **Mu**ltiple **m**yeloma **1** protein (MUM-1, also known as the interferon regulatory factor 4), is a lymphocyte-specific transcriptional activator expressed in the final differentiation stage of intra-germinal center B-lymphocytes. MUM-1 also plays a role in the differentiation of plasma cells, T-lymphocytes, myeloid cells, and dendritic follicular cells. MUM-1 is also a marker for post-germinal center B-cells (late centrocytes), memory B-cells in the marginal zone, and nongerminal/activated B-cell phenotype lymphomas (see modified Hans Algorithm 16.1). MUM-1 is also an essential marker for plasma cells and plasma cell neo-



Algorithm 16.1 Modified Hans algorithm of DLBCL [38]. *GCB* germinal center B-cell type, *ABC* activated B-cell type

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

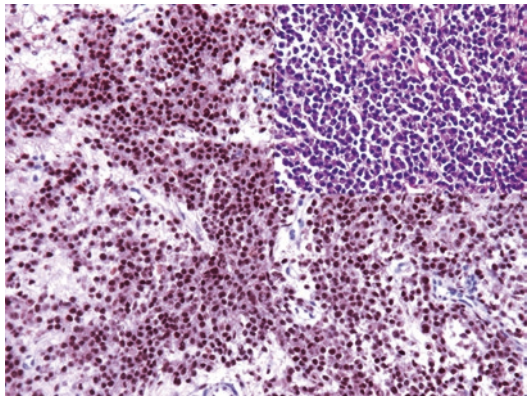


Fig. 16.21 Strong nuclear MUM-I expression in multiple myeloma cells

plasm (Fig. 16.21). Furthermore, MUM-1 is expressed in a subset of T-cells (TFH) and related lymphoma types. The expression of MUM-1 is activated in EBV-infected lymphocytes, which is also a diagnostic marker for Hodgkin cells in classic Hodgkin lymphoma. MUM-1 is usually negative in the cells of nodular lymphocyte-predominant Hodgkin lymphoma. Bcl-6 positive B-cells usually lack the expression of MUM-1.

Diagnostic Pitfalls The expression of MUM-1 is not limited to plasma cell neoplasm or B-cell lymphomas. Weak MUM-1 expression can be noted in some types of T-/NK-cell lymphomas, namely, those originating from follicular helper T-cells such as angioimmunoblastic T-cell lymphoma. MUM-1 stains also the majority of anaplastic CD30-positive large-cell lymphomas, both ALK+ and ALK−. MUM-1 stains also a subset of malignant melanoma, which can also be positive for other plasma cell markers such as CD138 and VS38c. Because of the multilineage expression of the MUM-1 protein, the immunostaining must be carefully interpreted in combination with other more specific antibodies to exclude other possible differential diagnoses [41, 42].

16.3.5 VS38c

VS38c (plasma cell marker)		
Expression pattern: cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Plasma cell neoplasms – Lymphoma with plasmacytic differentiation	Rare carcinoma types of different origins, malignant melanoma, clear cell sarcoma of soft tissue, neuroendocrine tumors, osteosarcoma	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, also known as cytoskeleton-linking membrane protein 63) 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 cells, it is always important to keep in mind that other tumor types, such as melanocytic and neuroendocrine tumors, may be also positive for this marker [43]. Paratrabecular osteoblasts in trephine biopsies are also positive for VS38c. VS38c is also a sensitive but less specific marker for osteosarcoma.

16.3.6 Kappa and Lambda Light Chains

Each molecule of the five major classes of immunoglobulins consists of a 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 chains; 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 a monoclonal/neoplastic nature of this lymphocyte or plasma cell population. In routine histopathology, the expression of the light chains can be indicated either by conventional immunohistochemistry or 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 the bone – Extraosseous plasmacytoma – Monoclonal immunoglobulin deposition disease	**CD38**, **VS38c**, **CD138**, CD229, CD319, 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, CD20, CD31, CD33, CD117, steroid hormone receptors (ER)	CD19, CD22, PAX-5, E-cadherin
	Flow cytometry			
	CD38, CD138, CD27, CD29, CD44, CD54, CD86, CD126 **κ or λ Ig light-chain restriction**	CD56	CD31, CD117, CD200	CD19, CD45

16.4 Markers and Immunoprofile of T-Cell Neoplasms

16.4.1 Immunohistochemical Markers for T-Cell Lineage and T-Cell Lymphoma

CD2, CD3, CD4, CD5, CD7, CD8, CD30, CD34, CD43, TdT, ALK, TCL-1, LEF-1, ICOS, TCR, CXCL13, PD-1 [2, 10, 44].

16.4.2 CD2

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) encoded on chromosome 1 and appears in the early stages of T-cell development at the prothymocyte stage. CD2 is the ligand for CD59 and mediates the adhesion between T-lymphocytes and other cells, binding to CD48 and CD58 (LFA3) surface proteins expressed on the antigen-presenting cells, and plays an important role in the activation of memory T-lymphocytes. CD2 is an excellent marker for T-lymphocytes and NK-cells and labels T-cell lymphomas and the majority of NK-cell 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 expression of CD2 in mast cells is considered a criterion of malignancy (see Chap. 18).

16.4.3 CD3

CD3		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– T-cell lymphomas	NK-lymphoma (cytoplasmic stain)	Thymocytes, peripheral mature T-cells, activated NK-cells, Purkinje cells of the 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. In early embryogenesis, CD3 is expressed in the cytoplasm of the prothymocytes and persists through all differentiation stages of T-lymphocytes until mature cells. CD3 builds a complex with the T-cell receptor on the membrane of T-lymphocytes responsible for recognizing antigens, leading to the activation of both T-cytotoxic and T-helper immune response. CD3 is the most commonly 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 stain pattern using CD3ε specific antibodies.

16.4.4 CD4

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, tumors of the parathyroid gland	Thymocytes, T-helper/inducer cells, macrophages, granulocytes, Langerhans cells, parathyroid chief 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 different types of T-lymphocytes, including Th1, Th2, Th9, Th17, Th21, TFH, and Treg lymphocytes in addition to the majority of thymocytes and a subset of monocytes, macrophages, and dendritic cells. CD4 is a marker of lymphomas originating from these cells, which include the majority of peripheral T-cell lymphomas and cutaneous lymphomas, mainly mycosis fungoides and other histiocytic and myeloid neoplasms (See Chap. 19). T-lymphocytes with TCRγδ and tumors originating from these cells are usually negative for CD4.

Diagnostic Pitfalls CD4 can also be expressed on different hematopoietic precursors, including erythroid and myeloid precursors, in addition to megakaryocytes. In immunohistochemistry and flow cytometry, CD4 is used in a panel with CD3 and CD8 and CD19. CD4 can also be positive in subtypes of acute myeloid leukemia, namely, AML with monocytic differentiation and histiocytic neoplasms (Fig. 16.22).

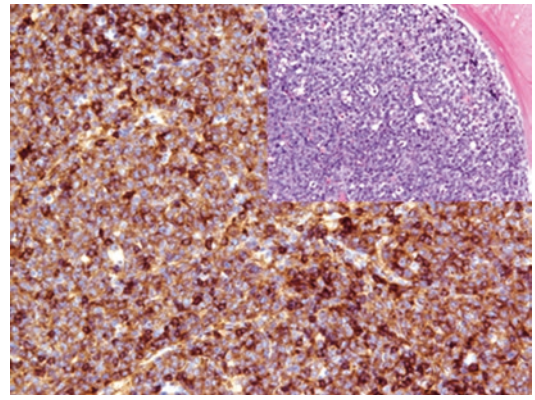


Fig. 16.22 Diffuse CD4 expression in myeloid blasts of AML (M5)

16.4.5 CD7

CD7		
Expression pattern: membranous		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– T-ALL and T-cell lymphomas	CML, AML, 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 membranous glycoprotein and a member of the immunoglobulin family involved in T-cell/B-cell interaction and activation of cytokine production. CD7 is expressed in early T-lymphocytes, thymocytes, NK-cells, and a subset of myeloid cells. The expression of CD7 persists in the majority of mature T-lymphocytes and in T-cell/NK-lymphomas derived from these cells, whereas the cells of adult T-cell lymphoma/leukemia and the cells of Sézary syndrome and mycosis fungoides usually lack the expression of CD7. Together with CD34 and CD117, CD7 labels the blasts of high-risk MDS.

Diagnostic Pitfalls CD7 is expressed in a subset of AML, mainly M4/5, in addition to CML. CD7 can also be positive in some carcinoma types, such as pancreatic and bile duct carcinomas [9].

16.4.6 CD8

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	Monomorphic epitheliotropic intestinal T-cell lymphoma, primary cutaneous aggressive epidermotropic cytotoxic T-cell lymphoma, T-PLL, CLL, mantle cell lymphoma	Suppressor/cytotoxic T-cells and subset of NK-cells
Positive control: appendix/tonsil		

Diagnostic Approach CD8 is a transmembrane disulfide-linked heterodimeric glycoprotein composed of either α- and β-chain or two α-chains functioning as a co-receptor for the T-cell receptor playing a role in the T-cell signaling cascade. CD8 is 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. 16.23).

Diagnostic Pitfalls CD8 is expressed in a small subset of B-cell lymphomas and should generally be a part of a panel with CD3, CD4, and CD20 [9]. The expansion of CD8-positive T-cell population is noted in lymph nodes associated with acute infectious mononucleosis.

16.4.7 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 lymphoma and some other lymphoma types, CD30 is a diagnostic marker for anaplastic large-cell lymphoma (Fig. 16.24). CD30 is listed in detail with the markers of Hodgkin lymphoma.

16.4.8 CD43

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, megakaryocytes, cutaneous mast cells
Positive control: appendix/tonsil		

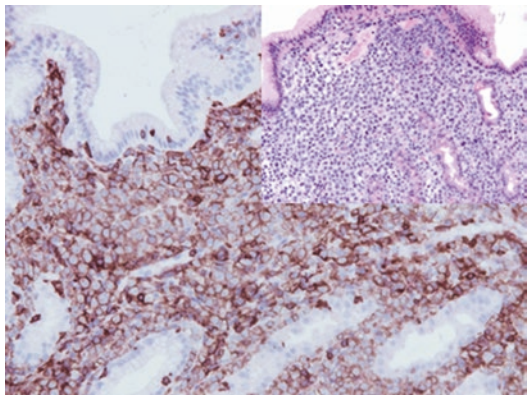


Fig. 16.23 Diffuse CD8 expression in cells of enteropathy-type T-cell lymphoma (type II)

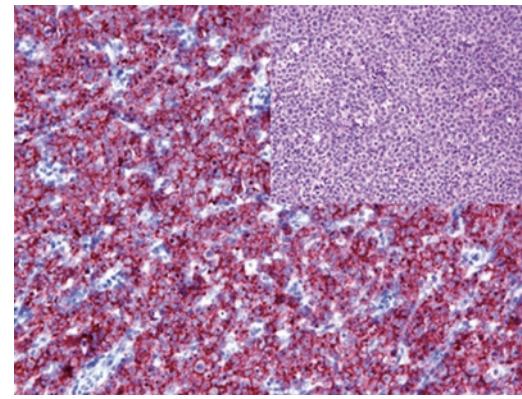


Fig. 16.24 Diffuse CD30 expression in anaplastic large-cell lymphoma

Diagnostic Approach CD43 (also known as Sialophorin or Leukosialin) is a sialoglycoprotein encoded on chromosome 16 functioning as an antiadhesion molecule. CD43 is expressed on the membrane and in the cytoplasm of the T-/NK-lymphocytes, different cells of the myeloid lineage, plasma cells, and neoplasms originating from these cells. CD43 is expressed in the majority of T-/NK-cell lymphomas, including T-ALL and a subset of B-cell lymphomas.

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 is to be considered 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 lymphoma/leukemia, including B-ALL and a subset of B-CLL and SLL, Burkitt lymphoma, mantle cell lymphoma, and nodal/extranodal marginal zone lymphoma in addition to diffuse large B-cell lymphoma [2]. 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.

16.4.9 CD103

CD103 is the alpha E integrin subunit of the heterodimeric αEβ7 integrin (also known as antihuman mucosal lymphocyte 1 antigen). CD103 is expressed in different types of T-lymphocytes mainly intestinal and intraepithelial CD8+ T-lymphocytes and mucosa-associated T-lymphocytes, cytotoxic and activated T-lymphocytes in addition to dendritic cells, and a small subset of B-lymphocytes. CD103 is a marker for enteropathy-associated T-cell lymphoma and monomorphic epitheliotropic intestinal T-cell lymphoma in addition to hairy cell leukemia.

16.4.10 Anaplastic Lymphoma Kinase

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 B-cell lymphoma – ALK rearrangement renal cell carcinoma – Therapy-related biomarker in pulmonary non-small-cell carcinoma	ALK-positive histiocytosis, malignant peripheral nerve sheath tumor, rhabdomyosarcoma, neuroblastoma, glioblastoma, Ewing's sarcoma/PNET, leiomyosarcoma, thyroid carcinoma, salivary gland carcinoma (intraductal and ductal carcinoma, myoepithelial carcinoma, secretory carcinoma)	Glial cells, neurons, endothelial cells, pericytes, T-lymphocytes
Positive control: anaplastic lymphoma/brain tissue/appendicular ganglion cells		

Diagnostic Approach Anaplastic lymphoma kinase (ALK) is a membrane-associated kinase encoded on chromosome 2p23 and clustered as CD246. ALK is expressed during embryogenesis, plays an important role in the differentiation of the nervous system, and remains positive in glial cells. In normal tissue, ALK is only detected in glial cells, neurons, endothelium, and pericytes. Other tissue types, including lymphoid tissue, usually lack the expression of ALK. The ALK expression is found in various tumor types due to the activation of the ALK gene transcription caused by the stimulation by a promotor of another gene due to different translocations or

gene rearrangements [45]. The t(2;5)(p23;q35) translocation is the most common genetic anomaly characteristic for ALK-positive anaplastic large-cell lymphoma and inflammatory myofibroblastic tumor [46]. The nucleophosmin (NPM) gene located on chromosome 5q35 is a housekeeping gene encoding a nuclear phosphoprotein which is the fusion partner of the ALK gene in this translocation generating the active NPM-ALK fusion gene, which in turn encodes a chimeric tyrosine kinase composed of the entire cytoplasmic ALK domain and a part of the NPM protein (known as p80). The unregulated expression of the NPM-ALK fusion protein causes the dysregulation of the tyrosine kinase regions in tumor cells. t(1;2)(q25;p23), inv. 2(p23;q35), t(2;3)(p23;q12.2), t(2;13)(p23;q34), t(2;17)(p23;q25), t(2;19)(p23;p13.1), t(2;22)(p23;q11.2), and t(X;2)(q11–12;p23) are further but less common genetic abnormalities associated with anaplastic large-cell lymphoma and other solid tumors.

The ALK molecule is the target for specific kinase inhibitors used to treat ALK-positive tumors, including pulmonary adenocarcinoma and ALK-positive anaplastic large-cell lymphoma. The immunohistochemical detection of ALK in tumor cells is a surrogate for a possible ALK gene rearrangement, which can be later confirmed by one of the molecular methods or FISH (see Chap. 3).

Diagnostic Pitfalls A strong ALK expression is also characteristic for ALK-positive large B-cell lymphoma. This rare lymphoma type lacks the t(2;5) translocation and is consistently CD30 negative (Fig. 16.25).

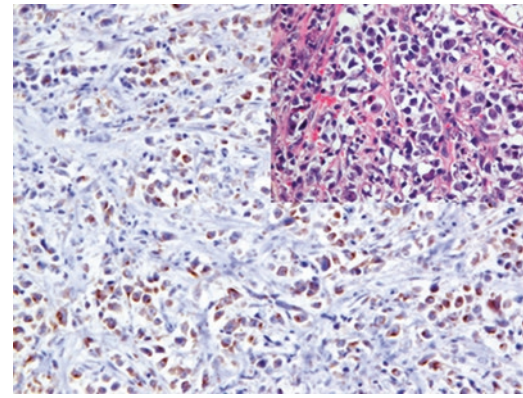


Fig. 16.25 Anaplastic large-cell lymphoma exhibiting ALK expression in lymphoma cells

16.4.11 T-Cell Leukemia Protein 1 (TCL-1)

T-**c**ell **l**eukemia protein **1** (TCL-1) is an oncoprotein encoded on chromosome 14q32.1 functioning as AKT kinase (an isoform of protein kinase B) coactivator involved in survival pathways by inhibiting the apoptotic cascades. TCL-1 is normally expressed in the early embryogenesis of lymphocytes in addition to nonneoplastic B-cells of the mantle zone, plasmacytoid dendritic cells, and testicular germ cells. TCL-1 is overexpressed in T-cell prolymphocytic leukemia as a result of the t(14;14)(q11;q32) rearrangement specific for this leukemia type, typically exhibiting a strong nuclear expression pattern. Other T-cell lymphoma types usually lack TCL-1 positivity. TCL-1 is a marker for plasmacytoid dendritic cell neoplasm but is negative in other histiocytic and myelomonocytic neoplasms. TCL-1 is expressed in different lymphoma types of B-cell origin, and a strong expression is found in Burkitt lymphoma. Follicular lymphoma, mantle cell lymphoma, CLL, hairy cell leukemia, and diffuse large-cell lymphoma show weak to moderate expression intensity, whereas marginal zone lymphoma, CD30+ anaplastic lymphoma, and plasma cell tumors are constantly negative for TCL-1.

The expression of TCL-1 is also one of the specific markers for testicular intratubular germ cell neoplasms (IGCN), seminoma, and ovarian dysgerminoma. TCL-1 is not a marker for other germ cell tumors.

16.4.12 Programmed Cell Death Protein 1 (PD-1)

Programmed cell death protein 1 (PD-1, clustered as CD279) is a type I membrane protein encoded by the PDCD1 gene on chromosome 2q37.3 and a member of the CD28/CTLA-4

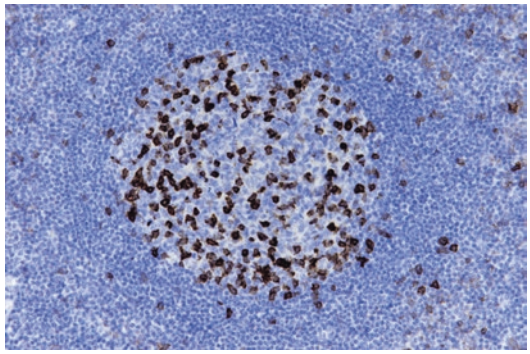


Fig. 16.26 Strong PD-1 expression in follicular helper T-lymphocytes and few peripheral T-lymphocytes

family of receptors. PD-1 binds to its two ligands PD-L1 and PD-L2, which are involved in the regulation of the immune response (see Chap. 31). The PD-1 expression is found on CD4+ follicular helper T-lymphocytes and activated T-lymphocytes in addition to a small subset of B-lymphocytes and myeloid cells (Fig. 16.26).

In routine immunohistochemistry, PD-1 is a marker for angioimmunoblastic T-cell lymphoma. PD-1 is also expressed in a subset of NOS peripheral T-cell lymphoma in addition to a subset of ALK + and ALK − anaplastic large-cell lymphoma.

PD-1 is a helpful marker for the diagnosis of both classic Hodgkin lymphoma and nodular lymphocyte-predominant Hodgkin lymphoma as it is strongly expressed on the T-lymphocytes surrounding the Hodgkin cells.

Both PD-1 and PD-L1 are the targets for different specific checkpoint inhibitors used in tumor therapy (see Chap. 31).

16.4.13 T-Cell Receptor (TCR)

The T-cell receptor (TCR) is a molecule that belongs to the immunoglobulin (Ig) superfamily, expressed on the membrane of T-lymphocytes, responsible for identifying the antigens bound to the major histocompatibility complex (MHC) molecules. Each TCR is composed of two different protein chains, whereas 95% of T-lymphocytes consist of alpha and beta chains (TCRα and TCRβ) and 5% are composed of gamma and delta chains (TCRγ and TCRδ; γ/δ lymphocytes). In routine immunohistochemistry, the expression of the TCR on lymphocytes confirms the T-cell lineage of these cells, and antibodies to the chains mentioned above can be helpful in classifying the T-cell lymphomas. NK cells and NK-cell lymphomas lack the expression of the TCR.

16.4.14 ICOS

ICOS (**i**nducible T-cell **co**-**s**timulator, clustered as CD278) is a member of the CD28 family that regulates the T-cell activity and immune responses and plays a role in the regulation of T-follicular helper cells. The ICOS molecule contains an extracellular, a transmembrane, and an intracellular domain and is primarily expressed on activated CD4+ and CD8+ T-cells. ICOS is a sensitive marker for T-cell lymphomas of follicular helper T-cell origin, mainly angioimmunoblastic T-cell lymphoma and peripheral T-cell lymphomas with T-follicular helper phenotype.

16.4.15 CXCL13 (CXC Motif Chemokine Ligand 13)

CXCL13 is a member of the chemokine family listed in Chap. 20. CXCL13 is strongly expressed on follicular helper CD4+ T-lymphocytes and follicular dendritic cells. CXCL13 is a marker for angioimmunoblastic T-cell lymphoma.

16.5 Markers and Immunoprofile of NK-Cell Neoplasms

16.5.1 Immunohistochemical Markers for NK-Cell Lymphoma

CD2, CD3, CD56, cytotoxic molecules (TIA-1, granzyme B, perforin), and EMA [2, 10].

16.5.2 CD56

CD56 (N-CAM; NKH1)		
Expression pattern: membranous		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– NK-cell lymphomas – Multiple myeloma – Acute and chronic myeloid leukemia – Neuroendocrine tumors (large-cell neuroendocrine carcinoma, small-cell carcinoma, carcinoid and Merkel cell carcinoma) – Pheochromocytoma – Neuroblastoma – Ovarian sex cord-stromal tumors	Synovial sarcoma, embryonal and alveolar rhabdomyosarcoma, angiosarcoma, solitary fibrous tumor, chordoma, epithelioid sarcoma, leiomyoma and leiomyosarcoma, Ewing's sarcoma/PNET, medulloblastoma, schwannoma and neurogenic sarcoma, astrocytomas, ependymoma, meningioma, retinoblastoma, paraganglioma, melanoma, mesothelioma, bile duct adenoma	NK-cells, activated T-cells, a subset of monocytes, cerebellum and brain cortex, neuromuscular junctions, neurons, intestinal ganglion cells, neuroendocrine tissue, thyroid follicular cells, 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 a 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.

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 multiple myeloma cells, whereas CD56-negative myelomas are found to have a poor prognosis. CD56 may also be expressed on other tumors with similar morphology, such as embryonal rhabdomyosarcoma, neuroblastoma, malignant melanoma, neurogenic sarcoma, and synovial sarcoma, which is to consider in the differential diagnosis [9, 47].

Granular cell tumor, neurofibroma, solitary fibrous tumor, and angiosarcoma lack the expression of CD56.

16.5.3 Cytotoxic Molecules (Granzyme B, Perforin, and TIA-1)

Cytotoxic molecules are a heterogeneous group of intracytoplasmic cytotoxic molecules found in the T-lymphocytes and natural killer (NK) cells. 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 commonly used cytotoxic molecules in routine immunohistochemistry.

16.5.4 Perforin

Perforin (complement 9 related protein, also known as cytolysin) is a cytolytic pore-forming protein found in the granules of cytotoxic T-lymphocytes and natural killer cells. It is able to

perforate a pore in the membrane of targeted cells to enable granzyme to enter the targeted cells.

16.5.5 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.

16.5.6 TIA-1

TIA-1 (**T**-cell restricted **i**ntracellular **a**ntigen-**1**, also known as nucleolysin) is a cytotoxic granule-associated protein expressed in NK-cells and cytotoxic T-lymphocytes. TIA-1 has nucleolytic activity against targeted cells, initiating apoptosis. TIA-1 is a marker for NK-cell lymphomas and is also used to label tumor-infiltrating lymphocytes. The expression of TIA-1 is also described in cutaneous mast cells.

Evolution of immunoprofile of nonneoplastic T-lymphocytes	
Cell type	Immunoprofile
Prothymocyte	CD2, cCD3, CD7, TdT, LMO2
Stage I thymocyte (subcapsular)	CD2, cCD3, CD5, CD7, CD10, CD34, TdT
Stage II thymocyte (cortical)	CD1a, CD2, cCD3, CD4, CD5, CD7, CD8, CD38, CD52, CD165, CD200, TdT
Stage II thymocyte (medullary) T-helper/inducer cells	CD2, cCD3, CD4, CD5, CD7, CD27, CD28, CD48, CD52, CD69, CD121a, CD127, CD155, CD165, CD200
Stage II thymocyte (medullary) T-suppressor and cytotoxic cells	CD2, cCD3, CD5, CD7, CD8, CD27, CD28, CD48, CD52, CD69, CD121a, CD127, CD155, CD165, CD200
Follicular T-helper	CD2, CD3, CD4, CD5, CD7, CD10, CD57, bcl-6, PD-1
Natural killer cell (NK cell/null cell/LGL)	CD11b, CD11c, CD16, CD48, CD56, CD57, CD69, CD94, CD122, CD158, CD159, CD161, CD200, CD226, CD224, CD247

Immunoprofile of T-cell and NK-cell neoplasms				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
T-lymphoblastic leukemia/ lymphoma: subtypes see below (a–d)	**CD2**, **TdT**, CD7, ERG Proliferation index (Ki-67): 40–80%	cCD3, CD10, CD4, CD5, CD8, CD33, CD34, CD99 Fli-1, LMO2	CD1a, CD13, CD15	PAX-5, CD19, CD20, MPO
	Flow cytometry:			
	CD7, CD38, TdT	cCD3, CD5, CD34	CD1a, CD11b, CD13, CD33	CD19, MPO
(a) Pro-T-cell precursor lymphoblastic leukemia	cCD3, CD7	CD34, CD2,	CD4, CD5, CD33, CD56, CD117, HLA-DR	CD1a, CD8, MPO
	Flow cytometry			
	CD7		CD5, CD8	CD1a, mCD3, CD4
(b) Pre-T-cell ALL	cCD3, CD34, TdT			**CD4**, **CD8**
(c) Cortical T-cell ALL	**CD1a**, cCD3, CD4, CD10, TdT	CD8	CD2	
(d) Medullary T-cell ALL	sCD3, CD4/CD8			CD1a

T-cell prolymphocytic leukemia	CD2, cCD3, CD5, CD7, CD43, **TCL-1**	sCD3, CD4	CD8, CD38	CD1a, CD10, CD25, CD28, CD30, CD56, TdT, cytotoxic molecules
	Flow cytometry			
	CD2, cCD3, CD4, CD7, CD8, CD43, CD52, TCL1	sCD3, CD26	CD52, CD38	CD1a, CD16, CD19, CD30, CD56, HLA-DR
NK-lymphoblastic leukemia/ lymphoma	CD34, **CD56**	TdT, CD2, cCD3, CD5, CD94	CD33, CD117	CD1a, mCD3, CD4, CD8, CD19, CD20, MPO
T-cell large granular lymphocytic leukemia	CD2, **CD3**, CD8 (in the common type), CD16, cytotoxic molecules (TIA-1, perforin, granzyme B)	CD5, CD38, CD57 (in the common and NK-cell types), TCRβ	CD56, CD4 (+ in rare types)	CD5, CD7, CD10, CD25
	Flow cytometry			
	CD3; CD5; CD7; CD8; CD16; CD57; CD122; CD158a, b, e; CD329			CD4, CD19
NK-large granular lymphocytic leukemia	CD2, CD8 (in the common type), CD16, cytotoxic molecules (TIA-1, perforin, granzyme B)	CD3, CD56		
	Flow cytometry			
	CD5; CD7; CD8; CD16; CD56; CD57; CD122; CD158a, b, e; CD329	cCD3, CD16		sCD3, CD4, CD19
Adult T-cell lymphoma/ leukemia (HTLV1+)	CD2, **CD3**, CD5, CD62, HLA DR	**CD4**, **CD25**, MUM-1	CD15, CD30, CD56	CD7, CD8, ALK
	Flow cytometry			
	CD2, CD4, CD5, CD25, CD27, CD52	CD3		CD1a, CD7, CD8, CD10, CD19, CD26
Aggressive NK-cell leukemia	CD2, cCD3ε, CD30 (only in large transformed cells), CD56, cytotoxic molecules (TIA-1, granzyme B), EBV	CD7, CD16, EMA	CD8, CD16	sCD3, CD4, CD5, CD8, CD57
	Flow cytometry			
	CD2, cCD3ε, CD7, CD56, CD16, CD29, CD43, CD54, CD122, CD161, HLA-DR		CD30	mCD3, CD4, CD5, CD8, CD19, CD57
Indolent T-cell lymphoproliferative disorder of the GI tract	CD2, CD3, CD8	CD5, CD7, TIA-1		CD4, CD30, CD56
Enteropathy-associated T-cell lymphoma	CD2, **cCD3**, CD7, CD103	CD30, cytotoxic molecules (TIA-1, perforin, granzyme B)	CD8	CD4, CD5, CD56
	Flow cytometry			
	cCD3, CD30, CD103, TCRγβ, TCRγδ	CD2	CD4	CD4, CD8, CD56

Monomorphic epitheliotropic intestinal T-cell lymphoma	CD2, CD3, CD7, **CD8**, **CD56**, TIA-1		CD20	CD4, CD5
	Flow cytometry			
	CD2, cCD3, CD7, CD8, CD56, CD103, TCRγβ, TCRγδ			CD30
Intestinal T-cell lymphoma NOS	CD3	Cytotoxic molecules (TIA-1, perforin)	CD30	CD56
EBV-positive nodal T- and NK-cell lymphoma	CD2, CD3, cytotoxic molecules (TIA-1, perforin, granzyme B), EBV	CD8, CD56	CD5, CD5	
Extranodal NK-/T-cell lymphoma (extranodal NK/T-cell lymphoma, nasal type)	**CD2**, **CD3ε**, CD43, **CD56**, CD94, **cytotoxic molecules** (TIA-1, perforin, granzyme B), EBV		CD4, CD7	**CD3**, CD5, CD8, CD57, CD161, TdT
	Flow cytometry			
	CD2, cCD3ε, CD25, CD26, CD38, CD56, CD94, HLA-DR			mCD3, CD4, CD5, CD7, CD8, CD16, CD57
Hepatosplenic γδ T-cell lymphoma	CD2, **CD3**, CD43, CD45RO, **TIA-1**	CD2, CD7, CD56	CD16, CD11c, CD11b, granzyme	CD4, CD5, CD8, CD30, CD57, perforin
	Flow cytometry			
	CD2, CD3, CD7, CD16, CD56, TCRγδ	CD56, TCRαβ	CD8, CD16	CD4, CD5, CD19, CD25, CD57
ALK-positive anaplastic large-cell lymphoma	**ALK**, **CD30**, CD4, CD43, MUM-1, clusterin[d], **cytotoxic molecules** (TIA-1, perforin, granzyme B)	CD2, CD25, CD45, EMA, galectin-3	CD3, CD5, CD7, CD15, Fascin, bcl-6	CD8, CD20, CD28, PAX-5
ALK-negative anaplastic large-cell lymphoma	**CD30**, clusterin[d], CD43, MUM-1, **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, bcl-2
Breast implant-associated anaplastic large-cell lymphoma	CD4, CD43, **CD30**, bcl-2 Proliferation index (Ki-67): >80%	CD2, CD3, CD5, EMA, clusterin, cytotoxic molecules (TIA-1, perforin, granzyme B), EMA	CD15	CD10, ALK
	Flow cytometry			
	CD2, CD4, CD25, CD30, CD38	CD13, CD33, TIA-1	CD15	
Nodal TFH-cell lymphoma, angioimmunoblastic type	CD2, **CD3**, CD4, CD5, CD7, **CD10**, CD28, **PD-1** (**CD279**), **ICOS**[g] (**CD278**), **CXCL13**[c] Expanded CD21, CD23, and CD35 positive meshwork of follicular dendritic cells	Bcl-6, CD38	CD8, CD30 EBV+ B-cell blasts	CD15
	Flow cytometry			
	CD3, CD4, CD5, CD10, CXCR5			CD8, CD20

