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

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

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

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

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

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

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

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

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

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

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

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

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

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

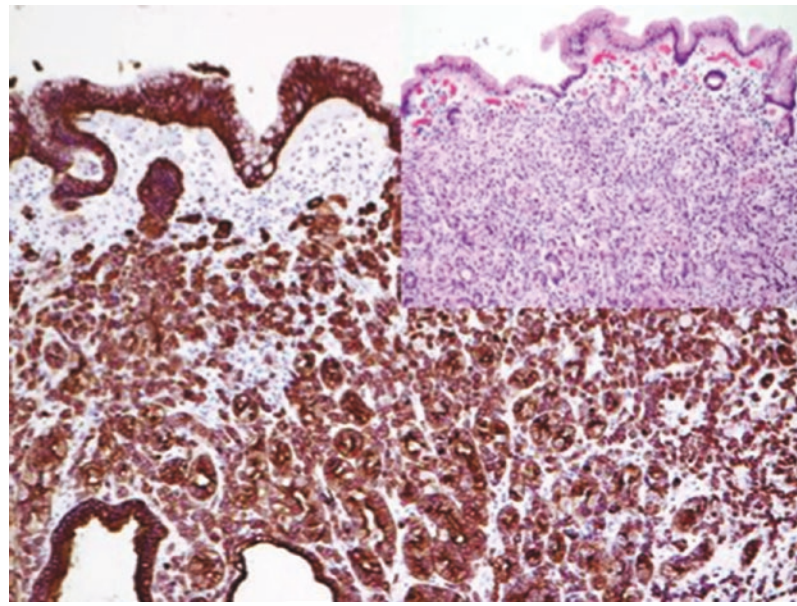

Fig. 2.1 Pan-cytokeratin (CK MNF116) highlighting the neoplastic cells in diffuse gastric adenocarcinoma

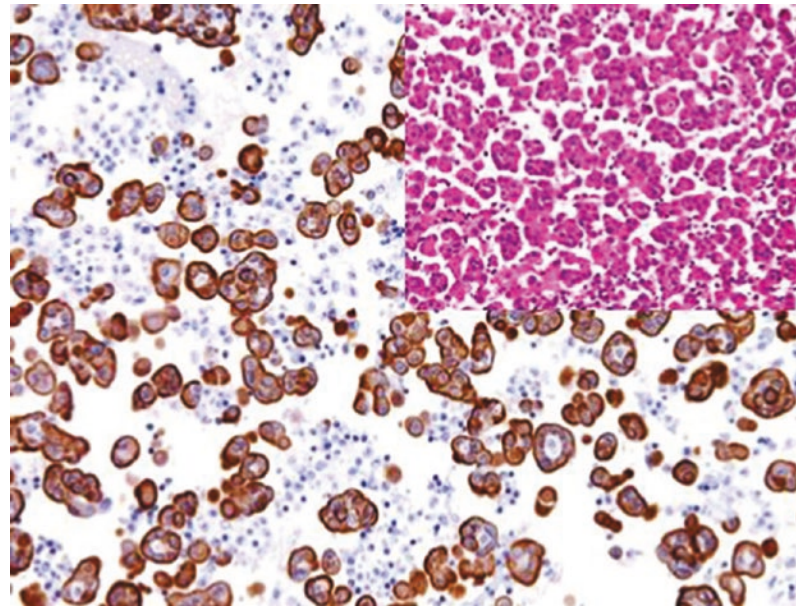

Fig. 2.2 Mesothelioma cells labeled by cytokeratin 5 in pleural effusion

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

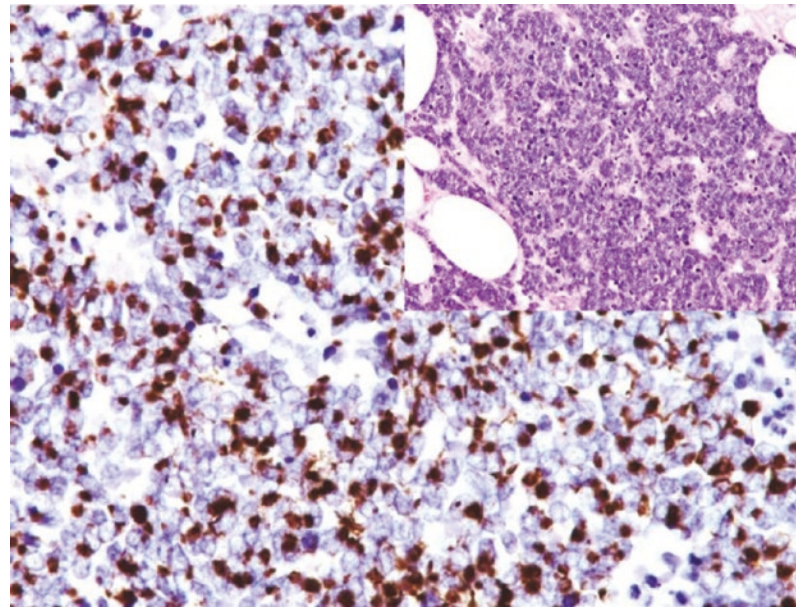

Fig. 2.3 Characteristic dot-like perinuclear expression of CK20 in Merkel cell carcinoma