Nodal TFH-cell lymphoma, follicular type	CD3, CD4, **CD10**, **PD1** (**CD279**), **bcl-6**, **CXCL13**[c]			
	Flow cytometry			
	CD4, CD10, CD57, CD200, CD278, CXCL13, CXCR5			
Nodal TFH cell lymphoma, NOS	CD3, CD4, CD10, PD1 (CD279), bcl-6, CXCL13[c]			
Peripheral T-cell lymphoma (NOS)	CD2, **CD3**, CD4	CD4, CD7, CD5, GATA-3	CD8, CD25, CD30, CD134	**ALK**, TdT, CD1a, CD10, CD15[a], CD19, CD20[b], bcl-6
	Flow cytometry			
	CD2, CD3, CD4,	CD5, CD7	CD8	CD19
Hydroa vacciniforme lymphoproliferative disorder (Hydroa vacciniforme-like T-cell lymphoma)	CD2, CD3, CD8, EBV	Cytotoxic molecules (TIA-1, perforin, granzyme B)	CD4	
Systemic EBV-positive T-cell lymphoma of childhood				
Primary cutaneous T-cell lymphomas				
Primary cutaneous CD4 positive small/medium T-cell lymphoproliferative disorder	CD2, CD3, **CD4**, CD38, bcl-6	PD-1	CD10	CD8, CD30
	Flow cytometry			
	CD4, CD38, CXCL13			CD10, CD30
Primary cutaneous acral CD8-positive lymphoma	CD2, CD8	CD3, CD5, CD7, cytotoxic molecule TIA-1	CD4	CD30, CD56, PD-1, EBV
	Flow cytometry			
	CD2, CD3, CD5, CD7, CD8, TIA-1			CD30, CD56
Mycosis fungoides/Sézary syndrome	CD2, **CD3**, CD4, CD5, TRβ Proliferation index (Ki-67): <5%		CD25	CD1a, CD7, CD8, CD10
	Flow cytometry			
	CD3, CD4, CD5			CD7, CD8, CD19, CD26
Primary cutaneous CD30-positive T-cell lymphoproliferative disorder: lymphomatoid papulosis	**CD30**[d,e], CD4, CD25	CD45, cytotoxic molecules (TIA-1, perforin, granzyme B)	CD2, CD3, CD5, CD7, CD56	Clusterin, CD8[f], CD15, EMA, CD246 (ALK, p80), PAX-5, EBV
Primary cutaneous anaplastic large-cell lymphoma: primary cutaneous anaplastic large-cell lymphoma	CD4	CD2, CD3, CD5, CD7, CD30	CD15	
Subcutaneous panniculitis-like T-cell lymphoma	CD2, CD3, CD7, CD8, CD43, CD45, cytotoxic molecules (TIA-1, perforin, granzyme B)	CD5, CD7, CD25	CD30	CD4, CD30, CD56
	Flow cytometry			
	CD3, CD5, CD7, CD8, TIA-1			CD4, CD56

Primary cutaneous gamma delta T-cell lymphoma	CD2, CD3, cytotoxic molecules (TIA-1, perforin, granzyme B), TCRγ, TCRδ	CD7, CD56	CD8	CD1a, CD4, CD5, CD57
	Flow cytometry			
	CD3, CD56, perforin, TIA-1, granzyme B			CD4, CD5, CD7, CD8
Primary cutaneous CD8-positive aggressive epidermotropic cytotoxic T-cell lymphoma	CD3, **CD8**, cytotoxic molecules (TIA-1, perforin, granzyme B)	CD5, CD7,	CD2, CD15, CD30	CD4, TCRγ, TCRδ
	Flow cytometry			
	CD3, CD8	CD7		CD2, CD4, CD5, CD56
Primary cutaneous peripheral T-cell lymphoma, NOS	CD2, CD3	CD4	CD8	

[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]CXCL13: CXC motif chemokine ligand 13; see Chap. 19 [48]
[d]Golgi stain pattern
[e]CD30 positive only in RS-like cells of type A lymphomatoid papulosis
[f]CD8 positive in type D lymphomatoid papulosis
[g]ICOS: inducible co-stimulator (CD278)

16.6 Markers and Immunoprofile of Hodgkin Lymphoma

Classical Hodgkin lymphoma is a malignant proliferation of lymphocytes originating from germinal center B-lymphocytes. Classical Hodgkin lymphoma includes four subtypes composed of neoplastic mononucleated Hodgkin cells and multinucleated Reed–Sternberg cells in a unique nonneoplastic microenvironment composed of different lymphocytes and inflammatory cells, while the malignant cells represent less than 2% of the total cell population. The Hodgkin cells have a characteristic immunoprofile diagnostic for these cells and are typically labeled by CD15, CD30, MUM-1, STAT-6, PAX-5, IMP3, PD-L1, and J-chain but are usually negative for CD45 and CD20. The surrounding T-cells show a PD-1 positivity.

Nodular lymphocyte-predominant Hodgkin lymphoma is another lymphoma type distinct from classical Hodgkin lymphoma, composed of large centroblasts with multilobulated nuclei (LP, popcorn cells) in a microenvironment exhibiting nodular or diffuse appearance composed of small lymphocytes and histiocytes. The LP cells are typically positive for CD45, CD20, bcl-6, and IMP-3 but negative for CD15, CD30, and bcl-2. The LP cells are usually surrounded by rosettes of CD3- and CD57-positive T-lymphocytes.

16.6.1 Diagnostic Antibody Panel for Classical Hodgkin Lymphoma

CD15, CD30, MUM-1, IMP3, PAX-5, STAT-6, PD-L1, Fascin, J-chain [49–51].

16.6.2 Diagnostic Antibody Panel for Nodular Lymphocyte-Predominant Hodgkin Lymphoma

CD19, CD20, PAX-5, J-chain, BOB.1, Oct-2, and EMA [49].

16.6.3 CD15

CD15		
Expression pattern: membranous/cytoplasmic and juxtanuclear		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Hodgkin lymphoma (Reed–Sternberg cells) – Myeloid leukemia/myeloid sarcoma	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 the kidney, intestinal Paneth cells
Positive control: appendix		

Diagnostic Approach CD15 (X hapten) is a cell surface granulocyte-associated glycoprotein involved in the regulation of neutrophil functions. CD15 is commonly used as a marker for normal and neoplastic myeloid cells and monocytes but is frequently lost in cells of AML. In combination with CD30, CD15 is a marker for Reed–Sternberg cells in classical Hodgkin lymphoma found in 75–85% of the cases (Fig. 16.27).

CD15 is also expressed on different carcinoma types but is usually negative in mesothelioma. Carcinomas positive for CD15 are reported to have a worse prognosis.

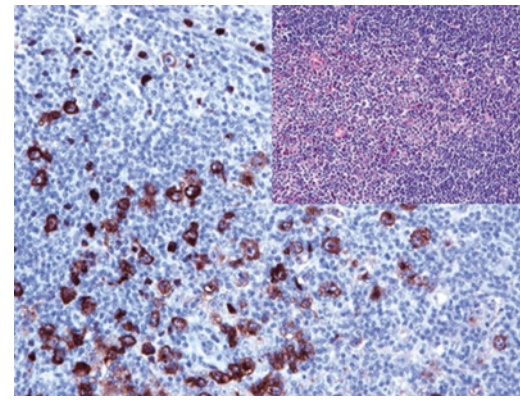


Fig. 16.27 Hodgkin and Reed–Sternberg cells in classical Hodgkin lymphoma with strong CD15 expression

Diagnostic Pitfalls Since CD15 is expressed in different hematopoietic and non-hematopoietic neoplasms, including adenocarcinomas, it is important to consider possible differential diagnoses and support the final diagnosis by other, more specific antibodies.

16.6.4 CD30

CD30		
Expression pattern: membranous/cytoplasmic paranuclear		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Reed–Sternberg cells in classic Hodgkin lymphoma – Systemic and cutaneous anaplastic large-cell 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-(immunoblasts), and NK-cells, a 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 with intracellular, transmembrane, and extracellular domains. CD30 is a member of the tumor necrosis factor superfamily (TNFRSF8), participating in the regulation of cell transformation, antibody response, and apoptosis. CD30 is normally expressed on activated B-, T-, and NK-cells but absent or minimally expressed in resting lymphocytes. CD30L and CD153 are the ligands that bind to the CD30 molecule and are expressed by histiocytes, granulocytes, and activated lymphocytes.

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 lymphoma (Fig. 16.28). 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 [52].

The expression of CD30 is not restricted to lymphoid tissue and lymphoid neoplasms but is also found in other different epithelial and mesenchymal tumors. 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 [53].

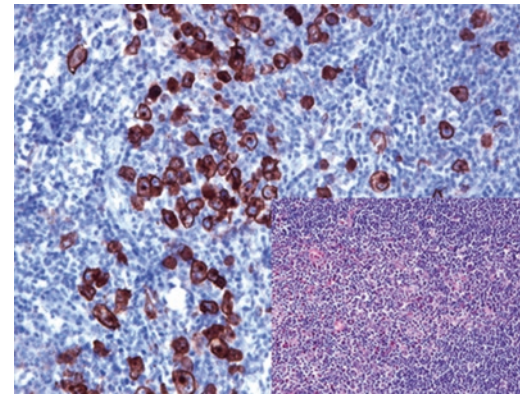


Fig. 16.28 Hodgkin and Reed–Sternberg cells in classical Hodgkin lymphoma with strong CD30 expression

CD30 is the therapeutic target for specific antibodies used to treat classical Hodgkin lymphoma, anaplastic large-cell lymphoma, peripheral T-cell lymphoma NOS, and systemic mastocytosis.

Diagnostic Pitfalls CD30-positive cells may be found in different T- and B-lymphoma types. CD30 stains also nonneoplastic activated T- and B-immunoblasts in reactive lymph nodes, spleen, thymus, and tonsil in addition to lymphocytes carrying EBV, HIV, or other oncogenic viral genomes; consequently, not all CD30-positive cells are Hodgkin cells.

16.6.5 Fascin

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 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; juvenile xanthogranuloma; 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 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 the normal epithelium but positive in many types of trans-

formed or neoplastic epithelium [54]. 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 immunostaining. In addition to Reed–Sternberg cells, Fascin positive cells in lymph nodes may be activated B-lymphocytes, cells of diffuse large B-cell lymphoma, or even disseminated cells of metastatic adenocarcinoma.

16.6.6 Insulin Like Growth Factor II mRNA-Binding Protein 3 (IMP3)

IMP3 is a cytoplasmic protein mediating RNA trafficking and cell growth, highly expressed in early embryogenesis. Benign adult tissue usually lacks the expression of IMP3 with the exception of fibroblasts, a subset of lymphocytes (mainly germinal center lymphocytes), ovarian and testicular tissue, placenta, and brain. IMP3 is expressed in different premalignant and malignant lesions and 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 (see Chap. 8). IMP3 selectively stains Hodgkin and Reed–Sternberg cells in both classical Hodgkin lymphoma and nodular lymphocyte-predominant Hodgkin lymphoma (Figs. 16.29 and 16.30).

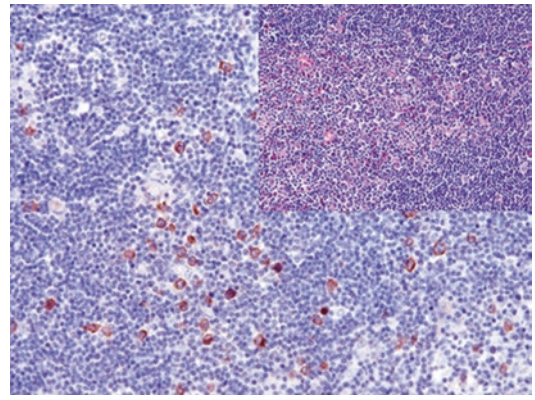


Fig. 16.29 IMP3 selectively labels the Hodgkin and Reed–Sternberg cells in classical Hodgkin lymphoma

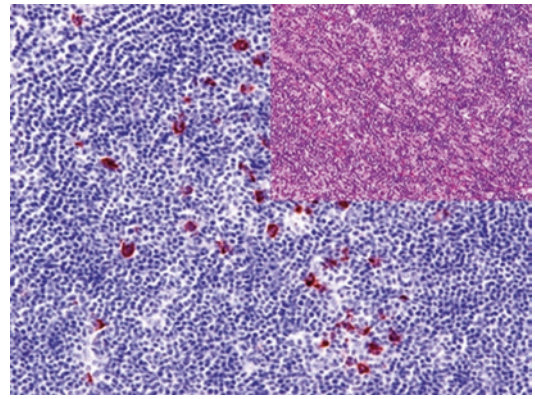


Fig. 16.30 Nodular lymphocyte-predominant Hodgkin lymphoma. IMP3 selectively labels the Hodgkin cells

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

16.6.7 STAT-6

STAT-6 is a member of the STAT family of cytoplasmic transcription factors listed in Chap. 23. STAT-6 also labels the nuclei of Hodgkin and Reed–Sternberg cells in classical Hodgkin lymphoma but is negative in nodular lymphocyte-predominant Hodgkin lymphoma [55].

In routine immunohistochemistry, STAT-6 has a specific nuclear expression pattern characteristic for different tumors, including solitary fibrous tumor and HRS cells of classical Hodgkin lymphoma.

Diagnostic Pitfalls A nonspecific cytoplasmic staining pattern found in different mesenchymal, histiocytic, and lymphoid cells (Fig. 16.31).

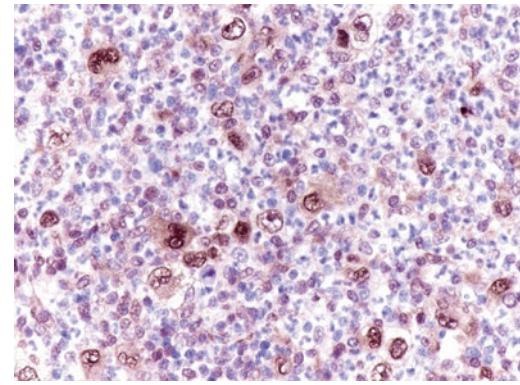


Fig. 16.31 STAT-6 highlighting the nuclei of Hodgkin and Reed–Sternberg cells in classical Hodgkin lymphoma. Lymphocytes and histiocytes in the background exhibit a nonspecific cytoplasmic expression pattern

Immunoprofile of Hodgkin lymphoma				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Classical Hodgkin lymphoma (Hodgkin and Reed–Sternberg cells[a]) in classical subtypes – Nodular sclerosis – Lymphocyte rich classic – Mixed cellularity – Lymphocyte-depleted – Unclassifiable	**CD30**, **PD-L1**, **PAX-5**, **MUM-1**, IMP3, Fascin	**CD15**, STAT-6, CD25, CE40, CD83, CD123, CD138, CD200, HLA-DR, p53 EBV (LMP1)	LMO2, HGAL, CD20, CD79	CD45, CD43, Oct-2, BOB.1, J-chain, PU.1, EMA, bcl-6, CD22, ALK
Main background cells in classical Hodgkin lymphoma	Nodular sclerosis: CD3 and CD4 positive T lymphocytes, macrophages, eosinophils, neutrophils, fibroblasts, plasma cells Mixed cellularity: lymphocytes, plasma cells, eosinophils, histiocytes Lymphocyte-depleted: fibroblasts Lymphocyte rich: mainly B lymphocytes, histiocytes, loose CD21 positive follicular dendritic cell meshwork			
Nodular lymphocyte-predominant Hodgkin lymphoma Lymphocyte-predominant cells (LP) or popcorn cells[a,b]	CD19, CD20, CD22, CD45, CD86, bcl-6, **Oct-2**, HGAL, PAX5, BOB.1, **J-chain**, IMP3	CD75, CD79a, CD40, PU.1, T-bet, **EMA**	MUM-1	CD10, CD15, CD30, CD138, CD200, bcl-2, p53, Fascin, ALK (p80), EBV, STST-6
Main background cells in nodular lymphocyte-predominant Hodgkin lymphoma	Small B-lymphocytes, T-lymphocytes LP cells surrounded by rosettes of CD3+, CD4+, CD57+, and PD-1+ activated lymphocytes			

[a]Usually, without IgH or TCR gene rearrangements
[b]Also known as lymphocytic/histiocytic Reed–Sternberg cells (L&H cells)

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Markers and Immunoprofile of Myeloid Neoplasms

17

Contents

In this chapter, the fifth revision of the World Health Organization classification of hematolymphoid neoplasms was considered. The final diagnosis of myeloid neoplasms must be made considering the histomorphology, immunophenotype (immunohistochemistry and flow cytometry), and molecular genetic analysis [1].

17.1 Diagnostic Antibody Panel for Myeloid Neoplasm

CD13, CD14, CD15, CD33; CD34, CD117, MPO, ERG [1, 2].

17.2 Diagnostic Antibody Panel for Megakaryoblastic Neoplasm

CD42b, CD61.

17.3 Diagnostic Antibody Panel for Erythroid Neoplasm

CD71, Glycophorin, E-Cadherin, Glut-1.

M. S. A. Tuffaha et al., *Immunohistochemistry in Tumor Diagnostics*,
https://doi.org/10.1007/978-3-031-45024-2_17

17.3.1 Myeloperoxidase

Myeloperoxidase (MPO)		
Expression pattern: cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– AML – CML	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 is detected in the early CD34+ myeloid precursors. MPO positivity is diagnostic for neoplasia of myeloid origin, whereas the lowest expression level is found in AML-M0 and M1. AML-M6 and M7 are negative for MPO. MPO is constantly absent in normal and neoplastic lymphoid tissue.

17.3.2 CD13 (Aminopeptidase N)

CD13 is a transmembrane metalloprotease involved in cell surface antigen presentation. Similar to CD33, CD13 is also a myeloid-associated antigen expressed on myeloid cells and myeloid precursors and appears before CD33 on the CD34-positive precursors. CD13 is also expressed on other non-myeloid cells such as monocytes, a subset of mast cells, fibroblasts, osteoclasts, endothelium, placenta, and various epithelial cells, including cells of proximal renal tubules, hepatocytes, bile canaliculi, and brush surface of enterocytes. Glands of acinar adenocarcinoma of the prostate often show the loss of CD13 expression in comparison with 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.

17.3.3 CD14

CD14 is a marker for monocytic differentiation and a helpful marker for the diagnosis of M4 and M5 acute myeloid leukemia. CD14 is listed in detail in Chap. 19.

17.3.4 CD15

CD15 is a cell surface granulocyte-associated glycoprotein and a further important marker for the myeloid lineage listed. CD15 is expressed on the majority of granulocytes, including neutrophils, eosinophils, and a subset of basophils, in addition to monocytes and related neoplasms. Mast cells lack the expression of CD15.

17.3.5 CD33

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, mast cell neoplasms	Monocytes, promyelocytes, myeloid blasts, dendritic cells, mast cells, liver Kupffer cells, alveolar macrophages, syncytiotrophoblasts
Positive control: bone marrow		

Diagnostic Approach CD33 is a transmembrane sialic acid-binding immunoglobulin-like lectin involved in cell-to-cell adhesion. CD33 is expressed in the early myeloid progenitor cells after CD34 and CD13 but is absent in stem cells [3]. The expression of CD33 persists during myelomonocytic differentiation and is weakly detectable on granulocytes, monocytes, mast

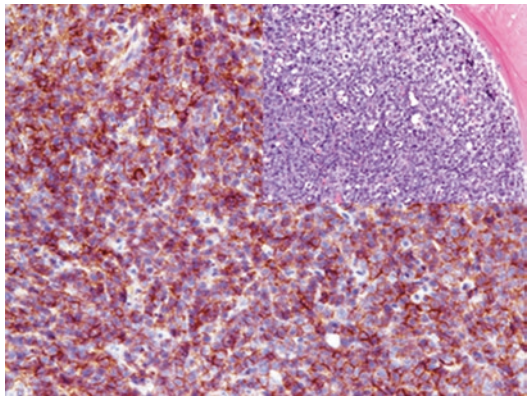

Fig. 17.1 Myeloid blasts in M5 AML with membranous CD33 expression

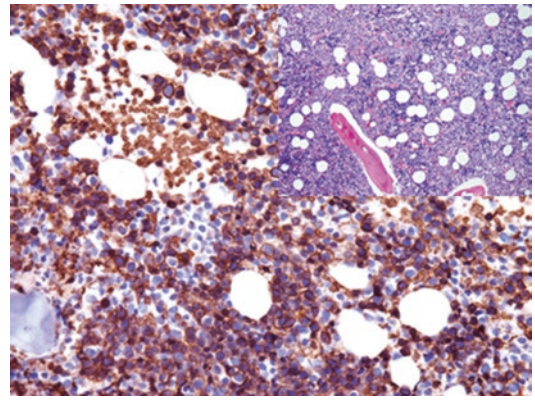

Fig. 17.2 Glycophorin expression in neoplastic erythroblasts of M6 AML

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. CD33 is the therapeutic target for specific antibodies used to treat AML and ALL.

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

17.3.6 Glycophorins

Glycophorins are a group of sialoglycoproteins found on 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 are considered specific markers for normal and neoplastic erythropoiesis, including acute erythroid leukemia (M6) (Fig. 17.2). Other leukemia types lack the expression of Glycophorins.

17.3.7 CD71

CD71 (p90, TFRC) is a transferrin receptor consisting of two transmembrane glycoprotein chains. CD71 is essential for iron transport into proliferating cells by mediating the uptake of iron-saturated transferrin complex to be transported to the endosomes and recycled to the apotransferrin-receptor complex.

CD71 is not a specific lineage marker and is expressed on all proliferating cells, including the erythroid precursors, reticulocytes, activated B and T lymphocytes, and macrophages, in addition to proliferating tumor cells of different origins. In routine immunohistochemistry and flow cytometry, CD71 strongly labels normal and neoplastic cells of erythroid lineage as they contain the highest concentration of transferrin receptor. CD71 intensively labels the cells of acute erythroid leukemia (Fig. 17.3).

The intense expression of CD71 in other tumors, such as carcinomas of the thyroid, lung, breast, colon, and liver, is usually associated with aggressive behavior.

17.3.8 E-Cadherin

E-cadherin is listed in detail in the chapter on epithelial markers. E-cadherin takes part in the

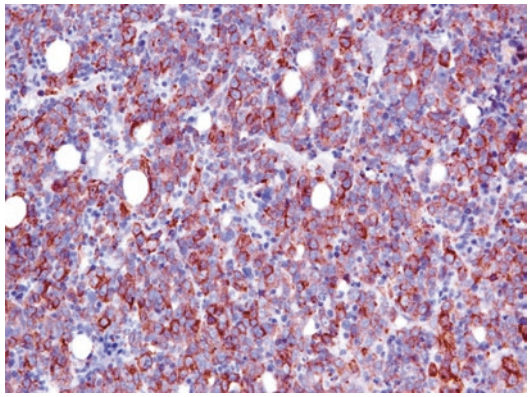

Fig. 17.3 Bone marrow infiltrated by acute erythroid leukemia (M6) with strong membranous CD71 expression

regulation of erythroid differentiation, and it is expressed in erythroid precursors (erythroblasts and normoblasts), while mature erythrocytes lack the expression of E-cadherin. E-cadherin is a marker for erythroblastic leukemia (M6).

17.3.9 CD42b

CD42b (platelet glycoprotein Ib, GPIb) is a membrane glycoprotein that links to other platelet glycoproteins and functions as a receptor for the von Willebrand factor, involved in platelet adhesion and aggregation in the process of thrombus formation. CD42b is expressed on megakaryocytes and platelets. It is also expressed on endothelial cells. CD42b is a marker for megakaryocytes and megakaryoblastic leukemia (M7).

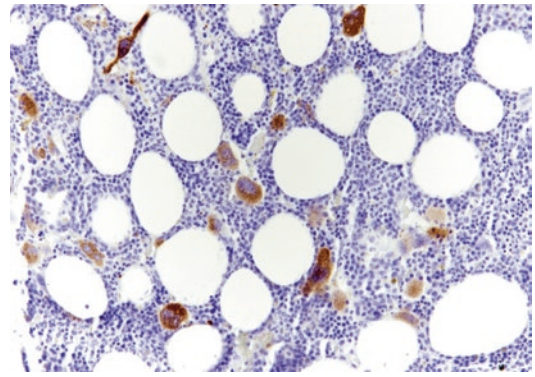

Fig. 17.4 Bone marrow with megakaryocytes strongly labeled by CD61

17.3.10 CD61

CD61 (integrin β-3 chain) is a cell surface glycoprotein strongly expressed on megakaryocytes and platelets in addition to cutaneous mastocytes, macrophages, and osteoclasts (Fig.17.4). CD61 is a marker for megakaryoblastic leukemia (M7) and partially expressed on M6 neoplastic cells. CD61 is also expressed on endothelial cells and a subset of myeloid progenitor cells.

17.3.11 CD117

CD117 (c-kit) is a member of the tyrosine kinase growth factor receptor type 3 family listed in a previous chapter. CD117 is a stem cell factor receptor also expressed on different hematopoietic precursors, including myeloid and erythroid lineages in addition to mast cells. Together with CD34, CD117 labels the myeloid blasts important for the interpretation of MDS cases.

Evolution of immunoprofile of nonneoplastic myeloid cells	
Cell type	Immunoprofile
Pluripotent stem cell	CD117, CD123, CD143, CDw338, HLA-DR
Myeloid stem cell	CD33, CD34, CD38, CD117, CD123, CDw131, CD176, CD228, CD280, HLA-DR
CFU-G	CD13, CD15, CD33, CD34, CD111, CD112, CD116, CDw123, HLA-DR
Myeloblast	CD13, CD33, CD34, CD114, CD116, CD117
Promyelocyte	CD13, CD33, CD89, CD116, CDw123, MPO
Myelocyte	CD13, CD15, CD33, CD65, CD89, CD91, CD114, CD116, CDw123, CDw131, MPO
Neutrophile	CD10, CD11b, CD13, CD15, CD16, CD17, CD24, CD32, CD35, CD43, CD65, CD66, CD89, CDw92, CD93, CD111, CD112, CD114, CD116, CDw123, CDw128, CD156, CD157, CD162, CD170, CD181, CD282, CD312, MPO
CFU-E	CD13, CD33, CD34, CD116, CDw123, CDw131
Myelocyte	CD11b, CD13, CD32, CD33, CD35, CD114, CD116, CDw131
Eosinophile	CD9, CD11b, CD15, CD24, CD32, CD35, CD43, CD114, CD116, CDw125, CDw131, CD193, CDw218, CD294
CFU-Bas	CD34, CDw123
Myelocyte	CD13, CD114, CDw123
Basophile	CD9, CD17, CD25, CD33, CD38, CD43, CD114, CDw123, CDw131, CD154, CD192, CD193, CD203c, CD294
CFU-M	CD13, CD15, CD33, CD111, CD112, CD115, CD116, CDw123, CDw131, HLA-DR
Monoblast	CD4, CD11c, CD13, CD15, CD33, CD36, CD64, CD115, CD116, CDw123, CDw131, HLA-DR
Promonocyte	CD4, CD11b, CD13, CD14, CD15, CD33, CD36, Cd64, CD111, CD112, CD115, CD116, CDw123, CDw131, HLA-DR, MPO
Monocyte	CD9, CD11b, CD11c, CDw12, CD13, CD14, CD15, CDw17, CD32, CD33, CD35, CD36, CD38, CD43, CD49b, CD49e, CD49f, CD63, CD64, CD65s, CD68, CD84, CD85, CD86, CD87, CD89, CD91, CDw92, CD93, CD98, CD101, CD102, CD111, CD112, CD115, CD116, CD119, CDw121b, CDw123, CD127, CDw128, CDw131, CD147, CD155, CD156a, CD157, CD163, CD164, CD168, CD171, CD172a, CD172b, CD180, CD184, CD91, CD192, CD195, CDw198, CD206, CDw210, CD213, CD226, CD277, CD281, CD282, CD300a, CD300c, CD300e, CD302, CD305, CD312, CD317, CD322, CDw328, CDw329, HLA-DR, MPO
Macrophage- and monocyte-derived dendritic cells	CD4, CD11c, CD14, CD15, CD16, CD26, CD31, CD33, CD32, CD36, CD45RO, CD45RB, CD63, CD64, CD68, CD71, CD74, CD87, CD88, CD101, CD119, CD121b, CD155, CD156a, CD204, CD206, CDw210, CD119, CD121b, CD155, CD156a, CD204, CD206, CDw210, CD312, HLA-DR (in activated macrophage also CD23, CD25, CD69, CD105
CFU-Meg	CD34, CD110, CDw123
Megakaryoblast	CD34, CD38, CD41, CD42, CD61, HLA-DR
Megakaryocyte	CD38, CD41, CD42, CD61, CD110, CDw123, CDw131, CD151, CD203c
Platelet	CD9, CDw17, CD23, CD31, CD36, CD41, CD42, CD49b, CD49f, CD60a, CD61, CD63, CD84, CD92, Cd109, CD147, CD151, CD173, CD226
BFU	CD33, CD34, CDw123, CDw131, CD297, CD324
CFU-E	CD36, CDw123, CDw131, CD175s, CD297, CD324
Erythroblast	CD36, CD71, CD117, HLA-DR, Glycophorin A, Glycophorin c
Normoblast	CD36, CD71, Glycophorin A, Glycophorin c
Reticulocyte	CD71, Glycophorin A
Erythrocyte	CD35, CD44, CD55, CD59, CD173, CD233, CD234, CD235a, CD235b, CD236, CD236R, CD238, CD239, CD240CE, CD240c, CD241, CD242, CD297, Glycophorin A, Glycophorin c

The following table includes the immunoprofile of myeloid leukemia of NOS type. The diagnosis and classification of leukemia types with recurrent genetic abnormalities or with myelodysplastic-related changes depend 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)	CD13, **CD34**, CD38, CD117	CD33, CD11b, CD43, MPO, HLA-DR	TdT, CD2, CD7, CD19, CD65	CD14, CD15
	Flow cytometry:			
	CD13, **CD34**, CD38	CD33, CD117, HLA-DR	CD2, CD19, CDw65	CD11b, CD14, CD15, CD20, CD38, CD61, CD64
Myeloblastic leukemia, without maturation (M1)	CD13, HLA-DR, **MPO**	CD33, CD34, CD117	CD7, CD56	
	Flow cytometry:			
	CD13, MPO, HLA-DR	CD15, CD33, CD34, CDw65, CD117	CD2, CD19, CD56,	CD20
Myeloblastic leukemia, with maturation (M2)	CD13, CD15, CD33, **MPO**, HLA-DR, CAE	CD43, CD117	CD56, CD34	
	Flow cytometry:			
	CD13, MPO, HLA-DR	CD15, CD19, CD33, CD34, CDw65, CD117	CD7, CD56	CD2, CD19
Myelocytic leukemia (M3)	CD13, CD33, MPO, CAE	CD43, CD64	CD15, CD65, CD117, CD56	CD34, HLA-DR
	Flow cytometry			
	CD13, CD33, MPO	CD64	CD15, CDw65, CD117	CD2, CD3, CD19, CD20, HLA-DR
Myelomonocytic leukemia (M4)	CD11b, CD13, CD33, CD64, CD68, MPO, HLA-DR	CD4, CD14, CD15, CD36, CD43, CD117	CD7, CD34, CD56	
	Flow cytometry			
	CD11c, CD13, CD14, CD33, CD64, CDw65, MPO, HLA-DR	CD2, CD14, CD15, CD117	CD34	CD3, CD19, CD20
Monoblastic/monocytic leukemia (M5a/M5b)	CD4, CD15, CD33, CD56, CD64, CD68, HLA-DR, CAE	CD11c, CD13, CD14, CDw65	MPO	CD34
	Flow cytometry			
	CD11c, CD14, CD15, CD33, CD64, HLA-DR	CD4, CD11b, CD11c, CD13, CD14, CDw65, CD68	CD7, CD117	CD2, CD3, CD19, CD20, CD34, MPO
Acute erythroid leukemia (erythroblastic leukemia; M6)	Glycophorin, hemoglobin A	CD33, CD71, Glut-1, E-cadherin, p53	CD117, CD56	CD34, CAE, CD13, MPO, TdT
	Flow cytometry			
	Glycophorin, E-cadherin, CD35, CD36	CD71		MPO, CD41, CD61

Immunoprofile of myeloid neoplasm				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Megakaryoblastic leukemia (M7)	CD61, CD41, CD42b, Spectrin	CD33	CDw65, CD13, HLA-DR	CD15, CD34, MPO, CAE
	Flow cytometry			
	CD36, CD41, CD61	CD13, CDw65, HLA-DR		CD34, CD38, MPO
Acute basophilic leukemia	CD13, CD33		CD22	CD117, MPO
	Flow cytometry			
	CD9, CD11b, CD13, CD25, CD33, CD123	CD9, CD11b, CD123	CD34	CD117, MPO
B. Chronic myeloid neoplasm				
Chronic myeloid leukemia (chronic phase)	CD11b, CD11c, CD14, CD15, MPO		CD117	
Chronic myeloid leukemia (blastic phase)	CD33, CD13, CD14, CD11b, CD11c, CD117	MPO, CD15	CD19, CD10, CD79a, PAX5, TdT	
Granulocytic sarcoma (myeloid sarcoma)[a]	**CD43**, MPO, vimentin, lysozyme	CD13, CD14, CD15, CD33, CD45, CD99, CD117, HLA-DR	CD34, CD56, CD68, CD5, CD7	CD3, CD20
Polycythemia vera	Glycophorin, CD71, E-cadherin			CD34
Essential thrombocythemia	CD61, CD42b			CD34

[a]Myeloid sarcoma MPN/CML type

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18 Markers and Immunoprofile of Mastocytosis

Contents

Mast cells are immune cells derived from the myeloid lineage in the bone marrow and released into the blood as mast cell progenitors, while the terminal differentiation and maturation take place only in the peripheral tissue under the influence of stem cell factors. Mature mast cells are present only in the tissue and contain many small cytoplasmic secretory granules, mostly with Tryptase, Histamine, Heparin, TNF-α, and in subtypes also Chymase. Mast cells are involved in the immune response and inflammatory cascade.