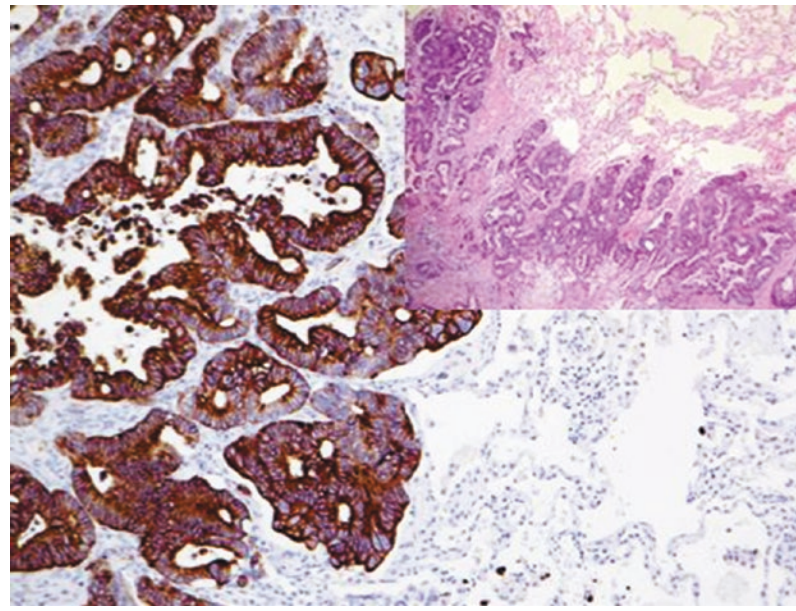

Fig. 2.4 Metastatic colorectal adenocarcinoma with strong CK20 expression

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

2.2 Mucins

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

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

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

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

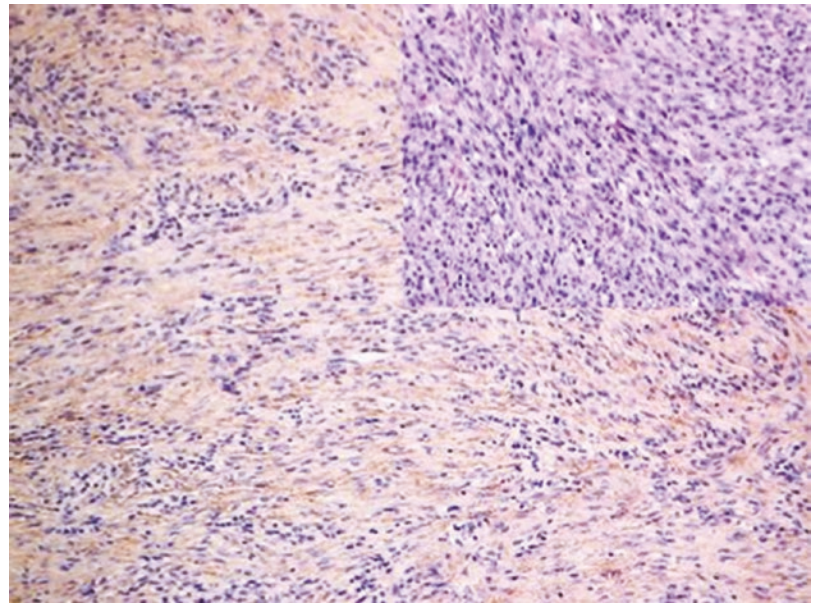

Fig. 2.5 EMA expression in atypical meningioma

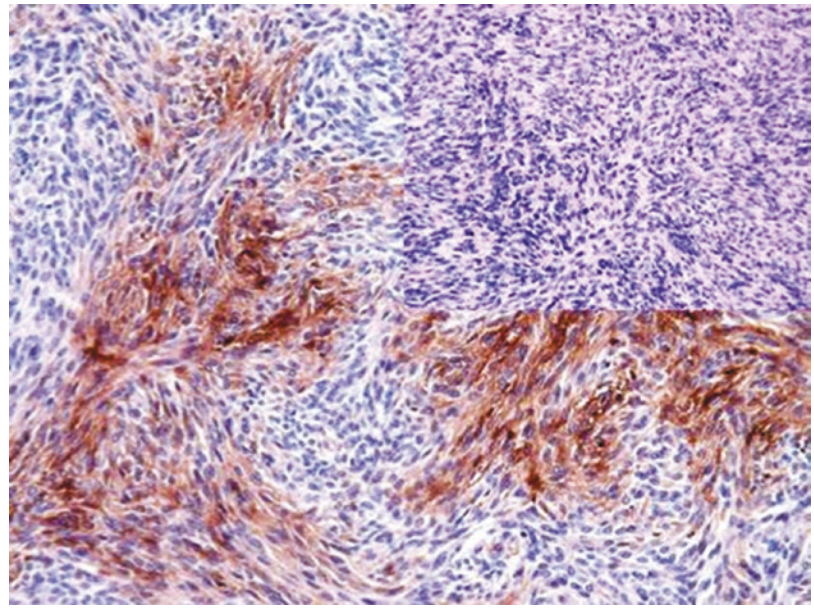

Fig. 2.6 Focal EMA expression in neurogenic sarcoma

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

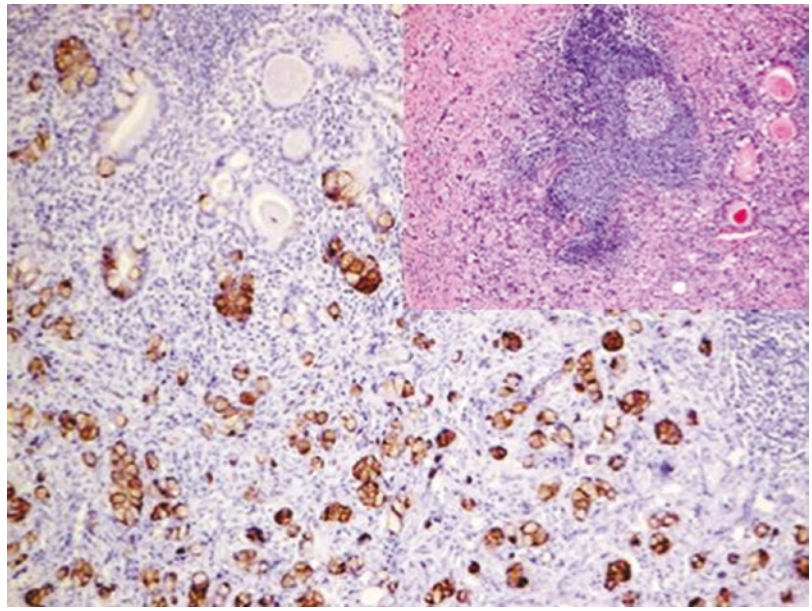

Fig. 2.7 MUC-2 highlighting tumor cells of appendicular mucinous carcinoma

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

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