18.1 Diagnostic Antibody Panel for Mast Cell Tumors

Mast cell Tryptase, CD117, CD2, CD25, CD123, CD30, and CD33 [1–3].

18.1.1 Mast Cell Tryptase

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		

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https://doi.org/10.1007/978-3-031-45024-2_18

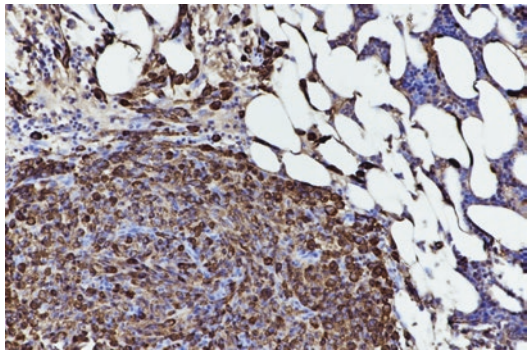

Fig. 18.1 Systemic mastocytosis; neoplastic cells with strong cytoplasmic expression of mast cell tryptase

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 basophils 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 (Fig. 18.1). The aberrant Tryptase expression is described in rare types of acute myeloid leukemia.

18.1.2 CD25

CD25, also known as p55, is a subunit (α-chain) of the interleukin-2 receptor, involved in the differentiation and activation of T lymphocytes, and is normally expressed in a subpopulation of T lymphocytes and monocytes 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. In mast cell disorders, the expression of CD25 is restricted to neoplastic mast cells and is usually negative in reactive mast cells (Fig. 18.2) [4].

In non-hematological lesions, CD25 is also a marker for the epithelial cells of bile canaliculi.

18.1.3 CD2

CD2 is a glycoprotein and adhesion molecule listed with the markers of T cell neoplasms (Chap. 16.4). CD2 is normally expressed in different stages of T cell development and T cell lymphomas but is 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 [5].

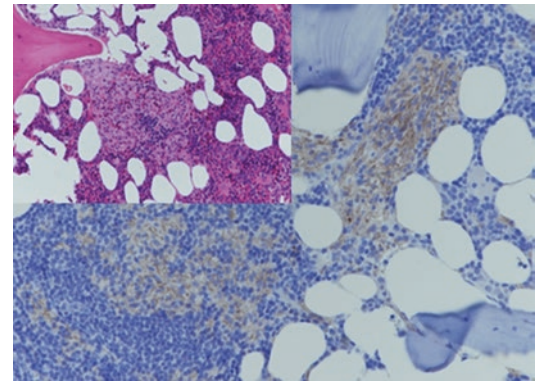

Fig. 18.2 Systemic mastocytosis with bone marrow involvement. CD25 highlights the neoplastic mast cells in the bone marrow

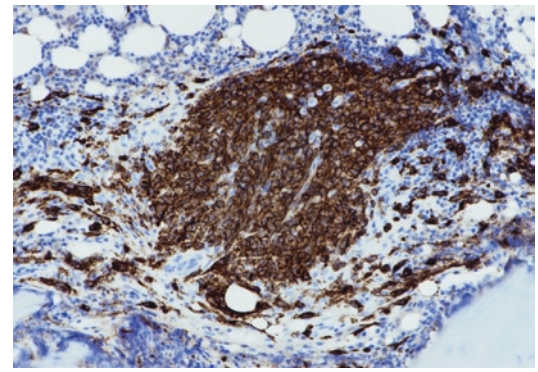

Fig. 18.3 Bone marrow with strong CD117 expression in neoplastic cells of systemic mastocytosis

18.1.4 CD117

CD117 (c-kit) is a member of the tyrosine kinase growth factor receptor type III family listed in Chap. 7.2. CD117 is a marker for normal and neoplastic mast cells (Fig. 18.3).

18.1.5 CD123

CD123 is a member of the cytokine receptor family listed in the next chapter. CD123 is negative in

normal mast cells, but the aberrant expression is found in indolent and aggressive systemic mastocytosis.

18.1.6 Toluidine Blue

Toluidine blue is a histochemical metachromatic stain that excellently labels the cytoplasmic granules of the mast cells. These granules composed of acidic molecules are colored metachromatically purple red by the alkaline dye toluidine blue (Fig. 18.4). Degranulated mast cells lose their metachromatic properties and stain pink with toluidine blue.

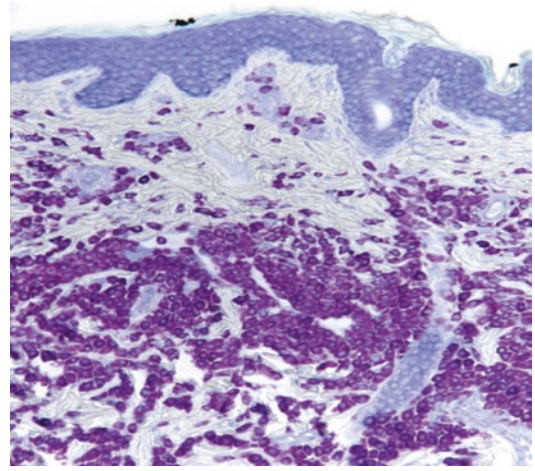

Fig. 18.4 Mast cells in subepidermal tissue labeled by Toluidine blue showing cytoplasmic metachromatic purple-red granules

Immunoprofile of nonneoplastic mast cells	
Positive	Negative
CD9, CD23, CD32, CD33, CD45, CD59, CD63, CD69, CD117, CD203c	CD2, CD14, CD15, CD16, CD25, CD34, CD123

Immunoprofile of mastocytosis				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Mastocytosis 1. Cutaneous mastocytosis 2. Systemic mastocytosis	**Tryptase**, **CD117**, CD45, CD33, **CD25**[a], CD68	**CD2**[b], CD30[c], Chymase	Calretinin	CD3, CD14, CD15, CD20, CD21, CD34, MPO
2.1 Indolent systemic mastocytosis 2.2 Systemic mastocytosis with associated hematological neoplasm 2.3 Aggressive systemic mastocytosis 2.4 Mast cell leukemia 3. Mast cell sarcoma 4. Extracutaneous mastocytoma	Flow cytometry CD2, CD117, CD59, CD63	CD14, CD25, CD30		CD11b

[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 [6, 7]

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19 Markers and Immunoprofile of Histiocytic and Dendritic Cell Neoplasms

Contents

19.1 Diagnostic Antibody Panel for Histiocytic and Dendritic Cell Tumors

CD1a, CD4, CD14, CD21, CD23, CD35, CD43, CD56, CD68, CD123, CD163, CD207 (Langerin), CXCL13, SSTR-2, TCL-1, ALK, Fascin, Sox-10, S100, BRAF-$_{v600E}$, Clusterin, Podoplanin (D2–40) [1, 2].

M. S. A. Tuffaha et al., *Immunohistochemistry in Tumor Diagnostics*,
https://doi.org/10.1007/978-3-031-45024-2_19

19.1.1 CD1a

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

Diagnostic Approach CD1 has five different isoforms divided into three groups—group I includes the isoforms a, b, and c; group II the isoform d; and group II the isoform e—all encoded by different genes located on chromosome 11q.

CD1a encoded on chromosome 1q23.1, expressed on the antigen-presenting cells and 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 is constantly negative in histiocytic, follicular dendritic, and interdigitating cell tumors (Figs. 3.11 and 19.1). CD1a is also expressed in some types of T cell lymphoma, chiefly cutaneous T cell lymphoma. CD1a is also a marker for lipid-laden macrophages (foam cells) in atherosclerotic plaques.

19.1.2 CD4

CD4 is a member of the immunoglobulin family normally expressed on different types of T lymphocytes, mainly T helper/inducer lymphocytes, in addition to monocytes, macrophages, and dendritic cells (see Sects. 16.2, 16.4 and Chap. 22). CD4 is an important marker for T cell lymphomas, listed in detail in. CD4 is also an informative marker for different histiocytic tumors (Fig. 19.2).

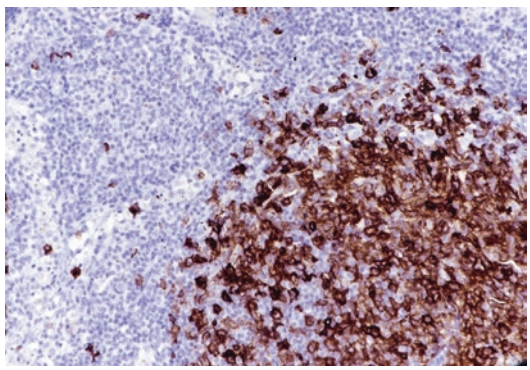

Fig. 19.1 CD1a highlighting the cells of Langerhans cell histiocytosis (lymph node). Histiocytic, follicular dendritic, and interdigitating cell tumors in the lymph node are negative for CD1a

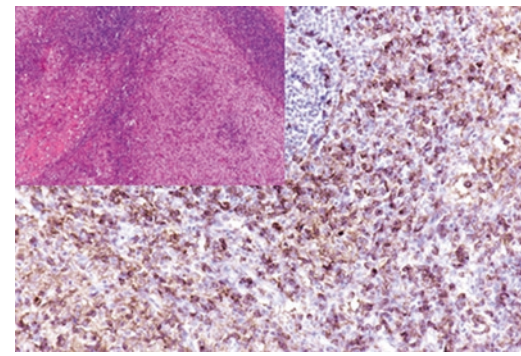

Fig. 19.2 CD4 staining the cells of Langerhans cell histiocytosis

19.1.3 CD14

CD14		
Expression pattern: membranous		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Langerhans cell histiocytosis and histiocytic sarcoma	Acute myelomonocytic leukemia (M4) and acute monoblastic/monocytic leukemia (M5)	Monocytes, histiocytes, Langerhans cells, follicular reticular cells, Kupffer cells, pleural and alveolar macrophages, neutrophils, endothelial cells, keratinocytes
Positive control: lymph node		

CD14 is a glycosylphosphatidylinositol-linked membrane glycoprotein and a member of the family of leucine-rich repeat (LRR) proteins existing in two forms, one anchored to the membrane (mCD14) and the second soluble form (sCD14) functioning as a receptor for bacterial lipopolysaccharides. CD14 is expressed on cells of the myelomonocyte lineage, including mature monocytes and histiocytes, Langerhans cells, and follicular reticular cells, in addition to Kupffer cells and pleural and alveolar macrophages. Weak expression intensity is found in neutrophils, endothelial cells, and keratinocytes. CD14 is a marker for histiocytic neoplasms, including Langerhans cell histiocytosis and histiocytic sarcoma, giant cell tumor beside AML with monoblastic/monocytic differentiation (M4 and M5), and chronic myelomonocytic leukemia.

19.1.4 CD21

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 tubuli, 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 (FDC) but is constantly negative in monocytes, granulocytes, and T lymphocytes. CD21 is positive in a subset of B cell lymphoma, namely, chronic lymphocytic lymphoma, and weak in mantle cell lymphoma and follicular lymphoma. CD21 is rarely expressed in a small subset of T cell lymphomas [3–6]. Generally, CD21, CD23, CD35, and Podoplanin are diagnostic markers for follicular dendritic cell tumors/sarcoma. The distribution pattern of the FDC highlighted by specific antibodies like CD21 is helpful for the diagnosis of different lymphoid neoplasms. Follicular lymphoma shows a dense FDC meshwork in follicular areas (Fig. 19.3), angioimmunoblastic lymphoma with FDC surrounding endothelial venules, and mantle cell lymphoma with FDC meshwork in residual germinal centers in addition to the hyaline vascular type of Castleman's disease.

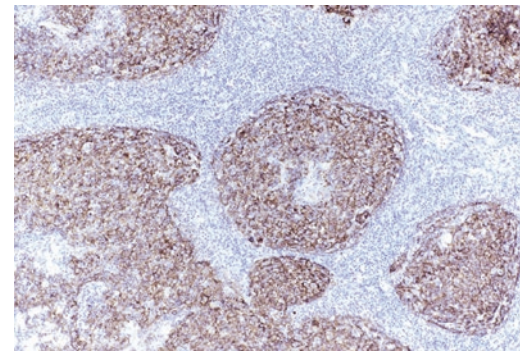

Fig. 19.3 Follicular lymphoma grade 2 with a dense meshwork of follicular dendritic cells in neoplastic follicular areas labeled by CD21

CD21 is usually negative in histiocytic, Langerhans cell, and interdigitating cell tumors. The expression of CD21 in pharyngeal and cervical epithelial cells must be considered in the interpretation of the immunostaining.

19.1.5 CD23

CD23 is a transmembrane glycoprotein involved in the regulation of IgE synthesis, listed in detail with the markers of B cell neoplasms. CD23 is also a marker for follicular dendritic cells and related neoplasms.

19.1.6 CD35

CD35 is the erythrocyte complement receptor 1 (CR1), a type I membrane glycoprotein functioning as a receptor for C3b and C4b. CD35 is expressed on erythrocytes, granulocytes, monocytes, follicular dendritic cells, and a subset of B and T lymphocytes, as well as on renal glomerular podocytes and a subset of astrocytes. Similar to CD21 and CD23, CD35 is a marker for normal and neoplastic follicular dendritic cells. CD35 is also expressed on a subset of T cell lymphomas and some carcinomas of different origins. Rarely CD35 stains Reed–Sternberg cells in classic Hodgkin lymphoma.

19.1.7 CD68

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, renal cell carcinoma, melanoma	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, cells of renal tubules
Positive control: appendix		

Diagnostic Approach CD68, also known as macrosialin, is a type I transmembrane glycoprotein encoded on chromosome 17, mainly expressed in late lysosomes and endosomes involved in the regulation of phagocytic activity of macrophages. CD68 is highly expressed in M1 and M2 macrophages, monocytes, microglia, osteoclasts, histiocytes, Kupffer cells, and myeloid dendritic cells, in addition to tumors arising from these cells [7]. Low expression of CD68 may be found in a subset of T and B lymphocytes, fibroblasts, and endothelial cells.

Diagnostic Pitfalls CD68 has a broad expression range and may be found in different hematologic diseases of B cell, T cell, NK cell, and myeloid lineage, in addition to a few other epithelial and melanocytic tumors.

19.1.8 CD123

CD123		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Blastic plasmacytoid dendritic cell neoplasm – Mast cell neoplasm	AML, hairy cell leukemia, mantle cell lymphoma, follicular lymphoma, Hodgkin lymphoma	Plasmacytoid dendritic cells, myeloid precursors, a subset of T and B lymphocytes, monocytes and macrophages, endothelial cells
Positive control: lymph node		

Diagnostic Approach CD123 is the α-chain of interleukin 3 (IL-3Rα), a member of the cytokine receptor family. CD123 is a marker for blastic plasmacytoid dendritic cell neoplasm. It is also expressed in the majority of Ph +/− ALL and hairy cell leukemia in addition to all types of AML, with the exception of M6 and M7. CD123 is not a marker of normal mast cells, but neoplastic mast cells, including indolent and aggressive systemic mastocytosis, show an aberrant CD123 expression. The CD123 expression is also found in a subset of mantle cell lymphoma and follicular lymphoma. The majority of Hodgkin-Reed-Sternberg cells of classic Hodgkin lymphoma are positive for CD123. CD123 is also expressed on endothelial cells. CD123 is the target for specific therapeutic antibodies [8].

19.1.9 CD163

CD163		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Histiocytic sarcoma – Rosai–Dorfman disease	AML, chronic myelomonocytic leukemia, myeloid sarcoma	Monocytes, macrophages
Positive control: skin		

Diagnostic Approach CD163 (also known as Ber-Mac3) is a member of the scavenger receptor cysteine-rich superfamily class B and is a scavenger receptor for the hemoglobin–haptoglobin complex. CD163 is expressed on most circulating monocytes and on the majority of tissue M2 macrophages (classically activated macrophages) such as splenic dendrocytes, alveolar macrophages, and Kupffer cells but not expressed on M1 macrophages (alternatively activated macrophages) including Langerhans cells and interdigitating reticulum cells and germinal center and mantle zone macrophages, while interfollicular macrophages and sinus histiocytes are strongly CD163 positive. CD163 is expressed in malignancies with monocytic/histiocytic differentiation, Rosai–Dorfman disease, and histiocytic sarcoma. CD163 is usually negative in immature monocytic/histiocytic neoplasia such as AML with monocytoid differentiation. CD163 is a good marker for tumor-infiltrating macrophages [9, 10].

19.1.10 Langerin

Langerin (CD207)		
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 [11]. CD207 is a specific marker for Langerhans cells and tumors arising from these cells, including Langerhans cell histiocytosis (histiocytosis X) and Langerhans cell sarcoma (Figs. 3.12 and 19.4).

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

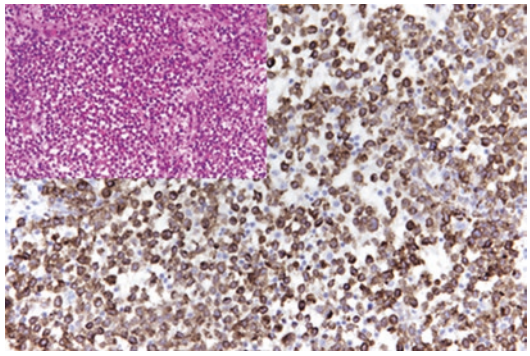

Fig. 19.4 CD207 staining the neoplastic cells of Langerhans cell histiocytosis

19.1.11 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].

19.1.12 Clusterin

Clusterin (apolipoprotein J) is a disulfide-linked heterodimeric glycoprotein and a member of the heat shock protein family. Clusterin is a highly sensitive and specific marker for follicular dendritic cell tumor and anaplastic large cell lymphoma in addition to tenosynovial giant cell tumor. Clusterin is also expressed in many well-differentiated neuroendocrine tumors of different origins, but the expression disappears in poorly differentiated neuroendocrine carcinomas. The positive immunohistochemical stain shows a cytoplasmic granular Golgi expression pattern.

Immunoprofile of histiocytic and dendritic cell tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Blastic plasmacytoid dendritic cell neoplasm	CD4, CD43, CD56, **CD123**, CD303 (BDCA-2), CD304, **TCL1**	CD68	CD2, CD7, CD33, CD38, CD117	CD1a, CD3, CD5, CD8, CD14, CD19, CD20, CD21, CD23, CD25, CD30, CD34, CD138, PAX-5
Tumors of Langerhans cell type – Langerhans cell histiocytosis (histiocytosis X)	**S100**, **CD1a**, CD68, **CD207 (Langerin)**, CD74, CD86 Proliferation index (Ki-67): 2–25% (median 10%)	CD2, CD4, CD11c, CD14, CD25, CD45RB, BRAF-$_{V600E}$, HLA-DR, PLAP	CD45	CD3, CD15, CD20, CD21, CD30, CD34, CD35, CD163, MPO, PAX-5, EMA
Langerhans cell sarcoma	**CD68**, CD45, HLA-DR Proliferation index (Ki-67): 10–60% (median 22%)	CD4, CD11c, CD14, CD15, **CD163** lysozyme, PU.1	S100, CD31	CD1a, CD3, CD20, CD21, CD23, CD33, CD34, CD35, CD30, CD207, Fascin, MPO, SOX10, Pan-CK
Interdigitating dendritic cell tumor/sarcoma	**S100**, CD45RB, **Fascin**, SOX10, vimentin Proliferation index (Ki-67): 10–20% (median 11%)	CD11c, CD75	CD4, CD15, CD33, CD45, CD68, MUM-1	CD1a, CD2, CD3, CD20, CD21, CD23, CD30, CD34, CD35, CD163, MPO, EMA, Pan-CK
Indeterminate dendritic cell tumor	CD1a, CD68, S100			CD21, CD23, CD30, CD35, CD207

Immunoprofile of histiocytic and dendritic cell tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Histiocyte and macrophage neoplasms – Juvenile xanthogranuloma	CD4, CD68, CD163	F XIIIa, Fascin		CD1a, CD207 (Langerin)
Histiocyte and macrophage neoplasms – Erdheim–Chester disease	CD4, CD68, CD163	F XIIIa, Fascin, CD14, BRAF-$_{V600E}$	S100	CD1a, CD207 (Langerin), ALK
Histiocyte and macrophage neoplasms – Rosai–Dorfman disease	S100	CD68, CD163, Oct-2, cyclin D1		CD1a, CD30, CD207 (Langerin), ALK
Histiocyte and macrophage neoplasms – ALK-positive histiocytosis	ALK, CD4, CD68, CD163		F XIIIa, Fascin, Oct-2, cyclin D1	CD1a, CD30, CD207 (Langerin)
Histiocyte and macrophage neoplasms – Histiocytic sarcoma	CD68, CD45, CD163, HLA-DR	CD4, CD15, CD11c, lysozyme, CD14, PU.1	S100, CD31	CD1a, CD3, CD20, CD21, CD23, CD33, CD34, CD35, CD30, CD207, Fascin, MPO, SOX10, Pan-CK

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20 Markers and Immunoprofile of Stroma-Derived Neoplasms of Lymphoid Tissues

Contents

This group is newly introduced in the fifth edition of the WHO classification of hematolymphoid tumors and includes tumors of different mesenchymal origin specific for the lymph nodes and spleen [1].

20.1 Diagnostic Antibody Panel for Histiocytic and Dendritic Cell Tumors

CD1a, CD4, CD21, CD23, CD35, CD43, CD56, CD68, CD123, CD163, CD207 (Langerin), CXCL13, Serglycin, FDC secreted protein (FDCSP), SSTR-2, Fascin, Sox-10, Clusterin, Podoplanin (D2–40), and S100 [2].

Most of the markers mentioned above were described in previous chapters.

20.1.1 CXCL13

CXCL13 (CXC motif chemokine ligand 13), also known as B lymphocyte chemoattractant, is a chemokine belonging to the CXC chemokine family, electively chemotactic for B lymphocytes bearing the CXCR5 receptor. CXCL13 is strongly expressed on follicular dendritic cells and follicular CD4+ T lymphocytes. In routine immunohistochemistry, CXCL13 is a specific marker for nodal T cell lymphomas with T follicular helper phenotype, namely, angioimmunoblastic T cell lymphoma, and is also a diagnostic marker for follicular dendritic cell sarcoma.

20.1.2 Serglycin

Serglycin is a hematopoietic proteoglycan core protein important for neutralizing hydrolytic enzymes, stored in the secretory granules of many hematopoietic cells and endothelial cells in addition to follicular dendritic cells and tumors originating from these cells.

M. S. A. Tuffaha et al., *Immunohistochemistry in Tumor Diagnostics*,
https://doi.org/10.1007/978-3-031-45024-2_20

Stroma-derived neoplasms of lymphoid tissues				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Follicular dendritic cell tumor/sarcoma	**CD21**, **CD23**, **CD35**, **CXCL13**, **SSTR2A**[a], Serglycin, FDCSP[b], Clusterin, PD-L1, Podoplanin (D2 40), KiM4p, Fascin, smooth muscle myosin, vimentin Proliferation index (Ki-67) FDC sarcoma: 5–70%	Desmoplakin, EGFR, HLA-DR, S100, CD14	CD4, CD30, CD45, CD68, CD163, EMA, actin, S100	CD1a, CD2, CD3, CD34, CD35, CD79a, CD163, MPO, Pan-CK
Fibroblastic reticular cell tumor	Fascin, actin, Caldesmon	Pan-CK, CD31, Tenascin c	EMA	CD1a, CD21, CD30, CD34, CD35, D2 40, S100
Intranodal palisaded myofibroblastoma	Actin	Calponin, D2–40, FXIIIa, cyclin D1	Desmin	Pan-CK, CD21, CD23, CD31, CD34, CXCL13
Spleen-specific vascular-stromal tumor – Littoral cell angioma	CD31, CD68, ERG	CD21, CD207		

[a]*SSTR2* somatostatin receptor type II
[b]FDC secreted protein

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Markers and Immunoprofile of Skin Tumors

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Contents

21.1 Diagnostic Antibody Panel for Keratinocytic (Epidermal) Tumors

Cytokeratin profile, p63/p40, EMA, epithelial specific antigen (Ber-EP4), p16, p53, HPV, Ki-67 [1].

21.2 Diagnostic Antibody Panel for Sweat Gland Tumors (Apocrine and Eccrine Differentiation)

Cytokeratin profile, p63/p40, CEA, EMA, CD15, GATA-3, S100, ER, PgR, androgen receptors, and GCFP-15. S100 is a marker for eccrine neoplasia negative in apocrine neoplasia; p63, ER, and GCFP15 are markers for eccrine neoplasia.

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 [2–4]. Furthermore, many sweet gland tumors have a similar morphology and immunoprofile as salivary gland tumors such as adenoid cystic carcinoma.

M. S. A. Tuffaha et al., *Immunohistochemistry in Tumor Diagnostics*,
https://doi.org/10.1007/978-3-031-45024-2_21

21.3 Diagnostic Antibody Panel for Hair Follicle (Pilar) Tumors

Cytokeratin profile, p63, EMA, HKN, HHK, Ber-EP4.

The hair-specific keratins, including the hair keratins (HKN) 5, 6, 7, and 15, in addition to the human hair keratin (HHK), are specific markers for pilar tumors.

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 the stratified epithelium is restricted to the basal cell layer. Sebaceous tumors usually lack the expression of CK15.

21.4 Diagnostic Antibody Panel for Sebaceous Tumors

Cytokeratin profile, EMA, Ber-EP4, CD10, CD15, D2 40, Androgen receptors, Adipophilin, Perilipin, and DNA mismatch repair proteins (MLH1, PMS2, MSH2, MSH6) [5, 6].

21.4.1 Adipophilin

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 the 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 morphology 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]. Characteristic for sebaceous carcinoma is the cytoplasmic annular expression pattern (Fig. 21.1). All other tumors with clear cell appearance, including squamous cell carcinoma, basal cell carcinoma, trichilemmoma, and clear cell hidradenoma, lack the expression of Adipophilin [5].

Adipophilin is also a marker of Burkitt lymphoma because of the presence of intracytoplasmic lipid vacuoles (see Fig. 16.18).

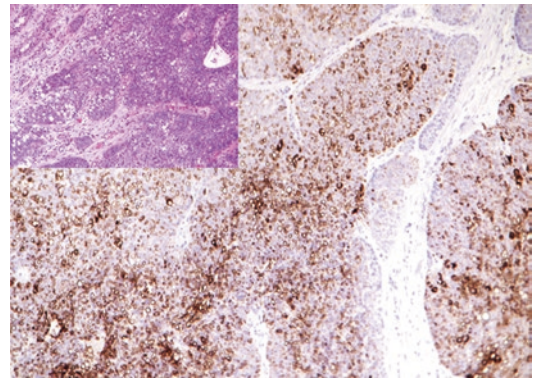

Fig. 21.1 Sebaceous carcinoma exhibiting strong annular cytoplasmic Adipophilin expression

21.4.2 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 in lipid metabolism. It is normally expressed in the cells of the adrenal cortex, Leydig cells, and brown and adult fat. Perilipin is expressed in about one-third of sebaceous tumors but lacks specificity as it can also be expressed in other tumors with clear cell morphology [8].

21.5 Diagnostic Antibody Panel for Melanocytic Tumors

See markers and immunoprofile of melanocytic tumors (Chap. 22).

21.6 Diagnostic Antibody Panel for Skin Neuroendocrine Tumors/Merkel Cell Carcinoma

Cytokeratin profile, Merkel cell polyomavirus, neuroendocrine markers (INSM-1, Chromogranin, CD56, NSE), EMA, SATB-2.

Merkel cell carcinoma is a primary cutaneous neuroendocrine carcinoma, whereas the exact histogenesis of Merkel cell carcinoma is not clarified, but the tumor could develop from skin-derived neuroendocrine precursors or dermal stem cells. Recently, pro- or pre-B lymphocytes have been discussed as the origin of Merkel cell carcinoma. Merkel cell carcinoma is generally associated with or induced by the Merkel cell polyomavirus, a double-stranded DNA virus and a member of the *Polyomaviridae* family, which can be detected by immunohistochemistry or molecular methods. Merkel cell carcinoma has a specific immunohistochemical profile with a characteristic paranuclear dot-like expression of cytokeratins, especially CK20 (Fig. 21.2), associated with the expression of different neuroendocrine markers, including ISM-1, in addition to the Merkel cell polyomavirus and frequently SATB-2 (Fig. 21.3) [9].

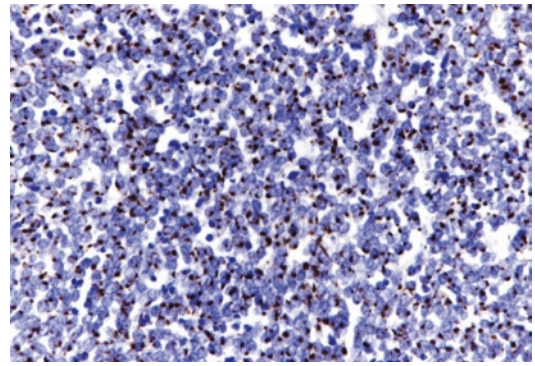

Fig. 21.2 Characteristic paranuclear dot-like CK20 expression in the neoplastic cells of Merkel cell carcinoma

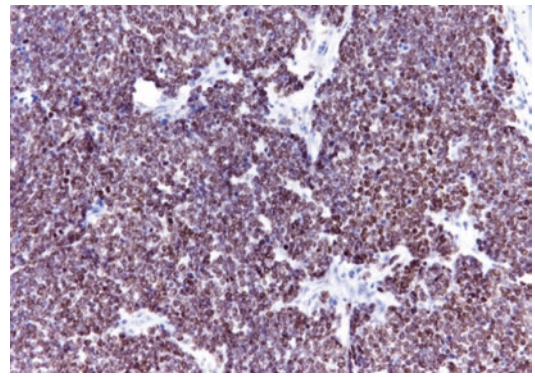

Fig. 21.3 Nuclear SATB-2 expression in the tumor cells of Merkel cell carcinoma

Immunoprofile of skin tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
A. Keratinocytic (epidermal) tumors				
Squamous cell carcinoma in situ/Bowen's disease	CK5/CK6/CK14, p40, p63, EMA	p53		
Squamous cell carcinoma	CK5/CK6, CK14, p40, p63, EMA			**Ber-EP4**, bcl-2, CK7, CK15, CK19, CK20
Basal cell carcinoma	*Epithelial cells*: CK5/CK6, CK14, **Ber-EP4**[a], p63, bcl-2 *Stroma cells*: CD10	Androgen receptor, GATA-3		**EMA**, CK7, CK19, CK15, CK20, CD44
B. Eccrine and apocrine sweat gland tumors	*Luminal (ductal) epithelial cells*: CK7, CK8, CD10, CK11, CK13, CK14, CK18, CK19, EMA, GATA-3 *Myoepithelial (basal) cells*: CK5/CK6/CK14, 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, GCFP15	EMA	CEA	
Microcystic adnexal carcinoma	*Luminal (ductal) epithelial cells:* CK15, CK7 *Myoepithelial (basal) cells:* CK5/CK6/CK14, p63		Ber-EP4, CD10, CK7	CD15, CK20-
Malignant mixed tumor	*Epithelial cells:* CK15 *Myoepithelial cells:* CK5/CK6/CK14, p63, actin	EMA	CEA	CK20
Porocarcinoma	*Luminal (ductal) epithelial cells:* CK7, CK15, p63	CK19, EMA	CEA	
Spiradenocarcinoma	CK15	EMA, GCFP-15		
Hidradenocarcinoma	*Luminal (ductal) epithelial cells:* CK15, CK7 *Myoepithelial (basal) cells:* CK5/CK6/CK14, p63	EMA		
Mucinous carcinoma	CK15, CK7 *Myoepithelial (basal) cells:* CK5/CK6/CK14, p63	ER, PgR		CK20, CDX-2
Digital papillary adenocarcinoma	*Luminal (ductal) epithelial cells:* CK7 *Myoepithelial (basal) cells:* CK5/CK6/CK14, p63	EMA, CEA		GCFP-15
Adenoid cystic adenocarcinoma	See the profile of equivalent in salivary gland tumors			
Apocrine cribriform adenocarcinoma	*Luminal (ductal) epithelial cells:* CK7, CK15 *Myoepithelial (basal) cells:* CK5/CK6/CK14, p63	GCFP-15		
Extramammary Paget disease	CK7, EMA, BerEP-4, CEA	GCFP-15, AMACR	Androgen receptors	CK5/CK6, CK20, ER

Immunoprofile of skin tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
C. 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	
Sebaceous tumors (ocular and extraocular sebaceous carcinoma)	**Adipophilin**[c], CK8/CK18, CK5/CK14, p63, PRAME, DOG-1[c], GATA-3	**Androgen receptors**, EMA[b], CD15	Perilipin, BerEP4, CK7, CD10, D2 40	CK15, CK19, CK20, S100, GCFP-15
D. Merkel cell carcinoma	Pan-CK[d], **CK20**[d], EMA, **INSM-1**, **Islet-1**, NSE, **Merkel cell polyomavirus/ CM2B4**[e], E-cadherin, p53, p16	**CD56**, Fli-1, SATB-2, neurofilaments, chromogranin, CK8, CK18, CD99[d]	CK7, Pax-5, TdT	S100, HMB45, CEA

[a]See Fig. 21.4
[b]The expression of EMA is more characteristic for malignant tumors
[c]See Fig. 21.5
[d]Paranuclear dot-like expression pattern
[e]CM2B2 antibody to MCPyV large T antigen

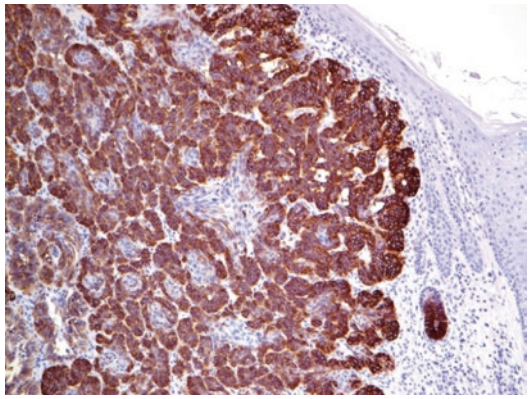

Fig. 21.4 Basal cell carcinoma with strong EPCAM (clone Ber-EP4) expression. Note negative stain of epidermal cells

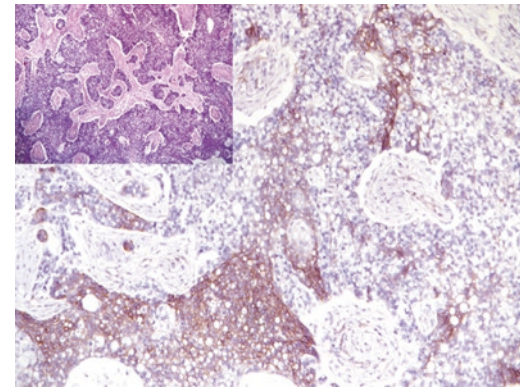

Fig. 21.5 DOG-1 expression in sebaceous carcinoma

Marker profile squamous cell carcinoma vs. basal cell carcinoma							
	CK5/CK14, p63	EMA	BerEp4	BCL-2	Sm-Actin	CD44	Androgen receptor
Squamous cell carcinoma	+	+	–	–/focal +	–	+	–
Basal cell carcinoma	+	–	+	+	+/–	–	+/–

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Contents

Melanoma is a highly malignant tumor developed from melanocytes/melanoblasts that originate from the neural crest precursor cells and migrate during embryogenesis to the skin, uvea, inner ear, leptomeninges, and ectodermal mucosa and can appear in different anatomical localizations. Melanomas have an 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 routine diagnostic pathology, it is always advisable to rule out the manifestation of malignant melanoma in metastatic tumors with ambiguous morphology. Examining tumors of unknown primary, it is important to consider that melanomas can occasionally be positive for different epithelial markers, including pan-cytokeratin, EMA, and E-cadherin, in addition to other lymphoid and hematopoietic markers such as CD10, CD15, CD20, CD21, CD30, CD43, CD56, CD68, CD99, CD117, and CD138.

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https://doi.org/10.1007/978-3-031-45024-2_22

22.1 Diagnostic Antibodies for Melanocytic Tumors

HMB45, Melan A (MART-1), Tyrosinase, Sox-10, Microphthalmia transcription factor (MITF), S100, CD63 (NK-C3).

22.2 Complementary Markers for the Evaluation of Malignant Transformation in Superficial Cutaneous and Mucosal Melanocytic Lesions

PRAME, IMP3, WT-1, p21, p16, cyclin D1, PHH3, Ki-67 [1].

22.3 Therapy-Related Markers

BRAF-$_{V600E}$, NRAS $_{Q61R}$, ALK, NTRK, ROS, PD-L1, PTEN.

22.3.1 HMB-45

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, cardiac rhabdomyoma	Fetal melanocytes and 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 **45M**; 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 the fetal retinal pigment epithelium and fetal melanocytes but absent in mature melanocytes and intradermal nevi (Fig. 22.1). HMB45 is a marker for melanocytic tumors and tumors with melanocytic differentiation, including different types of malignant melanoma, dysplastic nevi, junctional, Spitz and blue nevi, as well as clear cell sarcoma (Figs. 22.2 and 22.3). Furthermore, HMB45 is a diagnostic marker for PEComa, including renal angiomyolipoma, lymphangiomyomatosis, and sugar tumor of the lung.

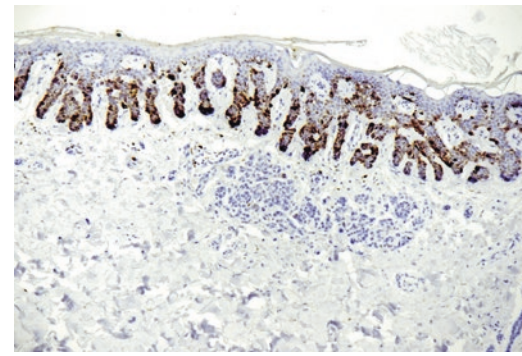

Fig. 22.1 HMB45 stains intradermal melanocytes, whereas subepidermal nevus cells are negative for HMB45

Diagnostic Pitfalls About 10% of malignant melanomas (more frequently amelanotic melanoma, desmoplastic and spindle cell melanomas) lack HMB45 expression. An antibody cocktail containing different anti-melanoma markers (usually HMB45, MART-1, and Tyrosinase) will markedly increase the sensitivity. Additionally,

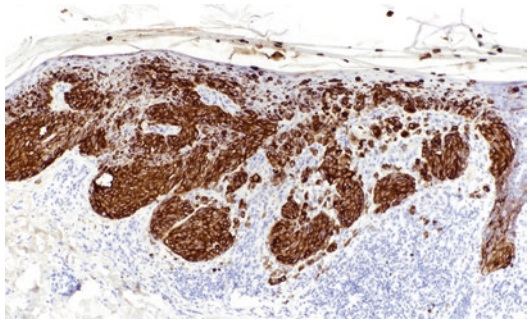

Fig. 22.2 Superficial spreading melanoma. HMB45 staining both intradermal and invasive subdermal cells of malignant melanoma

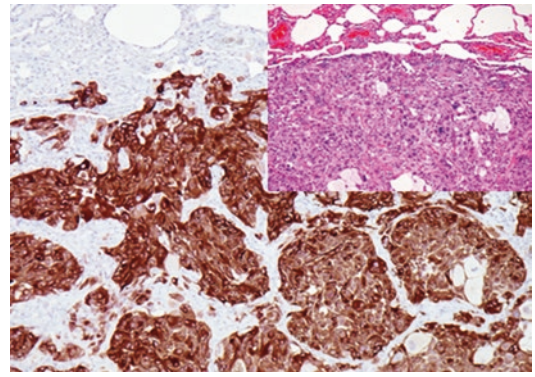

Fig. 22.3 Metastatic melanoma. Melanoma cells with strong HMB45 expression

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.

22.3.2 Melan A

Melan A (MART-1)		
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 Melan A (also known as MART-1) is a melanocyte antigen and a 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 in tumors derived from these cell types. The Melan A antigen is recognized by cytotoxic T lymphocytes. Desmoplastic melanoma usually lacks the expression of Melan A.

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

22.3.3 Tyrosinase

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

Diagnostic Approach Tyrosinase is a copper-containing enzyme catalyzing melanin synthesis from tyrosine in melanocytes. Tyrosinase is a very specific melanoma marker expressed in more than 80% of melanomas, including amelanotic melanoma, 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 a pan-melanoma cocktail. This pan-melanoma cocktail gives good results in diagnosing epithelioid, desmoplastic, and spindle cell melanomas and effectively detects micrometastases in sentinel lymph nodes.

22.3.4 Sox-10

Sox-10		
Expression pattern: nuclear		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Melanoma – Schwannoma – Granular cell tumor – Triple-negative and metaplastic breast carcinoma – Interdigitating dendritic cell sarcoma – Salivary gland tumors Acinic cell carcinoma Adenoid cystic carcinoma Myoepithelial carcinoma Polymorphous adenocarcinoma Mammary analog secretory carcinoma	Clear cell sarcoma, neurofibroma, neuroblastoma, paraganglioma, MPNST, nerve sheath myxoma, alveolar rhabdomyosarcoma, astrocytoma, medulloblastoma, myoepithelial tumors, skin adnexal tumors, basaloid squamous cell carcinoma, embryonal carcinoma, serous and clear cell ovarian carcinoma	Epidermal melanocytes, Schwann cells, autonomic ganglia, myoepithelial cells, acinar cells of salivary glands
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. 22.4) [2].

Compared with other conventional melanoma markers used in routine histopathology, Sox-10 is a very efficient marker that labels melanoma cells and micrometastases in sentinel lymph nodes (Fig. 21.4) [3].

Furthermore, Sox-10 is a marker for triple-negative and metaplastic breast carcinomas [4, 5]. Strong Sox-10 expression is found in myoepithelial cells and myoepithelial tumors, including different types of salivary gland tumors (see salivary gland tumors) and a subset of basaloid squamous cell carcinoma [6, 7]. Additionally, a subset of high-grade serous and clear cell carcinomas is reported to be positive for Sox-10 [8]. Sox-10 stains also astrocytic and oligodendroglial tumors.

Diagnostic Pitfalls Sox-10 is an excellent melanoma marker but lacks specificity as it stains other benign and malignant tumors such as schwannoma, including melanotic schwannoma, neurofibroma, granular cell tumor, and interdigi-

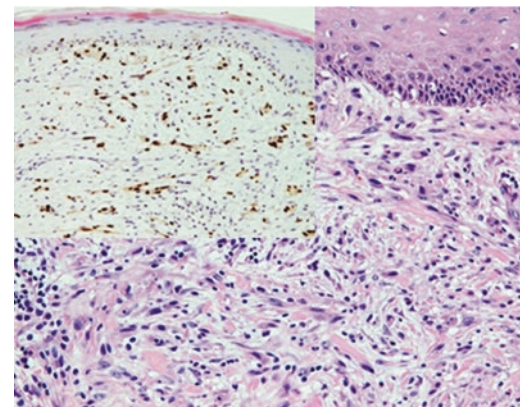

Fig. 22.4 Desmoplastic melanoma exhibiting strong nuclear Sox-10 expression in the tumor cells

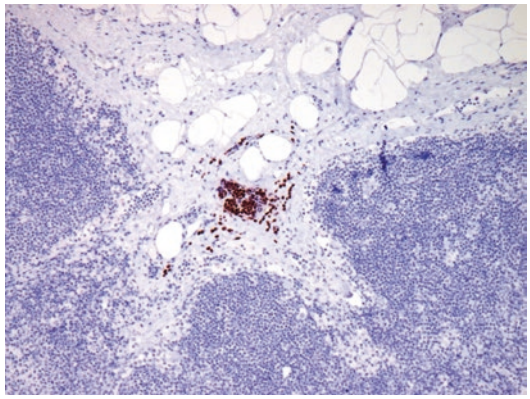

Fig. 22.5 Lymph node with intracapsular Sox-10 positive melanocytic nevi

tating dendritic cell sarcoma, and is found in up to 60% of malignant peripheral nerve sheath tumors [9, 10]. In doubtful cases, other more specific melanoma markers should be used to confirm the diagnosis.

Sox-10 is a sensitive marker to label metastatic melanoma cells in sentinel lymph nodes. To consider in the interpretation of sentinel lymph nodes are Sox-10 positive benign nodal melanocytic nevi, which are usually localized within the lymph node capsule or fibrous trabeculae but negative for HMB-45 and show in the Ki-67 stain a very low proliferative activity stain (Fig. 22.5).

22.3.5 Microphthalmia Transcription Factor

Microphthalmia transcription factor (MITF, also known as the melanocyte-inducing transcription factor) is a transcription factor considered as the master regulator of the development and differentiation of melanocytes, which also regulates melanin synthesis. MITF is also involved in the differentiation of osteoclasts and mast cells.

Diagnostic Pitfalls MITF is a sensitive and specific marker for melanocytes and melanoma; nevertheless, it is also commonly expressed in non-melanocytic cell and tumor types such as histiocytes, follicular dendritic cells, Schwann cells, fibroblasts, smooth muscle cells, and tumors originating from these cells [11]. It is also expressed in clear cell sarcoma and perivascular epithelioid cell neoplasms (PEComa).

22.3.6 PRAME

PRAME		
Expression pattern: nuclear		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Melanoma – Adenoid cystic carcinoma – Endometrioid/serous carcinoma	Thymic carcinoma, seminoma, breast carcinoma, sebaceous tumors, solitary fibrous tumor, neuroblastoma, synovial carcinoma, osteosarcoma, liposarcoma, leukemia	Seminiferous tubules, including Sertoli cells and cells of early spermatogenesis, proliferative endometrium, ovary, placenta, sebaceous glands, adrenal glands
Positive control: testis		

PRAME (**pr**eferentially expressed **a**ntigen in **me**lanoma) is a tumor-associated antigen and a member of the cancer D testis antigen family. PRAME is typically expressed in normal testis, including Sertoli cells and cells of early spermatogenesis in addition to proliferative endometrium, ovary, placenta, sebaceous glands, adrenal glands, and endometrium. Strong PRAME expression is found in more than 90% of primary and metastatic melanoma, including lentigo maligna melanoma, superficial spreading melanoma, acral melanoma, and nodular melanoma (Fig. 22.6). PRAME is expressed in minor cases (<15%) of benign nevi such as Spitz nevi, acquired nevi, and dysplastic nevi [12, 13]. Recently, antibodies to PRAME have been used as a marker to differentiate between benign and malignant melanocytic tumors.

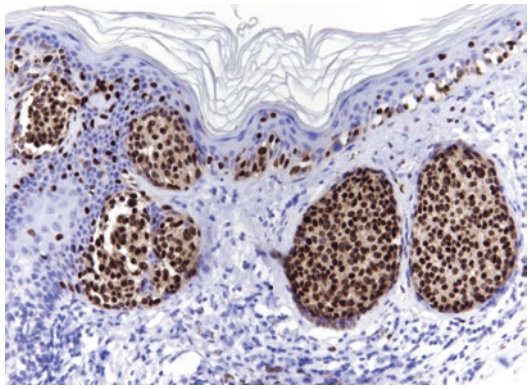

Fig. 22.6 Low-cumulative sun damage melanoma (superficial spreading melanoma) with nuclear PRAME expression in malignant melanoma cells

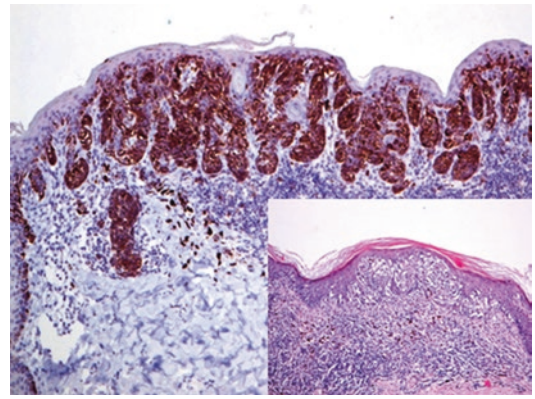

Fig. 22.7 IMP3 staining malignant cells in low-cumulative sun damage melanoma (superficial spreading melanoma)

Diagnostic Pitfalls PRAME is also expressed in other malignant epithelial and mesenchymal tumors, including some leukemia types, Hodgkin's lymphoma, synovial sarcoma, malignant peripheral nerve sheath tumor, osteosarcoma, liposarcoma, solitary fibrous tumor, and different carcinoma types including sebaceous carcinoma, breast carcinoma, endometrial carcinoma, and thymic squamous cell carcinoma [14, 15]. In many tumors, the expression of PRAME is associated with aggressive behavior.

22.3.7 Wilms Tumor Protein (WT-1) and IMP3

WT-1 is already listed in previous chapters (Chaps. 11, 26 and 33) and can be used as a complementary marker in the diagnosis of malignant melanoma [16]. Similar to HMB45, WT-1 can be informative in discriminating between malignant and benign melanocytic lesions. The majority of malignant melanocytes are usually positive for WT-1, whereas benign melanocytes lack the expression of this marker. It is also to consider that most Spitz nevi and about one-third of dysplastic nevi are positive for WT-1.

Cyclin D1 is a further marker showing a similar expression pattern in benign and malignant melanocytic tumors.

IMP3 is an additional marker that labels malignant melanocytes and is found in the majority of malignant melanocytic tumors (Fig. 22.7). IMP3 is not detected in either benign or dysplastic nevi, including Spitz nevi (see also Chap. 15).

22.3.8 p16

The p16 protein is a cyclin-dependent kinase inhibitor A2 listed in later chapters (Chaps. 11, 26 and 33). p16 plays an important role in preventing the cell cycle from progressing from G1 to the S phase, acting as a tumor suppressor gene. In melanocytic tumors, the expression of p16 is preserved in most benign nevi. The malignant transformation causes the deletion or inactivation of the p16 gene, and the p16 expression is lost in a large percentage of malignant or premalignant melanocytic lesions; however, p16 is not an absolute marker for malignancy in melanocytic tumors (Fig. 22.8).

22.3.9 BRAF

BRAF is a serine–threonine kinase that plays an important role in the RAS–RAF–MAPK kinase signaling pathway listed in detail with the markers of thyroid tumors (Chap. 14.3). Activating

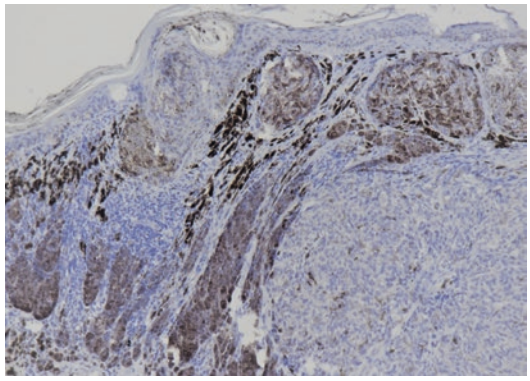

Fig. 22.8 Melanoma arising from preexisting nevus. Benign nevus cells with strong cytoplasmic and nuclear p16 expression (left), while the expression is lost in transformed cells of malignant melanoma (right)

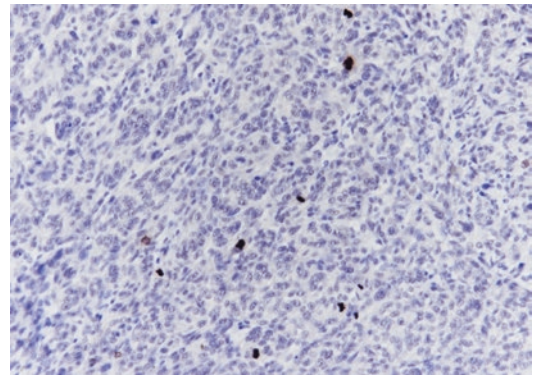

Fig. 22.9 PHH3 highlighting mitotic figures in malignant melanoma cells

BRAF mutations are found in ~50% of all cutaneous melanomas, including premalignant melanocytic lesions. The BRAF-V600E mutation makes up ~90% of all BRAF mutations. Other BRAF mutations such as V600K, V600D, V600M, V600R, and nonV600 are also described in melanomas. The BRAF mutations are found in about 15% of mucosal melanomas but are absent in uveal melanoma.

The mutated BRAF-V600E can be detected by immunohistochemistry using a specific antibody and is considered a diagnostic marker and a therapeutic target.

Diagnostic Pitfalls The BRAF mutations can also be found in benign cutaneous nevi and intracapsular lymph node melanocytic nevi. ~5% of Spitz nevi also have BRAF mutations.

The available antibodies can only detect a specific mutated amino acid sequence (BRAF-V600E), and to detect other possible mutation variants, the molecular sequencing of the complete BRAF gene is required.

22.3.10 RAS

The Ras proteins (KRAS, HRAS, and NRAS) are a group of closely related proteins with high sequence homology expressed in all mammalian cells and encoded by different genes discussed in Chaps. 14.3 and 35. Different RAS mutations, mostly in the NRAS gene, are found in 15–25% of melanomas, mainly radiation-induced cutaneous melanomas, whereas the NRAS-Q61R mutation makes up ~35% of all NRAS-mutated melanomas. Uveal melanomas usually lack NRAS mutations. The mutated NRAS-Q61R protein can be detected by immunohistochemistry using specific antibodies as a diagnostic marker and a specific therapeutic target.

22.3.11 Phosphohistone H3

Phosphohistone H3 (PHH3) is a nuclear core histone protein whose phosphorylation begins in the late G2 phase and reaches its maximal level during the mitotic (M) phase. The immunohistochemical staining of PHH3 using one of the specific antibodies to pHH3 is one of the most specific markers for mitosis. As no PHH3 phosphorylation occurs during apoptosis, the expression of pHH3 can distinguish between mitotic figures and apoptotic nuclei. PHH3 is an ancillary mitotic marker frequently used in the interpretation of melanocytic, meningeal, and neuroendocrine tumors in addition to GIST and breast carcinoma (Fig. 22.9) with a diagnostic and prognostic value [18, 19].

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, vimentin	**PRAME**[a], IMP3[a], BRAF-$_{V600E}$, nestin, CD10, bcl-2[a]	CD68, CD117, MUM-1, CD30, E-cadherin, p16[b]	Pan-CK[c], EMA, INSM-1[d], chromogranin[d], Synaptophysin[d]

[a]Usually negative in benign nevi
[b]Benign nevi show a marked nuclear and cytoplasmic p16 expression, whereas malignant melanocytic cells are usually negative or show a weak cytoplasmic p16 expression
[c]Diagnostic pitfall: A weak focal cytokeratin expression may be found in a small subset of malignant melanoma [17]
[d]Positive in rare melanomas with neuroendocrine differentiation

Immunoprofile of Spitz melanocytic neoplasms				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Spitz nevus	**HMB45**, Melan-A, Sox-10, Tyrosinase, S100, **p16** Proliferation index (Ki-67): <5%		ALK, NTRK and ROS (in ~10%) BRAF (in ~5%)	Pan-CK
Atypical Spitz tumor	Sox-10, Melan-A, Tyrosinase, S100 Proliferation index (Ki-67): 5–15%	**HMB45**	**p16** ALK, NTRK, ROS (in ~10%)	Pan-CK
Spitz melanoma	Sox-10, Tyrosinase, S100 Proliferation index (Ki-67): >20%		**HMB45**, Melan-A	Pan-CK, **p16**

Complementary markers to assist the diagnosis of malignant melanoma in borderline melanocytic lesions	
PRAME	Expressed in the majority of malignant melanocytic tumors; benign nevi lack the expression
IMP3	Expressed in the majority of malignant melanocytic tumors; benign nevi lack the expression
WT-1	Expressed in the majority of malignant melanocytic tumors; benign nevi lack the expression
P21	Expressed in the majority of malignant melanocytic tumors; benign nevi lack the expression
P16	Usually lost in malignant melanocytic lesions, preserved in most benign nevi
Ki-67	High proliferation index in melanoma, very low in benign nevi

The above-listed markers are used only for orientation and have many exceptions

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23 Markers and Immunoprofile of Fibroblastic, Myofibroblastic, and Fibrohistiocytic Tumors

Contents

23.1 Diagnostic Antibody Panel for Fibroblastic, Myofibroblastic, and Fibrohistiocytic Tumors

Vimentin, procollagen, Factor XIIIa, Actin, Desmin, CD34, CD68, NTRK, STAT-6.

23.1.1 Vimentin

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, epithelioid and sarcomatoid mesothelioma, meningioma, gliomas	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 the renal glomerulus, pancreatic acinar cells, melanocytes, lymphocytes, astrocytes, Schwann cells
Positive control: appendix		

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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 Peripherin. Vimentin is generally expressed in all primitive cells in early embryogenesis and is 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. 23.1) (see also Algorithm 1.5). Generally, poorly differentiated carcinomas may acquire Vimentin expression with loss of specific keratins, resulting in a sarcomatoid phenotype. For diagnostic purposes, Vimentin can be only used as a part of a diagnostic antibody panel.

23.1.2 Procollagen Type I

The synthesis of procollagen type I takes place in fibroblasts, and the molecules are processed in the extracellular matrix. Procollagen is a marker of fibroblasts and tumors derived from these cells, including fibroblastic and fibrohistiocytic tumors.

23.1.3 Factor XIIIa

Factor XIIIa (prototransglutaminase) is a member of the transglutaminase family functioning as a fibrin-stabilizing factor. FXIIIa is a fibrohistiocytic marker expressed in macrophages, megakaryocytes, and dendritic cells, including dermal dendrocytes and microglia. FXIIIa is a marker for benign and malignant dermatofibroma, calcifying fibrous tumor and neurofibroma [1]. Scattered FXIIIa-positive cells may also be seen in aggressive angiomyxoma, myofibroblastoma, and atypical fibroxanthoma. Tumor-associated stromal cells can also be positive for FXIIIa.

23.1.4 STAT-6

STAT6 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, a characteristic immunohistochemical marker for solitary fibrous tumor (Fig. 23.2). This chromosomal abnormal-

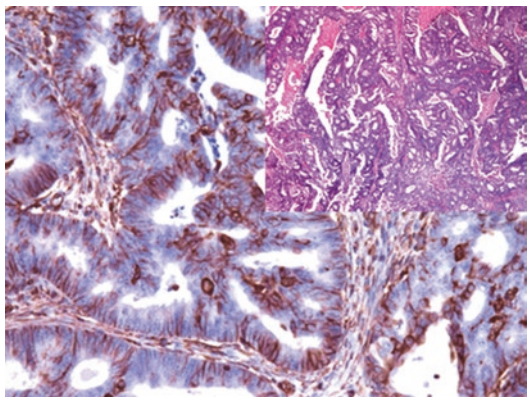

Fig. 23.1 Neoplastic glands of endometrioid carcinoma exhibiting strong Vimentin expression

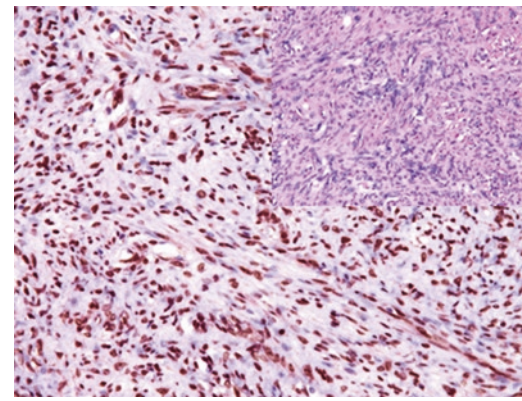

Fig. 23.2 Strong nuclear STAT-6 expression in the cells of solitary fibrous tumor

ity also affects 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 [2, 3]. The immunohistochemical stain with the STAT-6 specific antibody shows as well nuclear as cytoplasmic expression patterns, whereas the nuclear pattern is the specific one.

Diagnostic Pitfalls The overexpression of STAT-6 is also found in a limited number of other mesenchymal and lymphoid tumors, including meningeal hemangiopericytoma that carries the same genetic abnormality, subset of dedifferentiated liposarcoma, synovial sarcoma, desmoid tumor, and mediastinal large B- cell lymphoma in addition to HRS cells in classic Hodgkin lymphoma [4–6].

23.1.5 Mucin-4

MUC-4 is a transmembrane mucoprotein mentioned in a previous chapter with other mucins (Chap. 22). 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 [7, 8].

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, myoglobulin	Actin		Desmin, S100, pan-CK
Myofibroblastoma	Vimentin, D2-40, actin	FXIIIa, cyclin D1		Pan-CK, Desmin, S100
Angiomyxoid fibroma	Vimentin, sm-actin			Pan-CK
Giant cell angiofibroma	Vimentin, CD34			CD31, S100
Calcifying aponeurotic fibroma	Vimentin	CD68, CD99, S100		Actin, pan-CK
EWSR1-SMAD3-positive fibroblastic tumor	ERG			Actin, CD34
Angiomyofibroblastoma	Vimentin, Desmin	CD34, ER	Actin	Pan-CK, S100
Desmoid fibromatosis (abdominal and extraabdominal fibromatosis including desmoid 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
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, **glutamate receptor-2**	Actin, TLE-1, CD10, β-catenin [a]	Desmin, S100, pan-CK, EMA, CD56, CD68, CD117

Immunoprofile of fibroblastic and myofibroblastic tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Inflammatory myofibroblastic tumor (inflammatory pseudotumor)	Vimentin	ALK (p80), cyclin D1, actin	Desmin, CD68, bcl-2, pan-CK	EMA, CD34, CD117
Superficial CD34 positive fibroblastic tumor	CD34	Pan-CK		
Low-grade fibromyxoid sarcoma	**MUC-4**, vimentin	EMA	Actin, Desmin, bcl-2, CD34, pan-CK	S100, EMA
Infantile (congenital) fibrosarcoma	**NTRK**, EGFR, vimentin		Actin, Desmin, S100, CD34	Myoglobin, MyoD1, Myogenin, actin, CD34
Acral myxoinflammatory fibroblastic sarcoma (inflammatory myxohyaline tumor)	Vimentin		CD34, CD68	EMA
Fibrosarcoma (adult)	Vimentin			
Myxofibrosarcoma	Vimentin		Actin, CD34	Desmin, S100
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, CD10, α-1antitrypsin, vimentin	Actin	Desmin, CD34	S100
Plexiform fibrohistiocytic tumor	Vimentin	Actin		
Giant cell tumor of soft tissue	CD68, actin[a], vimentin			
Dermatofibrosarcoma protuberans	**CD34**, PDGF, p53, apolipoprotein D, 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 the skin)	CD10, Fli-1, S100A6, Procollagen-1 vimentin	CD68, actin	TLE-1	Pan-CK, Desmin
Localized giant cell tumor of the tendon sheath	CD68[b], CD45, vimentin			
Tenosynovial giant cell tumor	CD68[c], CD45, vimentin	CD31, CD34	Desmin	Actin, S100, h-Caldesmon, FVIII

[a] Only in the spindle cells
[b] Giant cell lacks Actin expression
[c] CD68 and CD45 expression only in multinucleated cells

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Markers and Immunoprofile of Muscle Tumors

24

Contents

24.1 Diagnostic Antibody Panel for Skeletal Muscle Tumors

Desmin, Myoglobin, Myogenin, Myosin MyoD1, EGFR, Fibrilin-2, and p-Cadherin [1, 2].

24.1.1 Desmin

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		

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https://doi.org/10.1007/978-3-031-45024-2_24

Diagnostic Approach Desmin is a type III intermediate filament protein involved in the contractility of muscle cells. Desmin presents in intercalated disks and Z-lines of the cardiac muscle, in Z-line of the skeletal muscle, and in cytoplasmic and sub-plasmalemmal dense bodies of the smooth muscle. Antibodies to Desmin label cardiac, skeletal, and smooth muscle cells and tumors derived from these cells. The intensity of Desmin expression correlates with the differentiation grade of muscle cells or muscle tumors. Desmin is an important diagnostic marker for all myogenic tumors and tumors with myogenic differentiation, whereas myoepithelial cells lack the expression of Desmin (Fig. 24.1).

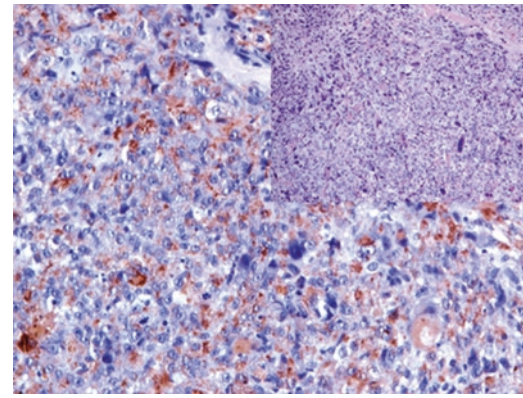

Fig. 24.1 Pleomorphic rhabdomyosarcoma with marked cytoplasmic Desmin expression

Diagnostic Pitfalls The expression of 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 also be included. 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.

24.1.2 Myoglobin

Myoglobin		
Expression pattern: cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Rhabdomyosarcoma – Tumors with skeletal muscle differentiation	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 the 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 Macrophages engulfing necrotic muscle cells are positive to myoglobin and can be misinterpreted as myoblasts. Weak myoglobin expression is reported in various carcinomas (e.g., breast, prostate, colon, head and neck), associated with hypoxia and steroid hormone receptor positivity.

24.1.3 Myogenin and MyoD1

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 part in the regulation of skeletal muscle differentiation in early embryonal stages and 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. 24.2 and 24.3) [3, 4].

Diagnostic Pitfalls Both myogenic transcriptional factors can be positive in nonneoplastic myoblasts found within regenerative and atrophic muscle lesions [5]. The expression of Myogenin and MyoD1 is also reported in some cases of desmoid tumors, infantile fibrosarcoma, mesenchymoma, and Wilms tumor. In the interpretation of Myogenin and Myo D1 stains, only the nuclear staining pattern can be considered positive; other patterns (cytoplasmic or membranous) are nondiagnostic artifacts.

24.1.4 PAX-5

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

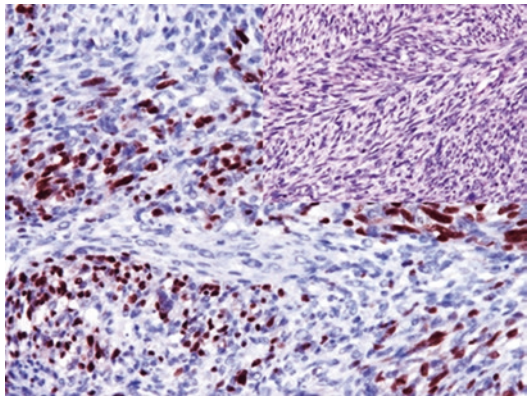

Fig. 24.2 Embryonal rhabdomyosarcoma showing strong nuclear Myogenin expression in the tumor cells

24.1.5 Epidermal Growth Factor Receptor-1

EGFR is a member of type 1 receptor tyrosine kinase family described in a previous chapter (see Chap. 23). 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 a diagnostic marker for embryonal rhabdomyosarcoma, discriminating it from other rhabdomyosarcoma types (Fig. 24.4) [7].

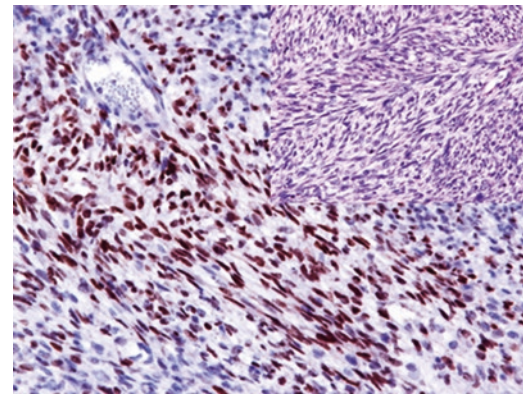

Fig. 24.3 Embryonal rhabdomyosarcoma with strong nuclear MyoD1 expression in the tumor cells

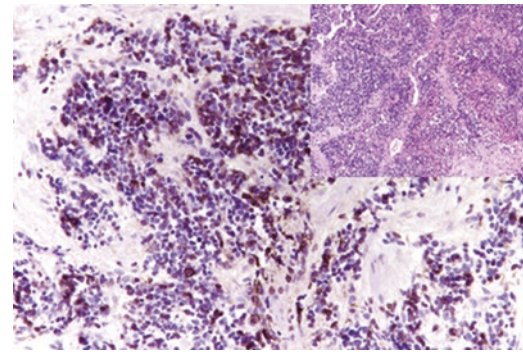

Fig. 24.4 Embryonal rhabdomyosarcoma exhibiting strong EGFR expression in the tumor cells

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**, **Fibrilin-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, PAX-7, CD56, bcl-2	PLAP, NSE	Pan-CK[a], Fibrilin-2, EGFR
Pleomorphic rhabdomyosarcoma	**Desmin**	**MyoD1**, **Myogenin**, Myf-5	Pan-CK	
Spindle cell/sclerosing rhabdomyosarcoma	**Desmin**, **Myogenin**	**MyoD1**, Myf-5, CD56	Pan-CK, S100	Actin, S100

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

24.2 Diagnostic Antibody Panel for Smooth Muscle Tumors

Desmin, sm-Actin, h-Caldesmon, Calponin, Smoothelin, Transgelin, Smooth muscle myosin heavy chain, and steroid hormone receptors [8].

24.2.1 Smooth Muscle Actin

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 endothelial cells
Positive control: appendix		

Diagnostic Approach Actins are a major cytoskeletal protein and a group of contractile microfilaments that include the 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 [2]. 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. 24.5).

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, GIST, and sarcomatous mesothelioma.

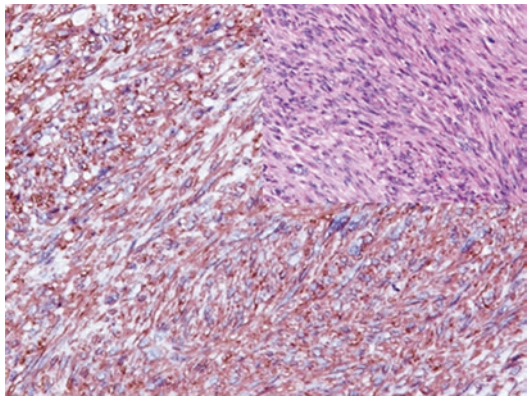

Fig. 24.5 Strong cytoplasmic expression of sm-Actin in the leiomyosarcoma cells

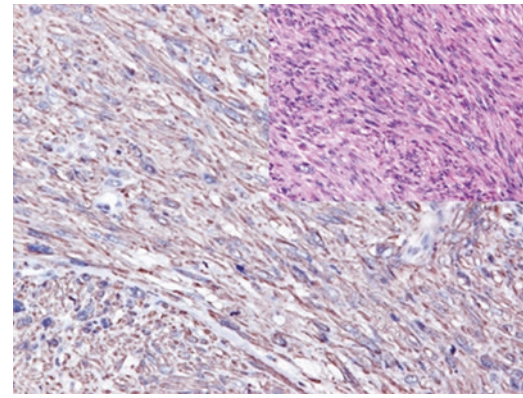

Fig. 24.6 Leiomyosarcoma exhibiting cytoplasmic h-Caldesmon expression in the tumor cells

24.2.2 h-Caldesmon

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		

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 the 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. 24.6). In contrast to Actin, myofibroblasts lack the expression of h-Caldesmon [9].

Diagnostic Pitfalls h-Caldesmon can be positive in non-smooth muscle lesions such as gastrointestinal stromal tumor and inflammatory myofibroblastic tumor in addition to pleural and peritoneal epithelioid mesothelioma, which is to consider in the differential diagnosis.

24.2.3 Calponin

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, myofibroblasts
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. Calponin also reacts with normal and reactive myofibroblasts and with myofibroblastic tumors. GIST usually lacks the expression of Calponin.

24.2.4 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 addition to myofibroblasts and related benign and malignant tumors [10, 11]. Transgelin labels also the epithelial tumor cells of triple-negative breast carcinoma of basal cell phenotype and a subset of malignant nerve sheet tumors [12]. Rhabdomyosarcoma, GISTs, and endometrial stromal tumors lack the expression of Transgelin [13].

Diagnostic Pitfalls The expression of Transgelin is also found in fibroblasts, myofibroblasts, and some epithelial cells.

24.2.5 Smoothelin

Smoothelin is a component of the cytoskeleton of differentiated smooth muscle cells and presents in 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 [14]. Myoepithelial cells, myofibroblasts, and skeletal and cardiac muscle 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. 24.7) [15]. Smoothelin shows two expression patterns. A cytoplasmic staining pattern is found in benign and malignant smooth muscle cells, whereas a cytoplasmic and nuclear staining pattern is mainly found in leiomyosarcoma (Figs. 24.8 and 24.9). Smoothelin is also a helpful marker to highlight the muscularis propria and muscularis mucosae for the interpretation of bladder and

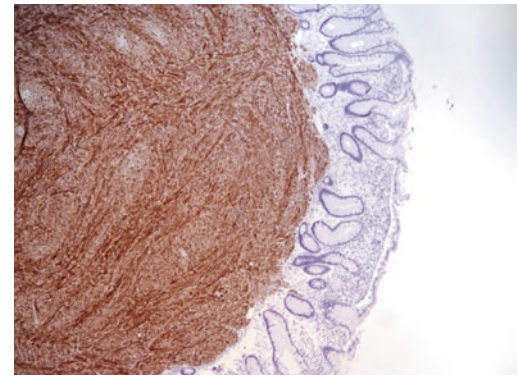

Fig. 24.7 Submucosal leiomyoma with strong cytoplasmic Smoothelin expression in myoma cells

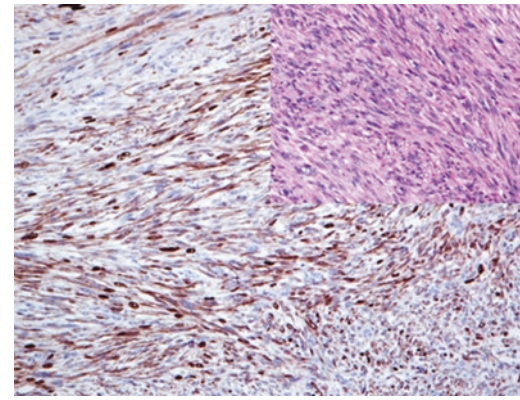

Fig. 24.8 Smoothelin highlighting the cells of leiomyosarcoma exhibiting a cytoplasmic and nuclear staining pattern

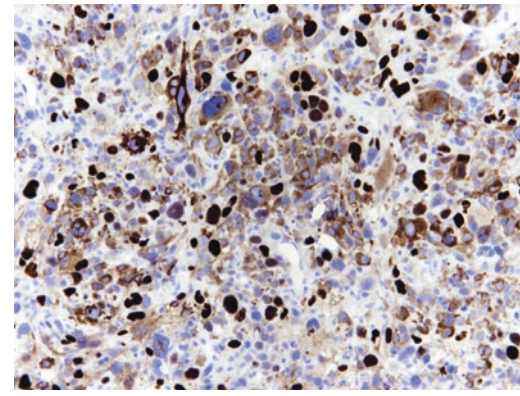

Fig. 24.9 Cytoplasmic and nuclear Smoothelin staining pattern in high-grade leiomyosarcoma

intestinal tumors. For the latter, the comparative use with sm-Actin is recommended. Sm-Actin stains both muscle layers equally strong, while Smoothelin tends to show a lesser staining of muscularis mucosae in comparison with muscularis propria.

24.2.6 Smooth Muscle Myosin Heavy Chain

Smooth muscle myosin heavy chain (SMMHC) is a structural protein encoded by the MYH 11 gene encoded on chromosome 16, which is a major component of the contractile apparatus in smooth muscle and myoepithelial cells. SMMHC is also expressed in follicular dendritic cells, whereas myofibroblasts lack the expression of this protein. SMMHC shows cytoplasmic and membranous expression patterns, whereas a nuclear stain is found in the cells of acute myeloid leukemia with inv.(16)(p13.1q22) or t(16;16)(p13.1;q22) (FAB: M4Eo) due to a specific translocation associated with this leukemia type (Fig. 24.10). In routine immunohistochemistry, Smooth muscle myosin is a marker for smooth muscle cells and related tumors. Also, it is an excellent marker to discriminate between malignant and benign breast and lung lesions later with preserved myoepithelial cells highlighted by the SMMHC immunohistochemical stain.

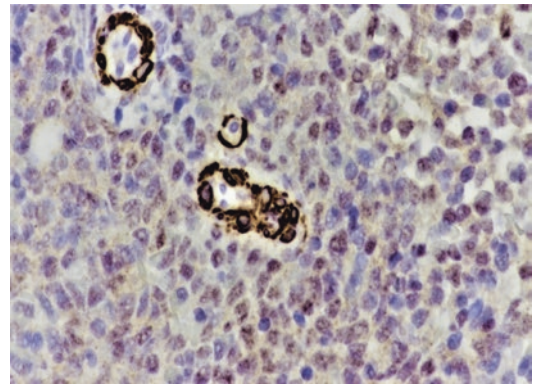

Fig. 24.10 Inv(16) associated AML M4Eo with nuclear SMMHC expression. Smooth muscle cells in blood capillaries with strong cytoplasmic SMMHC expression

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, CD56[a]	bcl-2	
Leiomyosarcoma	**Sm-actin**, h-Caldesmon, vimentin	Desmin, calponin, Smoothelin, Transgelin, CD56[a], p16, [b] CD146, D2-40	Pan-CK, CK8, CK18, CD34, bcl-2	CD117

[a] Harmful in vascular leiomyoma/leiomyosarcoma
[b] It can also be positive in smooth muscle tumors of uncertain malignant potential

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25 Markers and Immunoprofile of Vascular and Pericytic (Perivascular) Tumors

Contents

25.1 Diagnostic Antibody Panel for Vascular Tumors

CD31, CD34, Factor VIII, CD105, ERG, Podoplanin (D2 40), Thrombomodulin (CD141), Claudin-5, Fli-1 [1].

25.2 Diagnostic Markers for Lymphatic Endothelial Cells and Lymphangioma

Podoplanin (D2 40), Prox-1, LYVE-1.

25.2.1 CD31

CD31 (PECAM-1)		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**

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CD31 (PECAM-1)		
– Vascular/ endothelial tumors	Plasmacytoma, CLL, Langerhans cell histiocytosis and Langerhans sarcoma, granulocytic sarcoma, Ewing's sarcoma, rare carcinoma types	Endothelial cells, megakaryocytes/platelets, macrophages/monocytes, liver Kupffer cells, splenic sinusoidal 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 (Fig. 25.1) [1, 2].

Diagnostic Pitfalls Different expression levels of CD31 are reported in rare nonvascular tumors such as chronic lymphocytic lymphoma, plasmacytoma, Langerhans cell neoplasia, leiomyosarcoma, mesothelioma, melanoma, and glioma in addition to few carcinoma types such as carcinoma in situ and invasive carcinoma of the breast and papillary thyroid carcinoma (Figs. 25.2 and 25.3).

25.2.2 CD34

CD34		
Expression pattern: membranous		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
Vascular tumors, Kaposi sarcoma, GIST, dermatofibrosarcoma protuberans, solitary fibrous tumor, epithelioid sarcoma, AML (M0 and M7), granulocytic sarcoma, neurofibroma, liposarcoma	Pre-B-ALL, megakaryocytes in MDS, alveolar soft part sarcoma, congenital and infantile fibrosarcoma, inflammatory fibrous polyp of the gastrointestinal tract, breast fibroadenoma, giant cell fibroblastoma, juxtaglomerular cell tumor, superficial acral fibromyxoma	Hematopoietic progenitor cells (myeloid precursors, pro B cells, prothymocytes), endothelial cells, hepatic sinusoidal cells, interstitial cells of Cajal, thymic stromal cells, 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

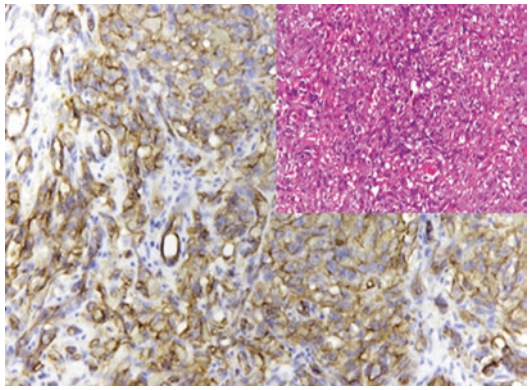

Fig. 25.1 CD31 highlighting neoplastic endothelial cells in angiosarcoma

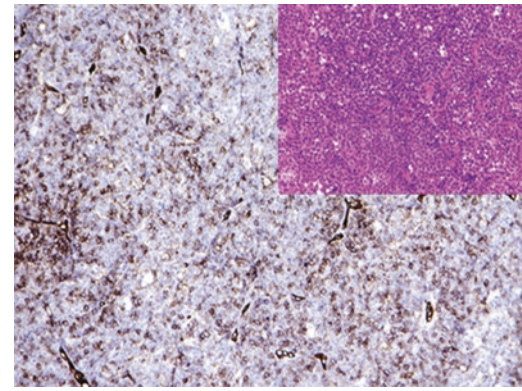

Fig. 25.2 CD31 expression in cells of malignant melanoma

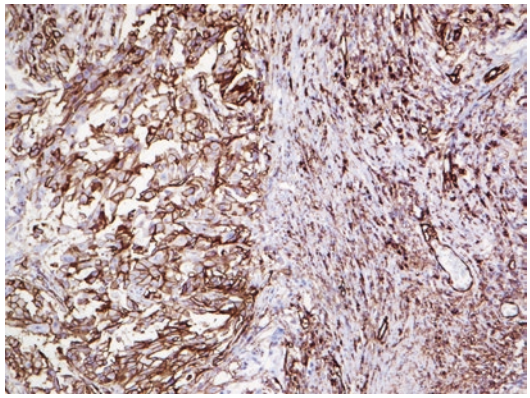

Fig. 25.3 CD31 staining epithelial tumor cells of invasive ductal carcinoma of the breast

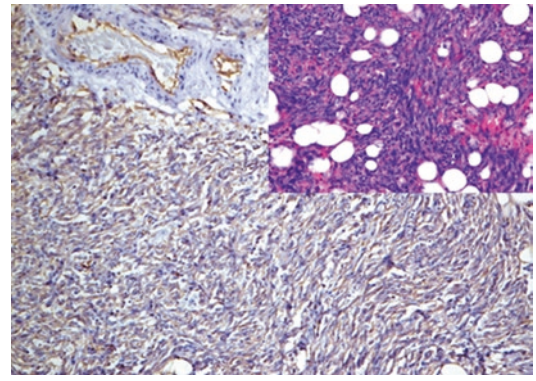

Fig. 25.4 CD34 highlighting neoplastic endothelium in angiosarcoma

widely used marker to highlight blood vessels and vascular tumors, but it is less specific than CD31 (Fig. 25.4) [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 labels 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].

25.2.3 Factor VIII (Von Willebrand Factor)

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		

Diagnostic Approach Factor VIII (von Willebrand Factor) is a glycoprotein complex consisting of four specific domains (3 A, 3 B, 2 C, and 4 D domains) 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 and alpha granules of platelets. 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 benign and malignant vascular tumors, including angiosarcoma (Fig. 25.5).

25.2.4 Erg

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		

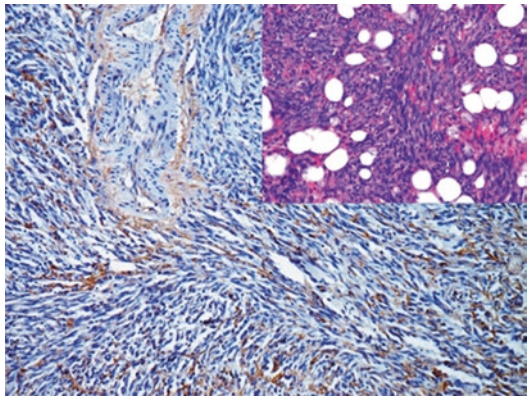

Fig. 25.5 Angiosarcoma with a diffuse expression of factor VIII

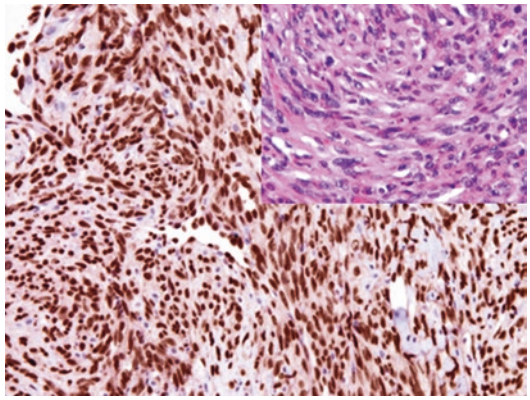

Fig. 25.6 Angiosarcoma of the myocardium, tumor cells exhibiting strong nuclear ERG expression

*E*26 *t*ransformation specific *r*egulator *g*ene1 (ERG) is an avian erythroblastosis virus oncogene homolog and a member of the ETS family of transcription factors listed in a previous chapter (see markers for prostatic carcinoma, Chap. 13). ERG is normally expressed in endothelial cells and plays a role in the regulation of angiogenesis and endothelial apoptosis. In addition to prostatic adenocarcinoma harboring the TMPRSS2-ERG translocation, ERG is a very sensitive and specific marker for endothelial neoplasia (Fig. 25.6) [4]. The expression of ERG is also found in a subset of immature hematopoietic cells and related neoplasia (see Chap. 17).

Diagnostic Pitfalls In mesenchymal tumors, the expression of ERG is reported in some other mesenchymal tumors with a morphology resembling vascular tumors, including solitary fibrous tumor, fibrous meningioma, and epithelioid sarcoma due to other genetic anomalies associated with these tumors [5, 6]. The expression of ERG is also found in a small subset of some lymphoma types.

25.2.5 Fli-1

Fli-1 gene (friend leukemia virus integration site 1) is a member of the ETS proto-oncogene ETS family functioning as a transcriptional activator highly expressed during embryogenesis (see Chap. 29). Fli-1 is also a marker for endothelial cells and endothelial tumors.

25.2.6 CD105 (Endoglin)

CD105 is a type I membrane glycoprotein expressed on the surface of endothelial cells, functions as a co-receptor for transforming growth factor (TGF β1 and β3), and has two isoforms L and S. CD105 is a proliferation-associated and hypoxia-inducible protein, which plays a critical role in angiogenesis. The expression of CD105 is mainly found on vascular endothelial cells and is markedly activated in neoangiogenesis. Weak CD105 expression is also found on the hematopoietic progenitor cells, including pre-B lymphocytes and a subpopulation of monocytes, fibroblasts, and vascular smooth muscle cells in addition to cells of malignant melanoma and prostatic carcinoma. CD105 is a marker for endothelial tumors and angiogenesis and can be used as a marker to estimate tumor-induced neoangiogenesis (Fig. 25.7).

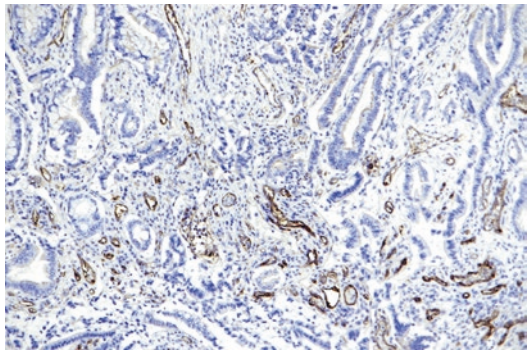

Fig. 25.7 CD105 highlighting the new blood vessels developed in the stroma of pancreatic ductal adenocarcinoma

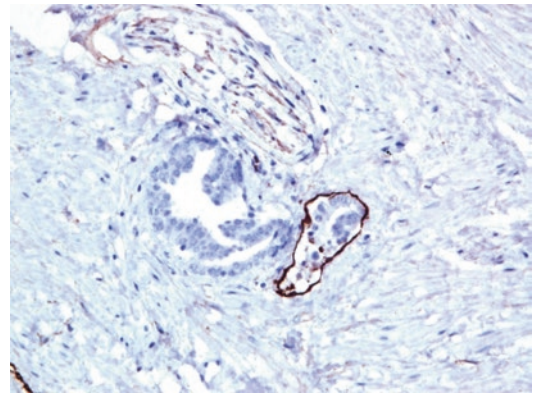

Fig. 25.8 D2-40 highlighting the endothelial cells of a lymphatic vessel exhibiting lymphangitic carcinomatosis. D2-40 is also staining the Schwann cells appearing in the upper part of the section

25.2.7 Podoplanin

Podoplanin (D2-40)		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Lymphangioma and tumors of lymphatic vessels – Mesothelioma and adenomatoid tumor – Meningioma – Germ cell tumors (dysgerminoma and seminoma)	Vascular tumors, Kaposi sarcomas, skin adnexal carcinomas, follicular dendritic cell tumors, adrenal cortical tumors, dermatofibroma, ependymoma, medulloblastoma, glioblastoma, choroid plexus tumors, GIST, craniopharyngioma, synovial sarcoma, leiomyosarcoma, desmoid, schwannoma, malignant peripheral nerve sheath tumor, epithelioid sarcoma, chondrosarcoma, serous ovarian carcinoma	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, choroid plexus epithelium, 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. 25.8). Furthermore, it is one of the important seminoma markers [7].

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, it must be used in a panel with other more specific antibodies [8].

25.2.8 PROX-1

PROX-1 (transcription factor *pro*spero homeobo*x* gene protein *1*) is a control gene encoding a nuclear transcription factor that regulates the differentiation of lymphatic endothelial cells and the formation of lymphatic vessels and embryonic veins. Antibodies to Prox-1 label the lymphatic vessels and tumors of lymphatic endothelium [9]. Prox-1 is expressed in the central nervous system, pancreatic endocrine cells, and liver, in addition to some other tumor types, including neuroendocrine tumors.

25.2.9 Lymphatic Vessel Endothelial Hyaluronan Receptor 1 (LYVE-1)

LYVE-1 is a receptor for extracellular matrix mucopolysaccharide hyaluronan, functioning as an adhesion molecule for dendritic cells and macrophages, regulating their migration into the lymph vessels and being involved in tissue remodeling. LYVE-1 is expressed on the endothelial cells of embryonic blood vessels and lymphatic vessels in addition to the sinusoidal endothelium of the liver and spleen. The expression of LYVE-1 is downregulated in the endothelium of mature blood vessels. In routine immunohistochemistry, LYVE-1 is a marker for lymphatic vessels, which also can be expressed in proliferating capillaries, infantile hemangioma, and Kaposi sarcoma.

25.2.10 Glut-1 and WT-1

Both are not endothelial markers but can be used to distinguish between neoplastic endothelium/hemangioma, usually positive for both markers, and nonneoplastic or reactive endothelial cells that remain negative for both markers (Fig. 25.9).

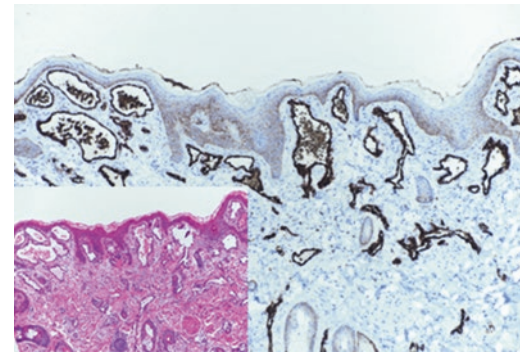

Fig. 25.9 Infantile hemangioma with strong Glut-1 expression in neoplastic endothelial cells

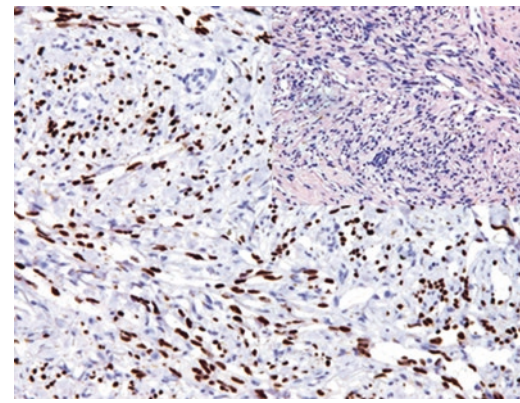

Fig. 25.10 Nuclear expression of HHV-8 (LNA) in neoplastic cells of Kaposi sarcoma

25.2.11 Human Herpes Virus Type 8

Human herpesvirus-8 (HHV-8) is a double-stranded DNA virus and a member of the *Rhadinovirus* subfamily of the herpes group.

HHV-8 is the etiological agent of different human neoplasms, including Kaposi sarcoma, primary effusion lymphoma, and multicentric Castleman's disease. The demonstration of latent nuclear antigen in tissue sections is a diagnostic marker for Kaposi sarcoma (Fig. 25.10) [10].

Immunoprofile of vascular tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Capillary hemangioma	**CD31**, **F VIII**, **CD34**, **ERG**, vimentin	Glut-1[a], WT-1		Pan-CK
Epithelioid hemangioma	**CD31**, **F VIII**, **CD34**, **ERG**, vimentin	Glut-1, WT-1[b], FOSB	Pan-CK	
Lymphangioma	**Podoplanin** (D2-40), **Prox-1**, CD31, F VIII, CD34, vimentin			Pan-CK
Retiform hemangioendothelioma	**CD31**, **CD34**, **FVIII**, **ERG**, vimentin			
Kaposiform hemangioendothelioma	**CD31**, **CD34**, **ERG**, vimentin			**HHV-8**, F VIII, Glut-1
Pseudomyogenic hemangioendothelioma	Pan-CK, Fli-1, ERG, FOSB	CD31, CD56	Actin	Desmin, Melan A, CD34
Epithelioid hemangioendothelioma	CD31, CD34, **ERG**, F VIII, **CAMTA-1**[c], Podoplanin Vimentin	Fli-1	TFE3[d], actin, pan-CK, CK8/18, ER, Melan A, HMB-45	EMA, S100
Epithelioid angiosarcoma	**CD31**, **CD34**, **F VIII**, **ERG**, Fli-1, vimentin	Pan-CK		EMA, S100
Kaposi sarcoma	**CD31**, **CD34**, CD105, **HHV-8**, ERG, D2-40, Fli-1, vimentin	bcl-2	MDM2	F VIII, CDK4
Angiosarcoma	CD31, CD34, CD105, F VIII, **ERG**, **Fli-1**, CD141 (thrombomodulin), vimentin	Laminin, CK1	Pan-CK, MDM2, CD117, inhibin A	D2-40, CD56, CDK4, HHV-8
Malignant endovascular papillary angioendothelioma (papillary intralymphatic angioendothelioma; Dabska tumor)	**CD31**, **CD34**, **ERG**, F VIII, VEGFR-3, D2-40, vimentin			Pan-CK, EMA, S100

[a] Glut-1 and WT-1 are usually positive in neoplastic endothelial cells (hemangioma) but negative in vascular malformation pyogenic granuloma and granulation tissue

[b] Positive in tumors associated with one of the different FOS/FOSB gene fusions characteristic for an epithelioid hemangioma

[c] Calmodulin-binding transcription activator 1 (CAMTA-1) is the fusion partner in the t(1,3)(p36.3;q25) (WWTR1-CAMTA1) translocation found in ~90% of EHE [11]

[d] TFE3 positive in a subset of EHE is associated with the YAP1-TFE3 gene fusion, whereas the majority of this tumor is associated with the WWTR1-CAMTA1 gene fusion [12]

Immunoprofile of pericytic (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/myofibroma	**Sm-actin**, h-Caldesmon, vimentin		Desmin, CD34	S100, pan-CK, EMA
Sinonasal glomangiopericytoma	Actin LEF-1, VEGF, vimentin	FXIIIa		Desmin, CD31, CD34, F VIII, EMA, pan-CK

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Markers and Immunoprofile of Adipocytic Tumors

26

Contents

26.1 Diagnostic Antibody Panel for Adipocytic Tumors

S100, CD34, MDM2, CDK4, DDIT3, and p16 [1, 2].

26.1.1 MDM2

MDM2		
Expression pattern: nuclear/cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Liposarcoma	Low-grade osteosarcoma, clear cell sarcoma, desmoplastic small round cell tumor, angiosarcoma, Kaposi's sarcoma, intimal sarcomas, epithelioid sarcoma, embryonal rhabdomyosarcoma, leiomyosarcoma, MPNST, adrenal oncocytoma, 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 encoded on chromosome 12q14-15 that interacts with p53 affecting the cell cycle and apoptosis. MDM2 gene amplification with overexpression of the MDM2 protein is noted in some tumors, while the main diagnostic use is to differentiate between well-differentiated liposarcoma and atypical lipomatous tumor with MDM2 gene

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amplification and benign adipocytic tumors lacking the amplification which can be detected by immunohistochemistry (Fig. 26.1) or FISH assay [3–5].

The overexpression of MDM2 is also a marker for intimal sarcoma and low-grade osteosarcoma but is absent in benign fibro-osseous lesions, which can be helpful in discriminating between the two identities.

Diagnostic Pitfalls As abovementioned, the expression or overexpression of MDM2 might be found in many sarcoma types, which is to consider 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. MDM2 is expressed in macrophages and necrotic fatty tissue that might mimic liposarcoma or atypical lipomatous tumor.

26.1.2 CDK4

CDK4 (*C*yclin-*d*ependent *k*inase *4*) is a 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 sheet tumor in addition to rhabdomyosarcoma; accordingly, CDK4 can be used as a marker 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.

26.1.3 p16

p16 (cyclin-dependent kinase inhibitor 2A) is a tumor suppressor protein expressed in a few carcinoma types, including HPV-associated squamous cell carcinoma of different origins (see Chaps. 11 and 33). p16 is a helpful marker to distinguish between well-differentiated liposarcoma/atypical lipomatous tumor and dedifferentiated liposarcoma, both positive for p16 and benign adipocytic tumors and normal fatty tissue lacking the expression of p16 (Figs. 26.2 and 26.3) [6].

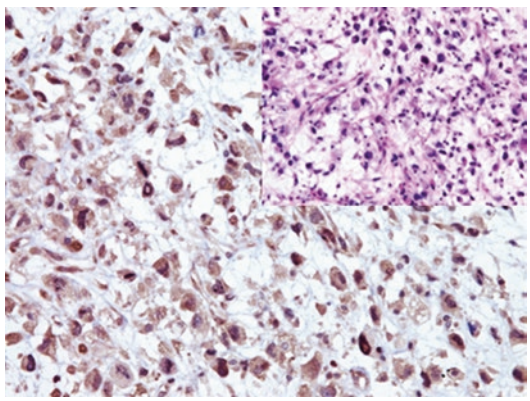

Fig. 26.1 MDM2 overexpression in neoplastic cells of dedifferentiated liposarcoma

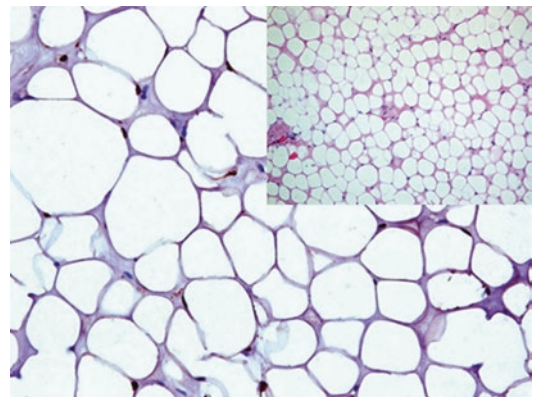

Fig. 26.2 Strong nuclear p16 expression in cells of atypical lipomatous tumor

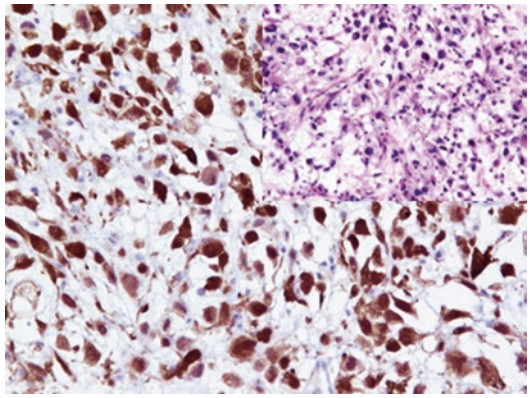

Fig. 26.3 Strong p16 expression in neoplastic cells of dedifferentiated liposarcoma

Diagnostic Pitfalls p16 is not a specific liposarcoma marker, as it is reported to stain other malignant mesenchymal tumors. The p16 positivity can also be found in areas with liponecrosis [7, 8].

26.1.4 DDIT3

DDIT-3 (*D*NA *d*amage-*i*nducible *t*ranscript *3)*, also known as CHOP, is a transcriptional factor involved in adipocytic differentiation, G1-S cell cycle progression, and growth arrest encoded on chromosome 12q13. The 12q13 region is also the location of other genes affected in lipomatous tumors, such as MDM2 and CDK4. DDIT3 is the fusion partner in the two main translocations associated with myxoid liposarcoma t(12;16)(q13;p11) and t(12;22)(q13;q12) [9]. These translocations cause the overexpression of DDIT-3 due to the activation by the promoters of the fusion partner genes. DDIT-3 is a specific diagnostic marker for myxoid liposarcoma found in more than 90% of the cases. A focal, very weak nuclear expression may also be found in a mall subset of pleomorphic and dedifferentiated liposarcomas [10].

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
Lipoblastoma	**S100**, **CD34**, CD56, 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**, **DDIT3**, **p16**, aP2, Ki-67 (clone K-2), vimentin	CD56, calretinin	S100	
Dedifferentiated liposarcoma	**CDK4**, **MDM2**, **p16**, aP2, Ki-67 (clone K-2), vimentin	S100	STAT-6[a]	
Pleomorphic liposarcoma	**S100**, aP2, Ki-67 (clone K-2), vimentin		MDM2	

[a] STAT-6 is a characteristic marker for solitary fibrous tumor but is also reported in a subset of dedifferentiated liposarcoma

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27 Markers and Immunoprofile of Peripheral Nerve and Nerve Sheath Tumors

Contents

27.1 Diagnostic Antibody Panel for Peripheral Nerve and Nerve Sheath Tumors

S100, CD56, PGP 9.5, Sox-10, Myelin basic protein, Glial fibrillary acidic protein (GFAP), Neurofilaments, Nerve growth factor receptor (NGFR, gp75), Claudin-1, Glut-1.

27.1.1 Myelin Basic Protein

Myelin basic protein		
Expression pattern: cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Neurogenic sarcoma – Neuroma, neurofibroma, and ganglioneuroma	Granular cell tumor	Cells of the white matter of the central and peripheral nervous systems
Positive control: brain tissue		

M. S. A. Tuffaha et al., *Immunohistochemistry in Tumor Diagnostics*,
https://doi.org/10.1007/978-3-031-45024-2_27

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 system and takes part in the 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.

27.1.2 Neurofilaments

Neurofilaments		
Expression pattern: cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Medulloblastoma – Retinoblastoma – Neuroblastoma – Ganglioglioma – Paraganglioma – Neurofibroma	Merkel cell carcinoma, 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) encoded by different genes. Neurofilaments 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 angiosarcoma, rhabdomyosarcoma, and epithelioid sarcoma and rare carcinoma types.

27.1.3 Protein Gene Product 9.5

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, melanoma, dermatofibrosarcoma protuberans	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 system and in neuroendocrine tissue. Antibodies to PGP 9.5 are good markers to highlight neuronal and neuroendocrine tumors (Fig. 27.1).

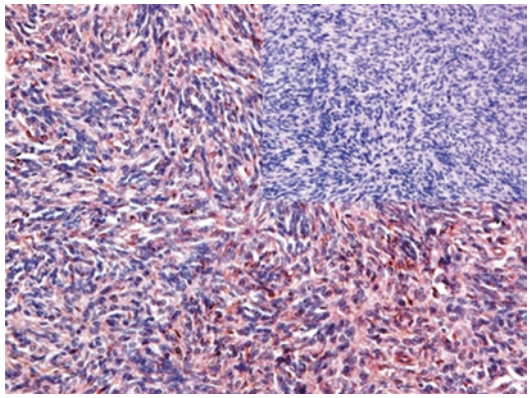

Fig. 27.1 Neurogenic sarcoma, tumor cells labeled with PGP 9.5

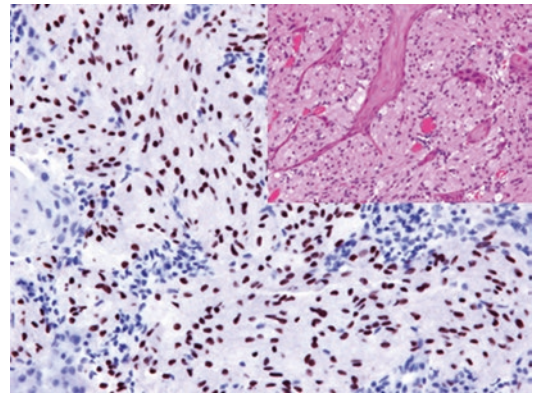

Fig. 27.3 Strong nuclear Sox-10 expression in cells of granular cell tumor

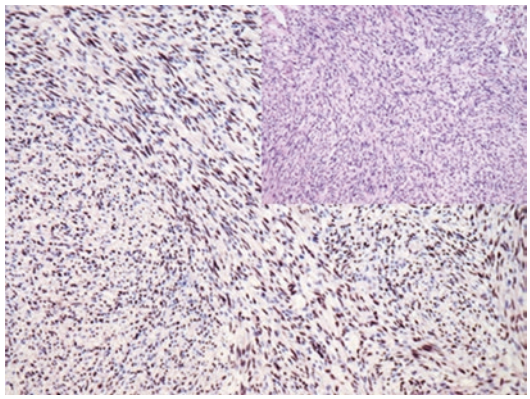

Fig. 27.2 Nuclear Sox-10 expression in the cells of neurofibroma

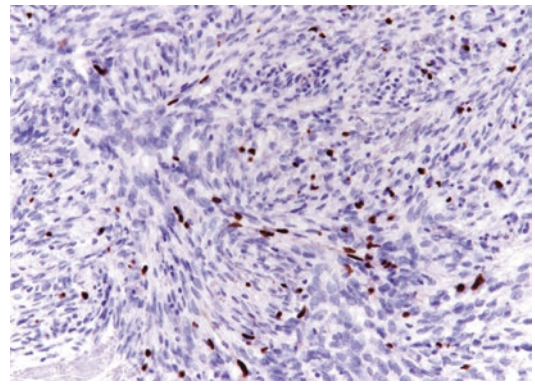

Fig. 27.4 Malignant peripheral nerve sheath tumor with focal nuclear Sox-10 expression

Diagnostic Pitfall PGP 9.5 has a low specificity and is found to be expressed in a number of non-neuronal tumors [1].

27.1.4 Sox-10

Sox-10 is a neural crest transcription factor involved in the maturation and differentiation of melanocytes, myoepithelial cells, and Schwann cells (see Chaps. 6 and 22). Sox-10 is normally expressed in melanocytes, Schwann cells, and myoepithelial cells. Besides melanocytic tumors, Sox-10 stains also schwannomas, neurofibromas (Fig. 27.2), granular cell tumors (Fig. 27.3), clear cell sarcoma, and myoepithelial tumors and is found in up to 60% of malignant peripheral nerve sheath tumors (Fig. 27.4) [2, 3].

27.1.5 Claudin-1

Claudin-1 is a member of the Claudin family of integral transmembrane proteins, listed in a previous chapter (see Chap. 23). Claudin-1 labels the perineural cells and is found in up to 90% of intestinal and up to 30% of soft tissue perineuriomas.

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, D2-40	EMA, CD56, calretinin
Neurolemmoma (schwannoma)	**S100**, **calretinin**, **Sox-10**, D2-40, collagen IV	CD56, leu7 (CD57), CK1, NGFR (gp75), TTF-1, TLE1, bcl-2	GFAP, CD34 (in Antoni B areas)	Neurofilaments, CK5/CK6, CK7, CK, CK18, CK20
Melanotic schwannoma	**S100**, **Sox-10**, collagen IV, HMB-45, Melan-A, tyrosinase			GFAP, EMA, pan-CK
Solitary circumscribed neuroma	**S100**, **Sox-10**			GFAP, EMA, pan-CK
Perineurioma	Claudin-1, Glut-1, EMA, collagen-IV	CD56		CD34, CD117, S100, Sox-10, actin, Desmin, GFAP
Paraganglioma	*Chief cells:* NSE, CD56, synaptophysin *Sustentacular cells:* CD56, S100 (in benign paraganglioma)	PGP 9.5, chromogranin, VIP, serotonin, somatostatin, bombesin, GATA-3 GFAP, S100 (in malignant paraganglioma)	GFAP, pan-CK[a]	S100, EMA Synaptophysin
Neurothekeoma (dermal nerve sheath 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	Inhibin, calretinin, PGP 9.5, CD68	CD56, TFE-3	Pan-CK, GFAP, neurofilaments, EMA, Desmin, HMB45
Pigmented (melanotic) neuroectodermal tumor of infancy	Pan-CK, HMB45	Sox-10, NSE	Synaptophysin	
Malignant nerve sheath tumor	Myelin basic protein, PGP 9.5 Proliferation index (Ki-67): 5–38% (main ~18%)	Sox-10, CD57 (leu7), NGFR (gp75), CD99, S100, SOX-2, bcl-2, c-MET	CD34, GFAP, SATB-2, EMA, EGFR, ALK, bcl-2, CD56, Desmin, CDX-2, p16 [4]	Pan-CK, HMB45, Melan A, ERG, actin

[a] Characteristic for paraganglioma arising in the cauda equine with a perinuclear pattern

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Markers and Immunoprofile of Central Nervous System Tumors

28

Contents

The modern classification of primary tumors of the central nervous system in the fifth edition of the WHO classification is based on histopathological appearance, anatomical localization, and immunophenotype besides the molecular and genetic alterations, which play a very important role in the new classification determining new genetically defined tumor subgroups. Some genetic anomalies can be detected by immunohistochemistry using specific antibodies to different mutated or normal proteins such as $BRAF_{V600E}$, $IDH1_{R132H}$, INI-1, and p53. The most commonly used immunohistochemical markers and the immunoprofile of the most common central nervous system tumors are listed in this chapter. For genetically defined subtypes, genetic analysis, including DNA methylation profiling by molecular methods, is essential.

28.1 Diagnostic Antibody Panel for Glial Tumors

GFAP, ATRX, Leu-7, MAP 2, NeuN, Olig-2, Neurofilaments, Synaptophysin, Pan-Cytokeratin, CD34, IDH1, Ki-67.

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https://doi.org/10.1007/978-3-031-45024-2_28

28.2 Therapy-Related Markers

IDH1 $_{R132H}$, Ki-67.

28.2.1 Glial Fibrillary Acidic Protein (GFAP)

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 – Neurolemmoma, neurothekeoma – MPNST	Salivary gland tumors (myoepithelial tumors, basal cell adenoma/carcinoma, pleomorphic adenoma), neuroblastoma, osteosarcoma, chondrosarcoma	Astrocytes, a subset of CNS ependymal cells, cells of choroid plexus, Schwann's 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 marker of neoplastic glial cells and glial differentiation (Figs. 28.1 and 28.2). A lower GFAP expression level is also found in neurilemoma and neuroblastoma.

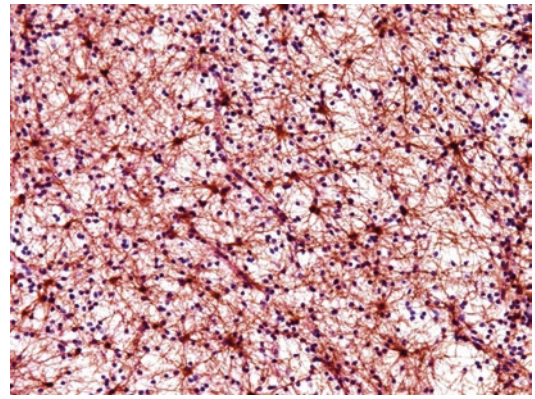

Fig. 28.1 Glioma grade II with strong GFAP expression in the tumor cells

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 components of different types of salivary gland tumors, osteosarcoma, chondrosarcoma, and angiosarcoma.

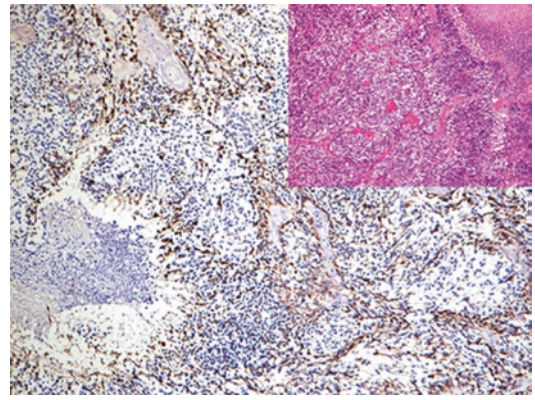

Fig. 28.2 Glioblastoma with various GFAP expression intensities

28.2.2 Microtubule-Associated Protein 2 (MAP 2)

MAP 2 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. MAP 2 labels

the cytoplasm of the neuronal cell body and basal dendrites and is considered an early marker for neuronal differentiation. In immunohistochemistry, MAP 2 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).

28.2.3 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.

28.2.4 Oligodendrocyte Lineage Transcription Factor 2 (Olig-2)

Olig-2 is a transcription factor involved in the regulation of neuroectodermal progenitor cells and the development and differentiation of oligodendrocytes and motoneurons. In normal brain tissue, Olig-2 is strongly expressed in oligodendroglial cells and oligodendroglioma derived from these cells. Olig-2 is not a reliable marker to distinguish oligodendroglioma from other gliomatous tumors, as the expression of Olig-2 is also found in different intensity levels in all other gliomas, including glioblastoma. Olig-2 expression is also reported in a small subset of neuroendocrine carcinomas and in central neurocytoma in addition to supratentorial ependymoma. Olig-2 is also found to be positive in rhabdomyosarcomas bearing the PAX3/7-FOXO1 fusion.

28.2.5 Alpha-Thalassemia/Mental Retardation Syndrome X-Linked (ATRX)

ATRX, also known as ATP-dependent helicase II, is a nuclear chromatin remodeling protein encoded by the ATRX gene on chromosome X and expressed in most normal tissue types. Initially, the ATRX gene was discovered in patients with the x-linked mental retardation syndrome (ATRX syndrome). In diagnostic histopathology, ATRX mutations with the loss of ATRX nuclear expression are detected in pancreatic neuroendocrine tumors (NET G1,2,3) and different types of high-grade sarcoma.

ATRX loss of function is also described in different gliomas due to the deletion or inactivation of ATRX gene by different mutations. Mutations and deletion of the ATRX gene are a marker for grade II–III gliomas and detected in ~60% of diffuse astrocytomas (grade II), ~ 50% of anaplastic astrocytomas (grade III), and ~10% of anaplastic oligodendrogliomas (grade III). In grade II/III astrocytomas, ATRX mutations are mostly associated with IDH1/IDH2 (isocitrate dehydrogenase) mutations (>90%). ATRX loss and 1p/19q codeletion are almost mutually exclusive. Mutations or deletions of the ATRX gene can be detected by immunohistochemistry. Tumors bearing ATRX mutations or ATRX gene deletion lack the nuclear ATRX stain in >90% of tumor cells, whereas the nuclei of nonneoplastic cells, including microglia, reactive astrocytes, lymphocytes, and endothelial cells, remain strongly positive.

28.2.6 IDH

The isocitrate dehydrogenase (IDH) family of enzymes is a group of metabolic enzymes catalyzing the reversible oxidative decarboxylation of isocitrate to α-ketoglutarate. It includes three isoforms, IDH1 located in the cytoplasm and IDH2 and IDH3 located in the mitochondria and

encoded by different genes on different chromosomes. Mutations in the IDH1 and IDH2 genes have been identified in multiple tumor types, including gliomas, acute myeloid leukemia, myelodysplastic syndrome, cholangiocarcinoma, and chondrosarcoma. The R132H mutation within the IDH1 gene is the most common in diffuse gliomas and accounts for ~90% of all IDH mutations that cause the conversion of arginine to histidine in the amino acid sequence of this enzyme. The IDH1 R132H mutation can be detected by immunohistochemistry using a specific antibody. A strong cytoplasmic immunoreaction correlates with the IDH1-R132H mutation (Fig. 28.3), whereas a weak diffuse staining fevers a wild type. Macrophages are used as a positive internal control. In immunohistochemically negative cases, further molecular sequencing is recommended to exclude other IDH mutations. The detection of the IDH1 R132H mutation can be helpful in distinguishing low-grade glioma from reactive gliosis or other tumors with wild-type IDH. In gliomas, the presence of IDH mutations is usually associated with a favorable prognosis.

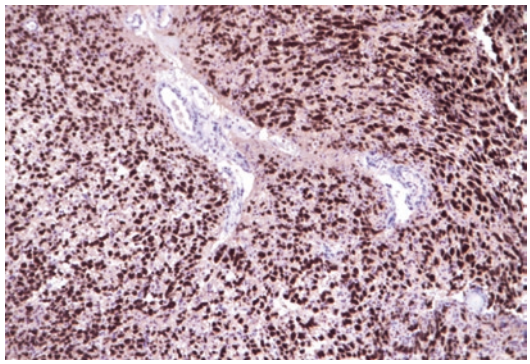

Fig. 28.3 IDH1-R132H immunostaining in astrocytoma Grade III, IDH mutant with strong cytoplasmic and nuclear IDH1 R132H stain

28.3 Diagnostic Antibody Panel for Choroid Plexus Tumors

Cytokeratin profile, Podoplanin (D2-40), Stanniocalcin-1, Kir7.1.

28.3.1 Kir7.1

Kir7.1 is a member of the inwardly rectifying potassium channel family of proteins encoded by the KCNJ13 gene. Kir7.1 is expressed in gastric and small intestine mucosa in addition to renal tubules and choroid plexus. In the central nervous system, Kir7.1 is a marker for the tumors of the choroid plexus.

28.3.2 Podoplanin

Podoplanin (D2 40) is a type I transmembrane mucoprotein listed in detail in a previous chapter (see Chap. 25). Podoplanin is strongly expressed on the epithelial cells of choroid plexus and choroid plexus tumors in addition to meningothelial cells and different meningioma types (see meningioma markers below) (Fig. 28.4).

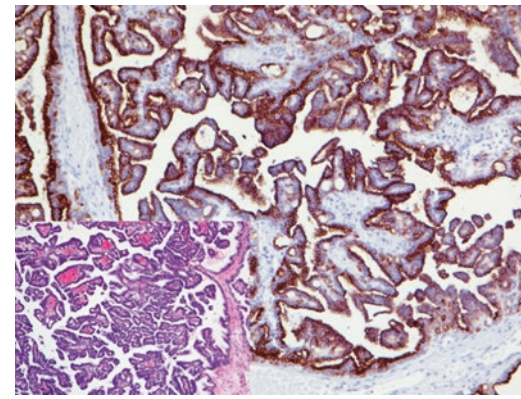

Fig. 28.4 Choroid plexus papilloma with strong Podoplanin expression

28.4 Diagnostic Antibody Panel for Tumors of the Pineal Region

Synaptophysin, Chromogranin, Neurofilaments, PGP9.5, Serotonin, NSE, β-tubulin.

These markers were listed in previous chapters.

28.5 Diagnostic Antibody Panel for Embryonal Tumors

INSM-1, CD56, Nestin, Synaptophysin, Chromogranin, OTX-2, PGP9.5, β-tubulin, MAP 2, NSE, Neurofilaments, GFAP.

28.5.1 Orthodenticle Homeobox 2 (OTX-2)

OTX-2 is a transcription factor involved in the early differentiation of the brain, craniofacial and sensory organs including the pineal tissue, pituitary gland, inner part of the ear and eyes, and optic nerve. OTX-2 is expressed in ~65% of medulloblastomas.

All other markers were described in previous chapters (Fig. 28.5).

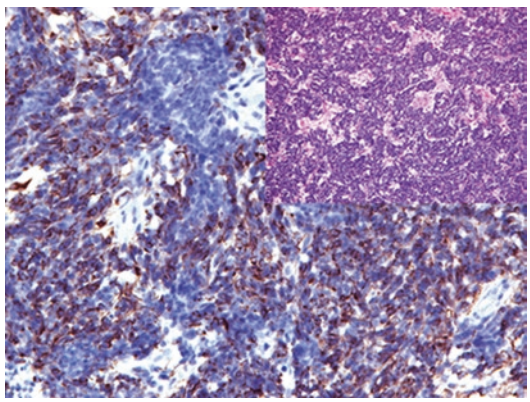

Fig. 28.5 Expression of neurofilaments in medulloblastoma

28.6 Diagnostic Antibody Panel for Meningeal Tumors

E-cadherin, Pan-Cytokeratin, Podoplanin (D2 40), EMA, Somatostatin receptor 2a (SSTR2a), S100, CEA, Progesterone receptor, Vimentin, Nestin, Ki-67.

Meningiomas are a group of tumors originating from meningothelial cells of the arachnoid layer. Characteristic for meningeal tumors is the co-expression of EMA, SSTR2, and E-cadherin in addition to Pan-Cytokeratin progesterone receptor and S100 (Figs. 28.6, 28.7, 28.8 and 28.9). The co-expression of Podoplanin (D2 40), SSRT2, and E-cadherin is also characteristic and more specific for meningeal tumors and helpful to confirm the diagnosis and to discriminate between aggressive meningeal tumor types such as atypical/anaplastic meningioma and other mesenchymal tumors, e.g., solitary fibrous tumor or different metastatic sarcomas (Figs. 28.8 and 28.9). To assess the tumor grade, the estimation of the proliferation (Ki-67) index and the mitotic index is essential. The intensity of progesterone receptor expression inversely correlates with the grade of tumor anaplasia. The CEA expression is characteristic for the pseudopsammoma bodies found in secretory meningioma (Fig. 28.10).

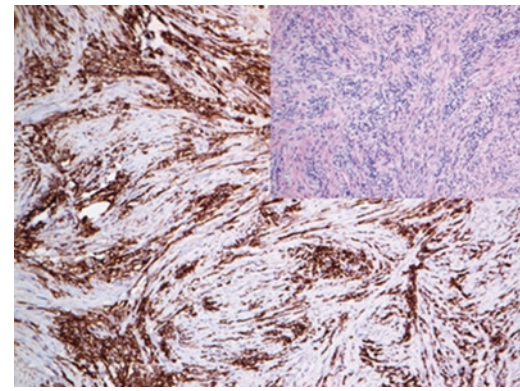

Fig. 28.6 Strong Podoplanin (D2-40) expression in anaplastic meningioma

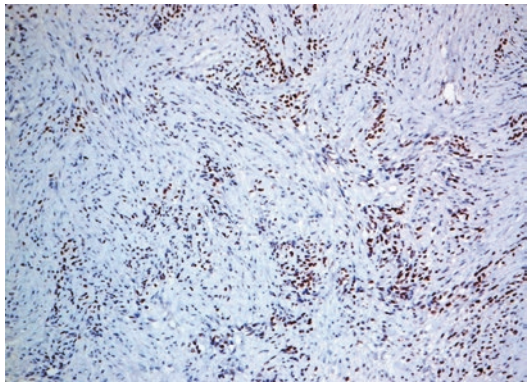

Fig. 28.7 Meningioma with strong nuclear expression of progesterone receptors in tumor cells

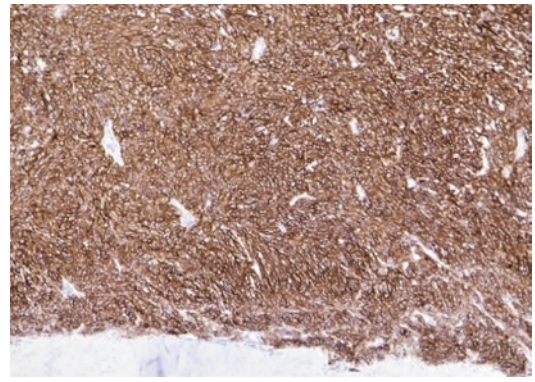

Fig. 28.9 Strong membranous SSRT2 expression in meningioma cells

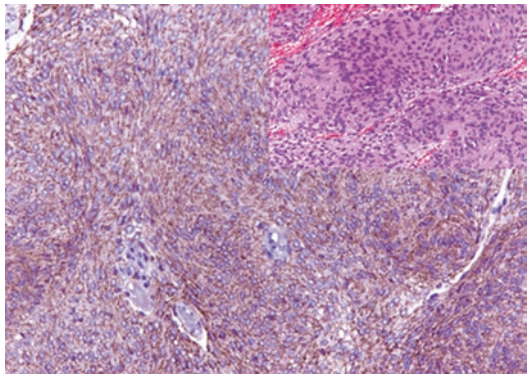

Fig. 28.8 Meningioma with strong membranous E-cadherin expression in the tumor cells

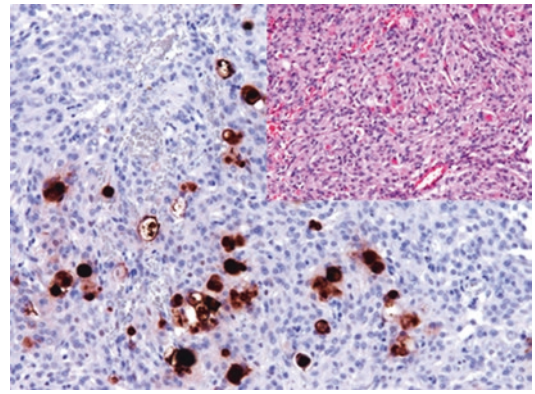

Fig. 28.10 Secretory meningioma with CEA-positive pseudopsammoma bodies

Immunoprofile of central nervous system tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
A. Gliomas				
Astrocytoma, IDH-mutant – Grade II – Grade III – Grade IV	GFAP, Olig-2, S100, vimentin Proliferation index (Ki-67): GII: <4%; GIII: 5–15%	CD56, CD99, ATRX, IDH-1 (R132H), HER-2	P53, synaptophysin	ChromograninCK7, CK20, neurofilament
Oligodendroglioma, IDH-mutant and 1p/19q-codeleted – Grade 2 – Grade 3	Olig-2, synaptophysin, MAP-2, ATRX, IDH-1 (R132H), Sox-10, S100 Proliferation index (Ki-67): GII: <5%; GIII: >10%			Pan-CK, Vimentin[a], EMA
Glioblastoma, IDH-wild type (grade 4)	GFAP, Olig-2, S100, vimentin Proliferation index (Ki-67): >15% (5–40%)	CD56, CD99, HER-2	Synaptophysin pan-CK, ATRX, IDH-1 (R132H)	IDH-1 (R132H), chromogranin CK7, CK20, neurofilaments
Diffuse astrocytoma MYB or MYBL-altered	GFAP, ATRX			Olig-2, CD34
Angiocentric glioma (grade I)	GFAP, S100 Proliferation index (Ki-67): <5%	TTF-1	EMA	Olig-2, synaptophysin, chromogranin
Polymorphous low-grade neuroepithelial tumor of the young	GFAP, Olig-2, ATRX Proliferation index (Ki-67): <2%	CD34	$BRAF\text{-}_{V600E}$	EMA, IDH-1 (R132H), synaptophysin, chromogranin
Diffuse low-grade glioma, MAPK pathway-altered	Olig-2	GFAP	CD34	IDH-1 (R132H)
Diffuse midline glioma, H3 K27-altered (grade IV)	CD56, Olig-2, MAP2, S100,	GFAP, H3K27M, p53	Synaptophysin	Chromogranin
Diffuse hemispheric glioma, H3 G34-mutant (grade IV)	MAP-2, FOXG1, p53	GFAP		ATRX, Olig-2
Diffuse pediatric-type high-grade glioma, H3-wild type and IDH-wild type (grade IV)		GFAP, Olig-2		
Infant-type hemispheric glioma		GFAP	ALK	
Pilocytic astrocytoma (grade I)	GFAP, Olig-2, MAP-2, SOX-10, S100 Proliferation index (Ki-67): <5%	CD56, CD99, p16, HER-2	Synaptophysin	Chromogranin pan-CK, IDH-1 (R132H), neurofilament
High-grade astrocytoma with piloid features			ATRX	

Immunoprofile of central nervous system tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Pleomorphic xanthoastrocytoma (grade II–III)	GFAP, S100, ATRX Proliferation index (Ki-67): Grade II <1%; grade III < 10%	Synaptophysin, neurofilaments, CD34, MAP2, BRAF-$_{V600E}$		
Subependymal giant cell astrocytoma (grade I)	GFAP, S100, Olig-2, TTF-1			CD34
Chordoid glioma (grade II)	GFAP, TTF-1, CD34 Proliferation index (Ki-67): <2%	S100	EMA	
Astroblastoma, MN1-altered	GFAP, S100, Podoplanin (D2-40)	Olig-2, EMA		
B. Glioneuronal and neuronal tumors				
Ganglioglioma/gangliocytoma (grade I)	*Neuronal/ganglion cells*: Neurofilaments, synaptophysin, MAP2 *Astrocytic cells:* GFAP, Olig-2, S100 Proliferation index (Ki-67): <5%	CD34	S100	GFAP, pan-CK
Desmoplastic infantile ganglioglioma/desmoplastic infantile astrocytoma (grade I)	*Leptomeningeal component:* Vimentin *Poorly differentiated neuroepithelial component:* GFAP, MAP2, synaptophysin, neurofilaments Proliferation index (Ki-67): 0.5–5%	*Leptomeningeal component:* GFAP	Actin	
Dysembryoplastic neuroepithelial tumor (grade I)	*Oligodendroglia like cells:* S100, Olig-2, PDGFRA Proliferation index (Ki-67): <8%	Neurofilaments, MAP2, β-tubulin	Synaptophysin BRAF-$_{V600E}$, GFAP	IDH-1 (R132H)
Diffuse glioneuronal tumor with oligodendroglioma-like features and nuclear clusters	Olig-2		MAP2	GFAP
Papillary glioneuronal tumor (grade I)	*Perivascular cells:* Pan-CK, GFAP, S100 *Neuronal cell component:* Synaptophysin, NeuN Proliferation index (Ki-67): <2%		Chromogranin	

Immunoprofile of central nervous system tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Rosette-forming glioneuronal tumor (grade I)	*Perivascular glial cells:* GFAP, Olig-2, S100 *Neurocytes and ganglion cells:* Synaptophysin, NeuN	MAP-2		GFAP, S100
Myxoid glioneuronal tumor (grade I)	GFAP, Olig-2, Sox-1, MAP2 Proliferation index (Ki-67): <5%			CD34
Diffuse leptomeningeal glioneuronal tumor	Olig-2, MAP2, S100	Synaptophysin, GFAP		IDH-1 (R132H)
Multinodular and vacuolating neuronal tumor (grade I)	Oli-2; doublecortin	Synaptophysin, MAP2		GFAP
Dysplastic cerebellar gangliocytoma (grade I)	Synaptophysin,			
Central neurocytoma (grade II)	Synaptophysin, NeuN Proliferation index (Ki-67): 2–4%	Calretinin	S100, TTF-1, L1CAM (CD171)	Pan-CK, GFAP ChromograninNeurofilamentOlig-2
Extraventricular neurocytoma (grade II)	Synaptophysin, NeuN Proliferation index (Ki-67): 2–3%	GFAP	Chromogranin	Pan-CK, NeurofilamentOlig-2
Cerebellar liponeurocytoma (grade II)	*Neuronal component:* Synaptophysin, MAP2, NeuN	GFAP		
C. Ependymal tumors				
Supratentorial ependymoma (grade II–III) Supratentorial ependymoma, ZFTA fusion-positive Supratentorial ependymoma, YAP1 fusion-positive Posterior fossa ependymoma Posterior fossa group A ependymoma Posterior fossa group B ependymoma Spinal ependymoma Spinal ependymoma, MYCN-amplified	Podoplanin, GFAP, CD56, S100, nestin For genetically defined tumor identities, the molecular analysis is required	EMA, [b] TTF-1	Synaptophysin Olig-2, pan-CK, CD99	Chromogranin

Immunoprofile of central nervous system tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Myxopapillary ependymoma (grade II)	GFAP, EMA, S100, Podoplanin (D2-40) Proliferation index (Ki-67): <2%	Pan-CK, CD56, CD99		Olig-2, MAP2
Subependymoma (grade I)	GFAP, EMA, ATRX Proliferation index (Ki-67): <1%	NSE, CD56, STAT-3		Olig-2
D. Choroid plexus tumors				
Choroid plexus papilloma: (grade I) Atypical choroid plexus papilloma (grade II) Choroid plexus carcinoma (grade III)	Podoplanin (D2-40), pan-CK, S100, Stanniocalcin-1 Proliferation index (Ki-67): Choroid plexus papilloma, 1–2%; atypical choroid plexus papilloma, 9% Choroid plexus carcinoma: ~8–42%	Transthyretin, S100, CK7, CD44, Kir7.1	GFAP, EMA, Synaptophysinp53	Chromogranin CD56, SOX10, CK20, CDX-2
E. Embryonal tumors				
Medulloblastomas, histologically defined	S100, INSM-1, CD56, MAP2, GAB-1, YAP-1, nestin, β-tubulin, vimentin	NSE, synaptophysin, PGP9.5, neurofilaments, PAX-8	GFAP, bcl-2, chromogranin	CD99, Sox-2, PAX-2
Medulloblastoma, WNT-activated Medulloblastoma, SHH-activated and TP53-wild type Medulloblastoma, SHH-activated and TP53-mutant Medulloblastoma, non-WNT/non-SHH	For genetically defined tumor identities, the molecular analysis is required			
Atypical teratoid/rhabdoid tumor (grade IV)	EMA, vimentin	GFAP, sm-actin, neurofilaments, pan-CK		INI-1, Desmin, AFP, PLAP
Cribriform neuroepithelial tumor	EMA	Tyrosinase, MAP2, synaptophysin, vimentin	GFAP	INI-1
Embryonal tumor with multilayered rosettes (grade IV)	*Neuroepithelial cells:* Nestin, vimentin *Neuropil-like areas:* Synaptophysin, NeuN, neurofilaments		Pan-CK, EMA, CD99	

Immunoprofile of central nervous system tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
CNS neuroblastoma, FOXR2 activated (grade IV)	Olig-2, S100, NSE, β-tubulin	Neurofilaments	Synaptophysin	GFAP
CNS tumor with BCOR internal tandem duplication	CD56,		GFAP; Olig-2	
CNS embryonal tumor NEC/NOS		Olig-2, synaptophysin		GFAP, vimentin
F. Pineal tumors				
Pineocytoma (grade I) Pineoblastoma (grade IV)	Synaptophysin, neurofilaments, NSE	β-Tubulin, PGP9.5, chromogranin, serotonin	S100	GFAP, pan-CK
Pineal parenchymal tumor of intermediate differentiation (grade II–III)	Synaptophysin, NSE	Neurofilaments, chromogranin, INSM-1, S100		
Papillary tumor of the pineal region (grade II–III)	Pan-CK, NSE, S100, MAP2, CD56, vimentin	EMA	Synaptophysin, chromogranin, EMA, GFAP	Stanniocalcin-1, Kir7.1, neurofilaments
Desmoplastic myxoid tumor of the pineal region, SMARCB1-mutant		CD34, EMA		INI-1
G. Mesenchymal tumors				
Solitary fibrous tumor	Vimentin, CD34, STAT-6, F XIIIa	bcl-2, CD99, glutamate receptor-2		
Hemangioblastoma (grade I)	*Endothelial cells:* CD31, CD34, ERG *Stromal cells:* CD56, inhibin, D2 40	*Stromal cells:* S100, NSE		*Stromal cells:* PAX-8, CD31, ERG, pan-CK
Primary intracranial sarcoma, DICER1-mutant		Desmin, actin	Myogenin, ATRX	GFAP, Olig-2
Ewing sarcoma CIC-rearranged sarcoma	See Chap. 29			
H. Meningeal tumors				

Immunoprofile of central nervous system tumors				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Meningioma (intra- and extracranial):	S100, Podoplanin, E-cadherin, SSTR2A, EMA, vimentin *Proliferation index (Ki-67)* (a) Meningioma (grade I): <4% (b) Atypical meningioma (grade II): 7–10% (c) Anaplastic meningioma (grade III): >10% *Progesterone receptor expression* (d) Meningioma (grade I): ~ 60–90% (e) Atypical meningioma (grade II): ~20–40% (f) Anaplastic meningioma (grade III): <20%	Nestin, Claudin-1, NSE, CD141, pan-CK, CK8/CK18, CD99, PgR, CEA, ERG	Osteonectin, CD34, CK7, bcl-2	GFAP, Sox-10, STAT-6, synaptophysin, chromogranin CD56, CK5/CK6, CK20, neurofilament

[a] See Fig. 28.11

[b] Cytoplasmic paranuclear dot expression pattern

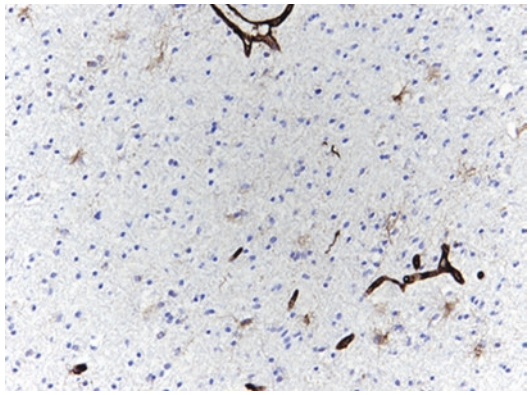

Fig. 28.11 Vimentin stain, oligodendroglioma cells lacking the expression of Vimentin

29 Markers and Immunoprofile of Ewing Sarcoma/Primitive Neuroectodermal Tumors (PNET) and Ewing-Like Sarcoma Tumors

Contents

29.1 Diagnostic Antibody Panel for Ewing/Primitive Neuroectodermal Tumors

CD99, Fli-1, NKX2.2, PAX-7, CD56, Chromogranin, Synaptophysin, WT-1, BCOR, ETV4, SATB-2.

29.2 Ewing Sarcoma

Ewing sarcoma and the primitive neuroectodermal tumor are small round blue cell tumors arising from a mesenchymal stem cell and harbor one of the specific translocations fusing a member of the RNA binding TET gene family—mostly EWSR1 or FUS gene—to a member of the ETS transcription gene family (Fli-1, ERG, ETV1, ETV4, or FEV). The t(11;22)(q24;q12) translocation generating the EWSR1-Fli-1 gene transcript is the most common translocation type found in about 90% of Ewing/primitive neuroectodermal tumors. The strong membranous expression CD99 is a characteristic immunoprofile for this tumor group.

29.3 Round Cell Sarcoma with EWSR1-Non-EST Fusions

This is a newly described tumor group that shares the small round blue cell morphology but lacks the Ewing sarcoma characteristic translocations.

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https://doi.org/10.1007/978-3-031-45024-2_29

This tumor group harbors a translocation between the EWSR1 gene and a second gene other than a member of the ETS transcription gene family such as NFATC2, PATZ1, SP3, and SMARCA5 genes [1]. These tumors have a different immunohistochemical profile than Ewing sarcoma and frequently lack the characteristic strong membranous CD99 expression but show the coexpression of myogenic and neurogenic markers. Tumors associated with EWSR1-NFATC2 translocation were reported to be positive for NKX3.1 as a characteristic marker but lack other adenocarcinoma markers [2].

29.4 CIC Rearranged Sarcoma

The CIC rearranged sarcoma is a further recently described Ewing-like sarcoma tumor group sharing the small round cell morphology but harbors different genetic abnormalities involving the CIC gene (Capicua transcriptional repressor) located on 19q13.2 and other gene partners, mainly the DUX4 gene located on 10q26 or 4q35. Other rearrangement partners are also rarely described, such as FOXO4, LEUTX, NUTM1, and NUTM2A. This tumor group shows a different immunohistochemical profile than Ewing sarcoma, frequently lacks the expression of CD99, and is usually positive for WT-1 and ETV4. Clinically this tumor is more aggressive than Ewing sarcoma and shows a poor response to the standard Ewing sarcoma regiments [3, 4].

29.5 Sarcoma with BCOR Genetic Alterations

Characteristic for this sarcoma group are genetic alterations involving the BCOR gene (bcl-6 interacting corepressor) located on Xp11.4 and other partner genes, including CCNB3, ZC3H7B, ITD, and MALM3. This tumor group also exhibits a different immunohistochemical profile but in ~50% of the cases shows the expression of CD99 and usually exhibits a positive immunohistochemical reaction with the antibodies to BCOR and SATB-2 [5, 6].

29.5.1 CD99

CD99 (MIC2)		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Ewing sarcoma/PNET – T and B ALL – Solitary fibrous tumor	T cell lymphoma; anaplastic large cell lymphoma; AML; GIST; various carcinomas including breast, prostate, and hepatocellular carcinoma; thymoma; ovarian and testicular 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		

Diagnostic Approach CD99 (known as MIC2 or E2 antigen) is a single chain type I cell surface glycoprotein expressed on the surface of cortical thymocytes and a subset of mature T and B lymphocytes. CD99 plays a role in T cell adhesion, leukocyte migration, and extravasation. CD99 has a broad expression spectrum and is found in a large number of normal and neoplastic cells. CD99 is widely used as a diagnostic marker for Ewing sarcoma/PNET tumor family. For this tumor group, characteristic is the membranous CD99 stain, while a cytoplasmic can be noted in other tumor types. A dot-like paranuclear stain is described in a subset of the Ewing sarcoma/

PNET tumors and also in other tumor types, such as Merkel cell carcinoma. CD99 is rarely expressed in Ewing-like sarcoma small round cell tumors and is negative in neuroblastoma (Fig. 29.1).

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 [7, 8]. A panel of more specific antibodies must always be used to confirm the diagnosis.

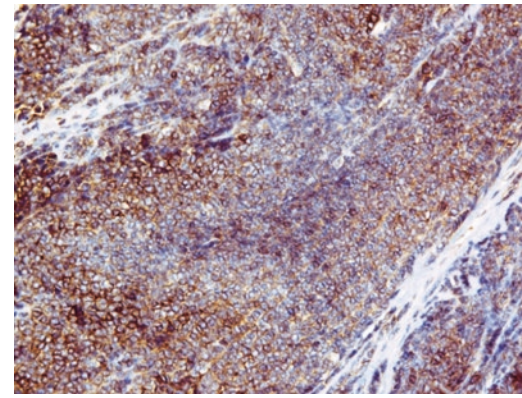

Fig. 29.1 Ewing sarcoma with strong membranous CD99 expression

29.5.2 Fli-1

Fli-1		
Expression pattern: nuclear		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Ewing sarcoma – Vascular tumors	Lymphoblastic lymphoma, anaplastic large cell lymphoma, angioimmunoblastic lymphoma, juvenile granulosa cell tumor, Merkel cell carcinoma, squamous cell carcinoma, melanoma, atypical fibroxanthoma, desmoplastic small round cell tumor, synovial sarcoma	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 ETS family functioning as a transcriptional activator highly expressed during embryogenesis. The Fli-1 gene is the translocation partner of the EWSR1 gene in the t(11;22)(q24;q12) translocation, the most common and most specific molecular marker for Ewing sarcoma/PNET family that is found in about 90% of the cases. Available antibodies to the Fli-1 gene product were found to be of high specificity for the PNET family (Fig. 29.2).

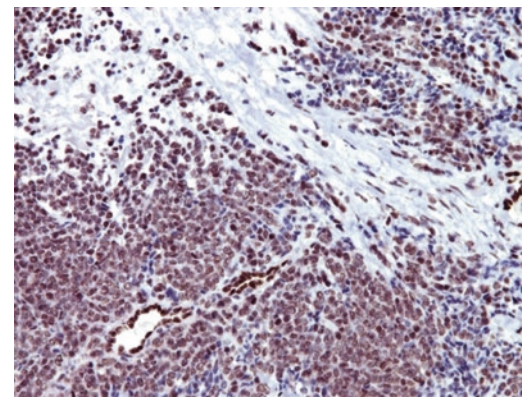

Fig. 29.2 Ewing sarcoma showing strong nuclear Fli-1 expression. FLI-1 also labels the endothelial cells

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 melanomas, mainly aggressive types, in addition to Merkel cell carcinoma [9, 10]. 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 the correct diagnosis [11].

29.5.3 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 to Ewing sarcoma. The expression of NKX2.2 was reported in more than 80% of Ewing sarcoma/PNET family (Fig. 29.3) [12–14]. NKX2.2 is normally expressed in pancreas islet cells and intestinal endocrine cells, as well as in the majority of neuroendocrine tumors of gastrointestinal and pancreatic origin.

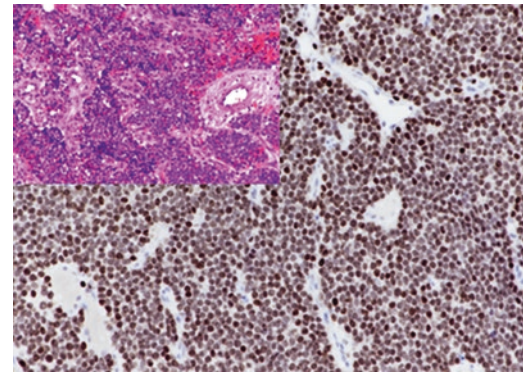

Fig. 29.3 Ewing sarcoma with strong nuclear NKX2.2 expression

29.5.4 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 a previous chapter as a marker for adrenocortical tumors. Due to the genetic alterations caused by the EWS/Fli-1 translocation that induce the expression of DAX-1, DAX-1 is overexpressed in Ewing sarcomas bearing this translocation (Fig. 29.4) [15, 16].

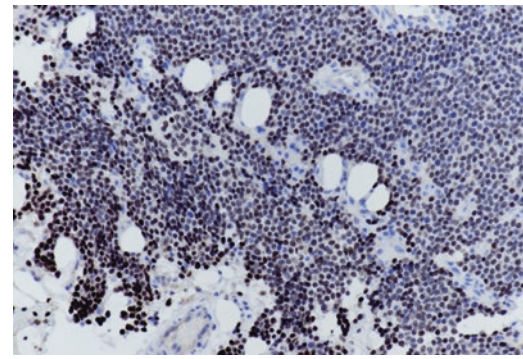

Fig. 29.4 EWSR1-Fli-1 translocation associated Ewing sarcoma with nuclear DAX-1 expression

Immunoprofile of primitive neuroectodermal tumors and related lesions				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Ewing sarcoma	**CD99**, **NKX2.2**, PAX-7, vimentin	**Fli-1**, vimentin	NSE, pan-CK, CD117	Synaptophysin, CD56, **WT-1**, **SATB-2**
PNET	**CD99**, **NKX2.2**, PAX-7, vimentin	**Fli-1**, NSE, CD56, S100, synaptophysin	Pan-CK, chromogranin, ALK	WT-1
Immunoprofile of Ewing-like sarcoma tumors				
Round cell sarcoma with EWSR1-non-ETS fusions	**NKX3.1**	CD99, Desmin, Myogenin, NKX2.2, MyoD1, Sox-10, GFAP, S100p, WT-1		Pan-CK
Sarcoma with NFATc2 rearrangement	**CD99**, **NKX2.2**		NKX3.1	NUT, S100
Sarcoma with BCOR genetic alterations	**BCOR**, **CCNB3**, CD56, cyclin B3, cyclin D1,	CD117, **SATB-2**, TLE-1	CD99, NKX2.2, SATB-2	Pan-CK, Sox-10, Desmin, NUT, MyoD1, **WT-1**
CIC rearranged sarcoma	**ETV4**, **DUX4**, **NUT**	**WT-1**, CD99		Pan-CK, Desmin, MyoD1, NKX2.2, **SATB-2**

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30 Markers and Immunoprofile of Extraskeletal Osseous and Cartilaginous Tumors

Contents

30.1 Diagnostic Antibody Panel for Osseous and Cartilaginous Tumors

S100, Osteocalcin, Osteonectin, Androgen receptors, SATB-2, Sox-9, Pan-Cytokeratin [1, 2].

30.1.1 Osteocalcin

Osteocalcin is a non-collagenous calcium-binding protein (also known as bone gamma-carboxyglutamic acid-containing 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.

30.1.2 Osteonectin

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, a subset of epithelial cells
Positive control: bone tissue		

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Diagnostic Approach Osteonectin (also known as basement-membrane protein 40) 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 specificity for bone tissue and bone tumors and must be a part of antibody panel.

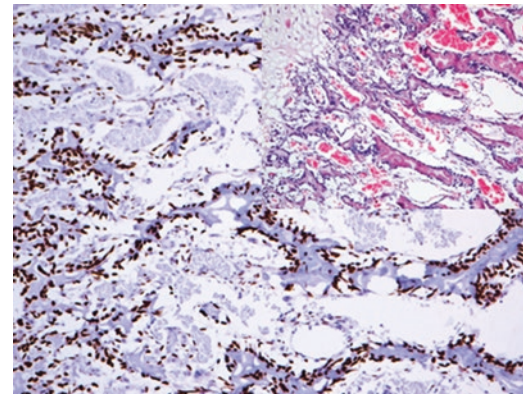

Fig. 30.1 Section of fetal bone showing osteoblasts exhibiting strong SATB-2 expression

30.1.3 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 the osteoblasts (Fig. 30.1), brain, liver, kidney, and colorectal epithelium (see also Chap. 7). SATB-2 labels neoplastic osteoblasts in both skeletal and extraskeletal osteosarcomas [3–5].

30.1.4 Sox-9

Sox-9 (*s*ex-determining region Y b*ox 9*) is a transcription factor involved in the regulation of chondrogenesis, including the differentiation of mesenchymal cells into chondrocytes. Sox-9 also regulates the differentiation of Sertoli cells and is also expressed in the cells of the neural crest. In diagnostic immunohistochemistry, Sox-9 is used as a marker for neoplasms with chondroid differentiation, including mesenchymal chondrosarcoma and chondroblastoma; nevertheless, the expression of Sox-9 can also be found in different types of osteosarcomas. It is also to consider that the expression of Sox-9 is also found in different thymoma types and malignant melanoma [7, 8].

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, **H3.3B**, Sox-9, vimentin	Pan-CK, CK8, CK18, CK19		
Mesenchymal chondrosarcoma	S100, Sox-9, ERG, vimentin	**NKX3.1**,[a] CD99[b], CD57	Actin	Pan-CK, EMA, Desmin, chromogranin, Osteonectin
Extraskeletal osteosarcoma	**Osteonectin**, vimentin	**SATB-2**, osteocalcin, androgen receptors, sm—actin, CD99	EMA, Desmin, CD117	S100
Small round cell sarcoma producing cartilage or bone matrix	**SATB-2**	CD99,	Pan-CK	

[a] See reference [6]
[b] CD99 is positive only in the small cell undifferentiated components

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31 Markers and Immunoprofile of Miscellaneous Tumors and Tumors of Uncertain Differentiation

Contents

31.1 Diagnostic Antibody Panel

Vimentin, Pan-Cytokeratin, Actin, Desmin, Sox-10, HMB45, S100, CD34, CD99, TLE-1, INI-1.

31.1.1 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 most characteristic for synovial sarcoma due to the tumor-specific translocation. However, the overexpression of TLE-1 is also reported in different soft tissue tumors, including endometrial stromal sarcoma, acral myxo-inflammatory fibroblastic sarcoma, solitary fibrous tumor, epithelioid sarcoma, lipoma and liposarcoma, leiomyosarcoma, neurofibroma, malignant nerve sheet tumor, chordoma, mesothelioma, BCOR rearranged sarcoma, and undifferentiated pleomorphic sarcoma. Because of the broad expression spectrum and low specificity, TLE-1 has limited diagnostic use in routine histopathology.

31.1.2 Transcription Factor-E3 (TFE-3)

TFE-3 is 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 a characteristic marker for alveolar soft part sarcoma in addition to the Xp11.2 translocation-associated renal cell carcinoma (Chap. 12) [5]. TFE-3 is also a marker for other epithelial and non-epithelial tumors, including

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solid pseudopapillary pancreatic neoplasms, granular cell tumor, and the majority of PEComas, including angiomyolipoma, clear cell sarcoma, and melanoma. In the later cases, the expression of TFE-3 is not associated with the t(X;17).

31.1.3 Brachyury

Brachyury is a member of the T box family and an embryonal 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 in addition to benign notochordal cell tumors (Fig. 31.1). Brachyury is negative in other tumors with chordoid or myxoid differentiation that mimic chordoma such as chondrosarcoma, chordoid meningioma, and clear cell and epithelioid sarcoma. The expression of Brachyury is also found in a subset of pulmonary adenocarcinoma, squamous cell carcinoma, small cell carcinoma, and subset of different germ cell tumors, including embryonal carcinoma, seminoma, and yolk sac tumor [6, 7].

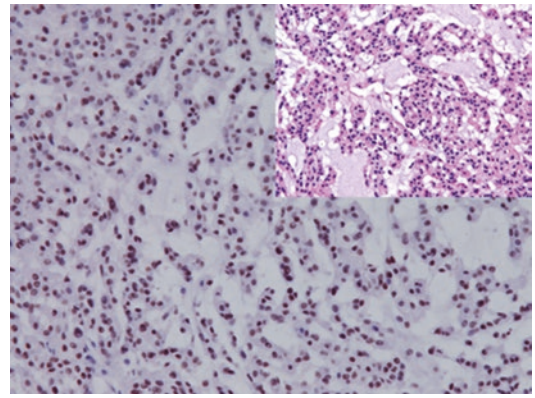

Fig. 31.1 Conventional chordoma; Brachyury highlighting the nuclei of chordoma cells

31.1.4 SMARCB-1 (INI-1)

SMARCB1 (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1) is also known as INI-1 (integrase interactor 1) or BAF47 and SNF5. INI-1 is a core subunit of the adenosine triphosphate (ATP) dependent SWI/SNF chromatin remodeling complex, encoded on 22q11.2 and constantly expressed in normal cells. INI-1 is involved in chromatin remodeling and regulation of the cell cycle. The loss of INI-1 expression occurs due to biallelic mutations or deletions within the encoding gene, which is characteristic for different tumors. The loss of INI-1 expression is distinctive for malignant rhabdoid tumor, atypical teratoid/rhabdoid tumor of the brain, epithelioid sarcoma, SMARCB-1-deficient renal cell carcinoma, and a subset of other tumors, including the following: epithelioid MPNST (~50%), myoepithelial carcinoma (~50%), parosteal osteosarcoma (~70), myxoid chondrosarcoma (~20%), intimal sarcoma, medulloblastoma, poorly differentiated and pediatric chordomas, and chorioid plexus carcinoma in addition to some other carcinoma and sarcoma types of different locations.

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], E-cadherin, 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

Immunophenotype of miscellaneous tumors and tumors of uncertain differentiation				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
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	CK8/CK18, CK19, CD34, ERG, Podoplanin	Actin, NSE, CK7, S100, CK5/CK6	INI-1, CD31, FVIII, CK20, Fli-1, ERG, CEA
Desmoplastic small round cell tumor	Pan-CK, CK8/CK18, EMA, **WT-1**, vimentin Proliferation index (Ki-67): ~30%	NSE, Desmin[c], CK19, CD15, CD56, CD57	CD99	CD34, CD117, CK5/CK6, CK20, S100, MyoD1, myoglobin, actin, h-Caldesmon
Extraskeletal myxoid chondrosarcoma	**INSM-1**, 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, SALL-4	CD34, Desmin, myoglobin, S100, INI-1
Alveolar soft part sarcoma	**TFE-3**, vimentin	Desmin	Sm-actin, S100, NSE, pan-CK, CD34	Synaptophysin, chromogranin, myoglobin, Myogenin, MyoD1, HMB45, Sox-10, EMA, CD 31, CD117, nestin
Pleomorphic hyalinizing angiectatic tumor	Vimentin	CD34	EMA	CD31, FVIII, actin, Desmin, pan-CK, S100
Myxoma (cutaneous, intramuscular, and juxtaarticular)	Vimentin, pan-CK[d]	Calretinin, CD34	Desmin, actin, CD68	S100
Myxoma of the jaw	S100, vimentin			Pan-CK, Desmin
Benign notochordal cell tumor	**Brachyury**, pan-CK	S100		
Chordoma: – Conventional – Chondroid – Poorly differentiated – Dedifferentiated	**Brachyury**[e], NSE, pan-CK, CK8/CK18, CK19[f], EMA, S100, vimentin	CK5/CK14, Sox-9, β-catenin	CK7, CEA, E-cadherin	Desmin, CK20, GFAP, D2-40, Sox-10
Aggressive angiomyxoma	Desmin, vimentin	Actin, CD34, ER	PgR	S100, pan-CK, Smoothelin
Myoepithelioma (mixed tumor of soft tissue, parachordoma)	Pan-CK, EMA, calponin, vimentin	Sox-10 S100, CK5/CK14	Desmin, sm-actin, GFAP	CK19
Angiomatoid fibrous histiocytoma	Vimentin	Desmin	CD99, EMA, CD68	CD31, CD34, MyoD1, pan-CK, Sox10, S100
Ossifying fibromyxoid tumor	Vimentin	S100, Desmin	Actin, GFAP	Pan-CK, EMA
Intimal sarcoma	**Sox-10**, S100, Osteopontin, **MDM2**, vimentin	CD56, actin	Desmin	Pan-CK, MelanA, HMB45, ERG, CD31, CD34, FVIII
NTRK-rearrangement spindle cell neoplasm	**NTRK** (pan-TRK), S100, CD34			Desmin, Sox-10

[a] Demonstration of specific t(X; 18) translocation is recommended to confirm the diagnosis
[b] Not a specific marker for synovial sarcoma
[c] Perinuclear stain
[d] Only in epithelioid components if present
[e] Lost in dedifferentiated chordomas and dedifferentiated areas
[f] Negative in parachordoma

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32 Immunohistochemistry and Biomarkers for Targeted Tumor Therapy

Contents

A large number of tumor-associated antigens are now the target for specific antitumor agents, including specific antibodies and specific inhibitors, including selective kinase inhibitors. As a morphology-based method that highlights the targets with its cellular localization, immunohistochemistry is a very useful tool that can detect many of these targets on sections from formalin-fixed paraffin-embedded tumor tissues. For many tumors with different histogenesis, several molecular targets are now established. The following list includes the most common targets that can be detected by immunohistochemistry.

- Lymphoproliferative neoplasia: CD19, CD20, CD22, CD30, CD33, ALK.
- Pulmonary non-small cell carcinoma: PD-L1, ALK, c-MET, ROS-1, NTRK, HER2.
- Breast carcinoma: ER, PR, AR, HER2, PD-L1, TROP-2, NTRK.
- Thyroid carcinoma: BRAF-$_{V600E}$, NRAS-$_{Q61R}$, TROP-2.
- Gastrointestinal carcinoma: microsatellite instability (MSI/MMRD), PD-L1, HER2, BRAF-$_{V600E}$, NRAS-$_{Q61R}$, NTRK.
- Pancreatobiliary adenocarcinoma: microsatellite instability (MSI/MMRD), PD-L1, HER-2, IDH1.

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- Carcinoma of the female genital system: ER, PR, HER-2, folate receptor alpha (FRα), microsatellite instability.
- Transitional cell carcinoma: PD-L1.
- Brain tumors: IDH1.
- Melanoma: BRAF-$_{V600E}$, NRAS-$_{Q61R}$, NTRK.
- Renal cell carcinoma (ALK rearrangement RCC): ALK.

The majority of the abovementioned antigens were listed in previous related chapters. In this chapter, PD-L1, BRAF, RAS, NTRK, and the mismatch repair proteins are listed as additional biomarkers for the assessment of personalized tumor therapy.

32.1 Mismatch Repair Proteins and Assessment of Microsatellite Instability (MSI)

DNA mismatch repair is a highly conserved biological pathway that plays a key role in maintaining genomic stability and preventing mutations from becoming permanent in dividing cells [1]. Microsatellites are short and tandemly repeated simple DNA sequences composed of 1–8 nucleotide bases scattered throughout the coding and noncoding human genome that can be up to 100 times repeated and consequently are liable for errors during the DNA replication due to endogenous or exogenous toxic agents. Two types of DNA mismatches are described within the microsatellites and include base/base mismatches and replication errors with deletion or insertion (indel). The mismatched DNA sequences can be identified and corrected by the mismatch repair protein orchestra, in mammalians including the MSH2, MSH3, MSH6, MLH1, and PMS2 proteins, as well as other proteins, including DNA polymerases and DNA ligase, exonuclease 1 (EXO1), proliferation cell nuclear antigen (PCNA), replication factor C (RFC), and regulation of replication protein A (PPA), which are encoded by different genes on different chromosomes. The MSH2, MSH3, and MSH6 proteins recognize and bind to the mismatched DNA sequence and form a heterodimeric complex, whereas the MLH1 and PMS2 proteins excise the mismatched nucleotides. The loss of one or more of the mismatch repair proteins (MMR proteins) usually occurs due to hypermethylation of the MLH1 promoter with epigenetic silencing or due to mutations within the genes encoding these proteins. The loss of these proteins leads to the accumulation of DNA replication errors in the areas of short repetitive DNA sequences, known as microsatellite instability. The microsatellite instability plays a causal role in HNPCC/Lynch syndrome and other related syndromes such as Muir-Torre syndrome, Turcot syndrome, and constitutional mismatch repair deficiency syndrome with colorectal and endometrial carcinomas and in many other sporadic malignant tumors, including skin and brain tumors. MMR protein deficiency and microsatellite instability (MSI) are detected in ~15% of all colorectal adenocarcinomas and ~40% of endometrial and ovarian endometrioid carcinoma. Colorectal adenocarcinomas with mismatch repair deficiency show distinct morphology with increased intratumoral-activated T lymphocytes due to the accumulation of mutated peptides. These carcinomas are commonly localized in the right hemicolon and usually show a good response to immune checkpoint inhibitors. Mucinous and medullary adenocarcinomas are frequently associated with microsatellite instability.

Two methods are now available for the detection of MMR protein deficiency/microsatellite instability in tumor tissue. The DNA-based molecular methods include PCR or NGS and immunohistochemistry. The molecular methods detect the changes in the DNA sequences, including insertion and deletion errors—compared with the DNA from normal tissue—caused by the loss of function of the MMR proteins. Immunohistochemistry is a good and low-cost alternative for molecular testing with high concordance based on detecting the MMR proteins in the tumor cells. The immunohistochemical reaction must be performed on well-fixed tissue, preferably preoperative biopsies. The MMR proteins show in stained sections a nuclear expression pattern which must be compared with the

expression in normal mucosal cells and stromal inflammatory cells, mainly lymphocytes, as a constant mandatory positive internal control for the precise interpretation (Fig. 32.1). A cytoplasmic or membranous staining pattern should be considered as an artifact. In routine immunohistochemistry, the four MLH1, MSH2, MSH6, and PMS2 mismatch repair proteins are the most informative targets and commonly used for the evaluation of mismatch repair deficiency in different tumor types:

32.1.1 Human Mut L Homolog 1 (MLH1)

Is a mismatch repair protein encoded by the MLH1 tumor suppressor gene located on chromosome 3. MLH1 heterodimerizes with PMS2, PMS1, or MLH3 to form MutLα, MutLβ, or MutLγ, respectively.

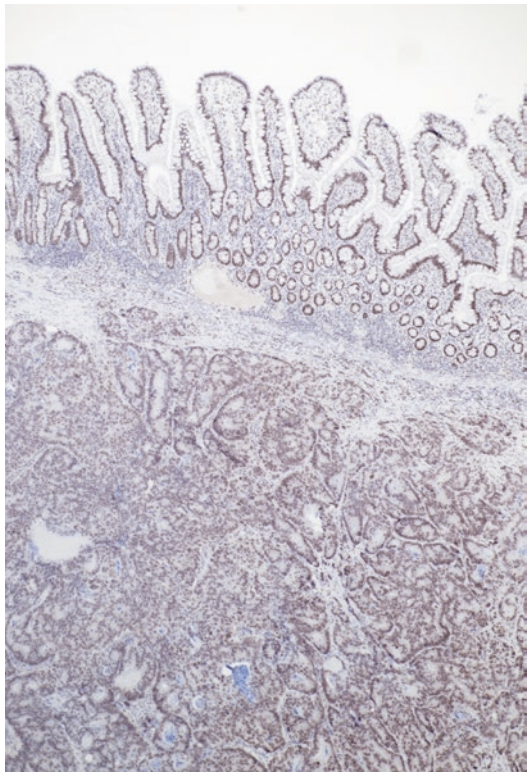

Fig. 32.1 MLH1 expression in primary adenocarcinoma of the small intestine. Nuclear MLH1 expression in normal mucosa and neoplastic cells

In examined tumor sections stained by immunohistochemistry, the loss of MLH1 is usually associated with the loss of PMS2, mainly due to MLH1 gene inactivation by hypermethylated gene promoter or mutation. The loss of MLH1 and PMS2 with the absence of the MLH1 promoter hypermethylation can be sporadic, as well as the manifestation of Lynch or related syndromes. The loss of both MMR proteins associated with hypermethylation of the MLH1 gene promoter is considered sporadic. The loss of MLH1 alone is also possible but rare and must be confirmed by more sensitive molecular methods.

In colorectal carcinomas, the combination of the loss of MLH-1 and BRAF mutation is suggestive of the sporadic nature of the neoplasia and most likely to be developed through the serrated pathway. The association between MLH1 deficiency and BRAF mutations is not a feature in gynecological carcinomas (Fig. 32.2).

32.1.2 PMS1 Homolog 2 (PMS2)

Is a mismatch repair endonuclease encoded on chromosome 7. The endonuclease activity of PMS2 causes single-strand breaks near the mismatch bases, presenting entry points for the exonuclease EXO1 to degrade the mismatched DNA sequence.

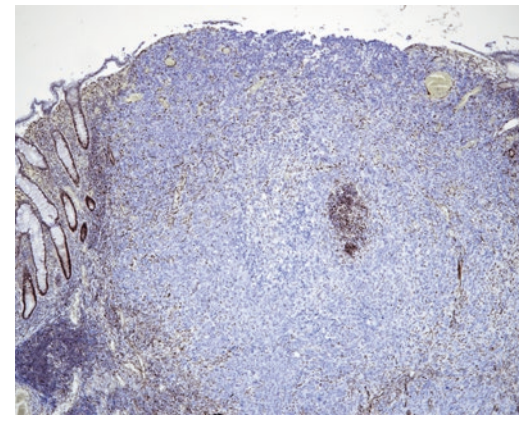

Fig. 32.2 Poorly differentiated colorectal adenocarcinoma. The tumor cells lack the expression of MLH1. Strong MLH1 expression is seen in normal mucosal and stromal cells

The loss of PMS2 is usually associated with the loss of MLH1. The isolated loss of PMS2 expression due to mutations within the PMS2 gene is also possible but less common and found in ~4% of tumors with MMR protein deficiency and can be associated with Lynch or related syndromes.

32.1.3 Human Mut S Homolog 2 (MSH2)

Is a DNA mismatch repair protein encoded on chromosome 2 and heterodimerizes with MSH6 or MSH3 to form the MutSα or MutSβ complex, respectively. These complexes recognize and bind to the mismatched dsDNA to initiate the repair of mismatched DNA.

In most cases, the loss of MSH2 occurs due to mutations within the MSH2 gene or promoter hypermethylation. Rarely, in up to 3% of the cases, the loss of MSH2 appears as a result of a germline deletion of the 3′ end of the EPCAM gene, located upstream of the MSH2 gene leading to gene silencing.

The loss of MSH2 is generally associated with the loss of MSH6 and usually appears as the manifestation of Lynch or related syndromes. The loss of MSH2 alone is rare and must be confirmed by more accurate molecular methods (Fig. 32.3).

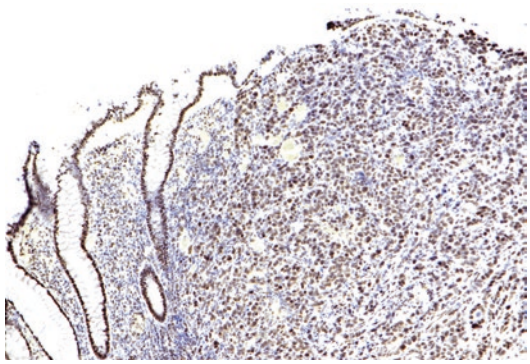

Fig. 32.3 Poorly differentiated colorectal adenocarcinoma with strong MSH2 expression in mucosal-stromal and tumor cells

32.1.4 Human Mut S Homolog 6 (MSH6)

Is a DNA mismatch repair protein encoded on chromosome 2 that heterodimerizes with MSH2 to form the MutSα complex.

In examined tumor sections, the loss of MSH2 is usually associated with the loss of MSH6; however, the loss of MSH6 alone due to mutations within the encoding gene is also common and can be associated with Lynch or other related syndromes [2].

The subclonal loss of the MMR proteins in the tumor sections can rarely be noticed in some tumor types, usually as a result of MLH1 promoter hypermethylation due to tumor progression, and must be mentioned in the final report [3]. The loss of MSH6 expression can be noted in tumors after neoadjuvant therapy causing false results.

The nuclear expression of the four proteins (MLH1, PMS2, MSH2, and MSH6) is the normal pattern indicating no evidence of mismatch repair deficiency. The loss of all MMR proteins must be considered an artifact, and the reaction must be repeated or retested by other molecular methods.

32.2 Programmed Death-Ligand 1 (PD-L1)

PD-L1 (clustered as CD274) is a member of the B7 family of cell surface ligands, a type I transmembrane protein composed of extracellular domains, transmembrane domain, and intracellular domains and expressed on activated immune cells and different tumor cells. PD-L1 is an immune checkpoint protein that plays an important role in the modulation of the immune reaction by binding to its receptor-programmed cell death protein 1 (PD-1) (see Chap. 16), expressed on activated CD4+ and CD8+ T lymphocytes, B lymphocytes, and myeloid cells. As a major immune checkpoint protein, PD-L1 mediates the antitumor immune response and eliminates the effects of the cytotoxic T lymphocytes that cause

the activation of the host immunity against tumor cells. In routine immunohistochemistry, the detection of PD-L1 in tumor tissue is widely used as an important biomarker to predict the clinical response to PD-1 and PD-L1 selective checkpoint inhibitors. The immunohistochemical detection of PD-L1 status (TPS, IC, CPS) is now required for the therapy of many malignant tumors, including squamous cell carcinoma of the head and neck, non-small cell lung carcinomas, mesothelioma, triple-negative breast carcinoma, gastrointestinal adenocarcinoma, hepatocellular carcinoma, renal cell carcinoma, and transitional cell carcinoma of the kidney and urinary bladder in addition to carcinomas of the uterine cervix [4, 5].

Different antibody clones with different specificity are now available for the immunohistochemical stain of the PD-L1 molecule. The choice of the antibody depends on the target tissue and the staining method [6]. The immunohistochemical reaction can be optimized and standardized using control tissue such as tonsillar or placental tissue and reference tumor slides. For adequate evaluation of the PD-L1 score by immunohistochemistry, a minimum amount of 100 well-preserved viable tumor cells must be present in the examined section. The heterogeneity of PD-L1 expression in different tumor parts must be considered when interpreting stained sections. To predict the response to the anti-PD-L1/PD1 checkpoint inhibitor therapy, different PD-L1 scores are required for different tumors and include the following scores:

- *Tumor proportion score (TPS)* is the percentage of viable tumor cells with partial or complete membranous PD-L1 staining of any intensity.
- *Immune cell score (IC)* is the proportion of tumor area occupied by all PD-L1-positive tumor-infiltrating immune cells (lymphocytes, macrophages, granulocytes, dendritic cells) of any staining pattern and any intensity.
- *Combined positive score (CPS)* is the amount of PD-L1-positive cells (invasive tumor cells with membranous staining in addition to lymphocytes, macrophages with any staining pattern) divided by the total number of viable tumor cells multiplied by 100.

$$\mathrm{CPS} = \frac{\text{Total amount of PD-L1 positive cells (tumor cells, lymphocytes, macrophages)}}{\text{Total amount of viable tumor cells}} \times 100$$

32.3 RAS

The Ras proteins are a group of closely related proteins that belong to the family of small G proteins with high sequence homology and overlapping functions with GTPase activity. These proteins are expressed in all mammalian cells and encoded by different genes located on different chromosomes [7, 8]. Mutations within the RAS genes cause the deregulation of the RAS-MAPK signaling pathway and uncontrolled kinase activity affecting cell proliferation and differentiation. As the RAS mutations are the most common mutations associated with human neoplasia, mutations within the encoded genes are used as therapy-related biomarkers, whereas KRAS, NRAS, and HRAS are the most common targeted biomarkers in this group.

- The KRAS gene (*K*irsten *ra*t *s*arcoma) located on chromosome 12p12.1 shows the most frequent mutation rate and driver mutations in this gene are commonly found in colorectal and pancreatic adenocarcinomas. Mutations within this gene are usually detected by molecular sequencing or NGS.
- The HRAS gene (*H*arvey *ra*t *s*arcoma) is located on chromosome 11p15.5. Mutations within this gene can be found in the urinary bladder, salivary gland, and thyroid carcino-

mas in addition to melanocytic tumors. Mutations can be detected by molecular sequencing or NGS.

– The NRAS gene (*N*euroblastoma Ras) is located on chromosome 1p13.2. Mutations within this gene are frequently found in melanomas, follicular thyroid tumors, and adrenocortical tumors. The NRAS 182 A > G mutation is one of the most common mutations found in the NRAS gene and encodes an anomalous amino acid sequence where glutamine is substituted by arginine at position 61 (NRAS-Q61R). NARAS mutations are found in 15–25% of melanomas, whereas the NRAS-Q61R mutation is found in ~35% of all NRAS-mutated radiation-induced cutaneous melanomas. RAS mutations are also described in up to 50% of thyroid tumors with follicular morphology, including follicular carcinoma and follicular variant of papillary thyroid carcinoma, in addition to ~20% of adrenocortical tumors, a subset of acute myeloid leukemia and multiple myeloma [9]. The NRAS-Q61K mutation is a further common mutation variant associated with the tumors mentioned above. Mutations within the RAS genes can be detected by molecular sequencing. In routine histopathology, the NRAS-Q61R protein can also be detected by specific antibodies with high sensitivity and specificity as a surrogate marker for this mutation.

32.4 BRAF

BRAF is a member of the RAF kinase family and a cytoplasmic serine-threonine kinase that plays an important role in the RAS-RAF-MAPK kinase signaling pathway. Different mutations within the BRAF gene are considered diagnostic, prognostic, and therapeutic biomarkers for various tumors, including melanoma and thyroid and colorectal carcinomas. BRAF is listed in detail in the chapters on thyroid and melanocytic tumors (see Chaps. 14 and 21). Only a few mutation variants of the BRAF gene, mainly $BRAF_{V600E}$, can be detected by routine immunohistochemistry using specific antibodies.

32.5 Neurotrophic Tropomyosin Receptor Kinase (NTRK)

*N*eurotrophic *t*ropomyosin *r*eceptor *k*inase (NTRK) A, B, and C are highly homologous proteins composed of extracellular, transmembrane, and intracellular domains encoded by three different genes, NTRK1, NTRK2, NTRK3, located on the chromosomes 1q, 9q, and 15q, respectively. Each TRK receptor binds to a specific member of the neurotrophin family of ligands that takes part in developing the central and peripheral nervous systems. The expression of all three TRK proteins may be activated by different genetic anomalies caused by the fusion of one of the NTRK genes to a second gene with a potent promotor, causing abnormal activation of the intracellular tyrosine-kinase domain of the TRK receptor. Nowadays, more than 80 NRTK fusion partners are described. Diagnostically important is the t(12;15)(p13;q25) translocation generating the ETV6-NTRK3 fusion transcript associated with congenital fibrosarcoma and cellular mesoblastic nephroma and secretory carcinoma of the breast and salivary glands, in addition to a subset of acute lymphoid and myeloid leukemia, mainly pediatric papillary thyroid carcinoma, gliomas, and inflammatory myofibroblastic tumor. TRK overexpression is also found in a small percentage of other different tumors, such as NSCLC, gastrointestinal and colorectal adenocarcinomas, cholangiocarcinoma, and melanoma, due to sporadic mutations in the promotor region, which can be used as targeted tumor therapy using one of the available selective TRK inhibitors [9].

The immunohistochemical detection of the TRK proteins in tumor cells is a surrogate for an NTRK gene fusion, which should be later con-

firmed by one of the molecular biology methods. In routine immunohistochemistry, a Pan-TRK antibody is used to stain the TRK molecules, which binds to all three TRK A, B, and C molecules. Tumors associated with NTRK1 or NTRK2 gene fusions usually have a cytoplasmic expression pattern, but rare perinuclear and nuclear membrane staining has been reported. Tumors harboring NTRK3 fusions show both cytoplasmic and nuclear expression pattern [10].

32.6 Anaplastic Lymphoma Kinase (ALK)

Anaplastic lymphoma kinase (ALK) is a membrane-associated receptor tyrosine kinase encoded on chromosome 2p23 and clustered as CD246 listed in previous chapters (see Chaps. 3 and 16). The ALK molecule is composed of extracellular, transmembrane, and intracellular domains playing an important role in the regulation of the cell cycle by activation of different cellular signaling pathways, including the *m*itogen-*a*ctivated *p*rotein *k*inases (MAPK; Ras and RAF), PI3K/AKT/mTOR, JAK, and STAT, which are responsible for the regulation of cell proliferation, transformation, and apoptosis. Multiple genetic mechanisms are discovered causing the abnormal activation of the ALK molecule, including translocations (anaplastic large cell lymphoma), inversions (non-small cell carcinoma), gene amplifications, and point mutations (neuroblastoma). Tumors harboring activating ALK genetic anomalies are sensitive to specific ALK tyrosine-kinase inhibitors. The expression of ALK in tumor tissue can be detected by immunohistochemistry using different specific antibodies.

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33 Markers to Assist in the Diagnosis of Dysplasia and Malignant Transformation

Contents

In routine immunohistochemistry, different markers are used to aid in the diagnosis of malignancy or malignant transformation, especially in lesions with unclear H&E histology. In this chapter, the most commonly used markers are listed, but to consider that none of these markers are an absolute marker of malignancy.

33.1 Ki-67

Ki-67 is a nonhistone nuclear protein in humans that is encoded by the MKI67 gene on chromosome 10q26.2 and is expressed in active cell cycles. The expression of Ki-67 begins in the G1 phase and persists during the active phases of the 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 (see also Chap. 16). In routine histopathology, Ki-67 is an important marker for the assessment of cell proliferation and for estimating the malignancy grade of tumors. The Ki-67 index is an important criterion for the classification of tumors (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 these cells to escape the

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cellular regulatory mechanisms. In stratified squamous epithelium, Ki-67 is expressed in the parabasal cell layer, and 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 lymphomas and gliomas.

33.2 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 (in human cells, 12 isoforms). 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 from passing through to the next phase of cell division, which can activate the transcription of different preapoptotic genes and initiate apoptosis. Normal p53 is an unstable molecule with a very short half-life (5–20 min) found in normal cells in minimal quantities.

Mutations within the Tp53 gene 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 Tp53 mutation status can be analyzed by one of the gene sequencing assays, including NGS, or by immunohistochemistry [1].

In routine immunohistochemistry, the p53 stain can show one of the following expression patterns:

- Normal wild-type pattern: The stain shows few moderately positive cells.
- Overexpression: strong nuclear expression in >50% of tumor cells. The p53 overexpression is generally considered a surrogate marker for p53 gene mutations producing stable p53 molecules.
- Complete negativity (null phenotype): The complete negative stain usually correlates with loss of function mutation/nonsense mutations.
- Subclonal pattern: heterogenous p53 expression noted in tumors with tumors associated with other mutations or with microsatellite instability (MMRD).
- Cytoplasmic pattern: abnormal cytoplasmic expression in the tumor cells besides normal nuclear expression in the stromal cells. This pattern appears as a result of the disruption of the nuclear domain due to indel and stop gain (frameshift with insertion or deletion) within the gene and can be noticed in a small portion of high-grade carcinomas.

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 and neoplastic changes, usually positive for p53 and reactive changes negative for p53.

The examples listed below demonstrate the role of p53 overexpression/complete loss of expression as a criterion for the diagnosis of malignant and premalignant lesions:

- Reactive urothelium vs. urothelial carcinoma in situ and transitional cell carcinoma (Fig. 33.1).
- Flat dysplasia and DALM of colonic mucosa vs. reactive hyperplasia.

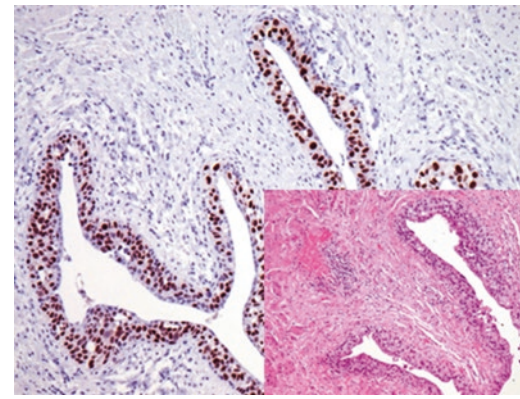

Fig. 33.1 High-grade dysplasia with carcinoma in situ of the ureter with strong p53 expression

- Reactive squamous epithelium vs. cervical/vulvar intraepithelial neoplasia (CIN/VIN).
- Normal ductal mucosa of the pancreas vs. mucinous cystic neoplasia.
- Dysplasia in esophageal columnar mucosa.
- Transformation of B-CLL/SLL to high-grade lymphoma (Richter's syndrome).
- Low-grade astrocytoma and secondary glioblastoma.
- p53 overexpression is a characteristic marker for serous endometrium carcinoma and high-grade ovarian serous carcinoma.

33.3 IMP3

IMP3 is a cytoplasmic oncofetal protein listed with mesothelioma markers (see Chap. 15). Benign adult tissue usually lacks the expression of IMP3 with the exception of the ovarian and testicular tissue, placenta, endocrine cells, mucinous cells, adenohypophysis, and brain. In routine immunohistochemistry, IMP3 is used to discriminate between malignant and reactive proliferative lesions. Similar to GLUT-1 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. IMP3 is a marker for malignant melanocytes and is found in the majority of malignant melanocytic tumors but is not detected in either benign or dysplastic nevi. Furthermore, IMP3 is a valuable marker to distinguish between benign and dysplastic and neoplastic epithelial lesions in the pancreatobiliary region [2].

Besides Alpha-methylacyl-CoA racemase (AMACR) and Hep Par-1, IMP3 is also a helpful marker to label dysplastic epithelium in Barrett's esophagus [3].

33.4 Glut-1

Glucose transporter 1 (Glut-1) 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 (see also Chap. 15). Generally, the overexpression of Glut-1 in tumors is associated with increased malignant potential and aggressive tumor behavior. In diagnostic histopathology, Glut-1 is a potential marker for malignant transformation as it is overexpressed in many types of malignant epithelial and non-epithelial tumors. It is a helpful marker to discriminate between benign and malignant pancreatic glands, between the reactive proliferation of mesothelial cells and malignant mesothelioma, or between benign and atypical endometrial hyperplasia. Glut-1 can also be helpful in differentiating between invasive and noninvasive implants of serous ovarian tumors.

33.5 BAP-1

BAP-1 is a nuclear ubiquitin hydrolase functioning as a transcriptional regulator and tumor suppressor listed in the mesothelioma chapter (Chap. 15). The genomic region is 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. 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.

33.6 Carcinoembryonic Antigen (CEA)

CEA is an oncofetal glycoprotein normally expressed by colonic mucosa of the fetal colon and to a lesser degree in adult colonic mucosa (see also Chap. 25). 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 of malignant transformation.

33.7 CD24

CD24 is a glycoprotein and cell adhesion molecule expressed on the surface of stem cells, most B lymphocytes mainly pre-B cells, mature granulocytes, squamous epithelium, renal tubules, and differentiating neuroblasts in addition to regenerating tissue [4].

In neoplastic lesions, CD24 plays a role as a mediator for proliferation, invasion, and immune evasion. Generally, the overexpression of CD24 in tumors is associated with aggressive behavior and poor prognosis. The overexpression of CD24 was reported in different tumor types, including colorectal carcinoma, cholangiocarcinoma, breast carcinoma, prostatic carcinoma, ovarian carcinoma, and carcinoma of the uterine cervix. The overexpression of CD24 detected by immunohistochemistry on paraffin sections is a putative marker for dysplasia in oral and cervical mucosa [5, 6].

33.8 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 from progressing from G1 to the S phase, acting as a tumor suppressor gene. The CDKN2A gene is the subject of different mutations or deletions seen in many epithelial and mesenchymal tumors. The expression of p16 is regulated by the activity of the retinoblastoma gene (Rb), which in turn is affected by the E7 oncogene, one of the HPV genes. 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 and adenocarcinoma of the endocervix (see Chap. 11). Additionally, p16 is a helpful marker to differentiate between urothelial carcinoma in situ strongly positive for p16 and reactive atypia, usually lacking the expression of p16.

p16 is a very useful marker to differentiate between benign lipoma negative for p16 and well-differentiated liposarcoma positive for p16 (see markers of adipocytic tumors; Chap. 25). p16 is also a marker for uterine leiomyosarcoma.

Since different tumors are associated with the deletion or inactivation of the p16 gene, the loss of p16 expression is helpful for the diagnosis of different tumors, such as melanoma and pancreatobiliary carcinomas. In such tumors, the expression of p16 decreases or disappears after malignant transformation.

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34 Recommendations for the Utility of Immunohistochemical Results in Tumor Diagnosis

Immunohistochemistry is a powerful and sensitive diagnostic tool for tumor diagnosis that requires a high level of practical and theoretical knowledge. Precise tumor diagnosis begins with the adequate processing of tissue samples and includes the standardized stain technique and optimal choice of diagnostic antibody panels and ends with the 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 considering the following points:

1. Initially, it is important to remember that careful histopathologic examination and clinical correlation remain the cornerstone of morphologic diagnosis. The immunoprofiling is to support or rule out one or more possible differential diagnoses.
2. The laboratory of immunohistochemistry must be under the supervision of a well-trained pathologist, highly skilled in methods and techniques of immunohistochemistry and who 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. An adequate panel of antibodies helps 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).
   - Short postoperative cold ischemia time, besides optimal and standardized tissue fixation and tissue processing, is essential for the stability of antigens. As a rule, bad H&E sections mean bad immunohistochemistry results.
   - Histopathologists deal with neoplasia with heterogeneous cell populations with high potential for genotypic and phenotypic variations; consequently, the reason for the atypical or heterogeneous 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.

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- Care must be exercised when using newly developed antibodies. New antibodies are 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 the used detection system.

6. Standardizing the immunohistochemical staining method is one of the essential factors for the 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. The quality and intensity of the stain and staining pattern must also be 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 many available antibodies, immunohistochemistry—as any method—has its 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 of where we need other methods to obtain a precise tumor diagnosis.

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