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

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

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

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

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

2.3 Claudins

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

2.4 Miscellaneous Epithelial Markers

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

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

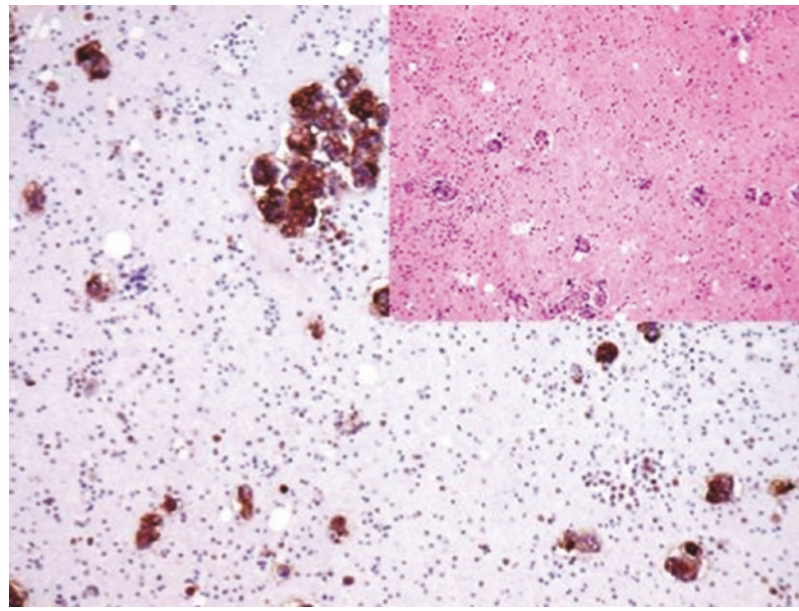

Fig. 2.8 Claudin-4 highlighting tumor cells of ovarian carcinoma in ascitic fluid

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

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

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

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

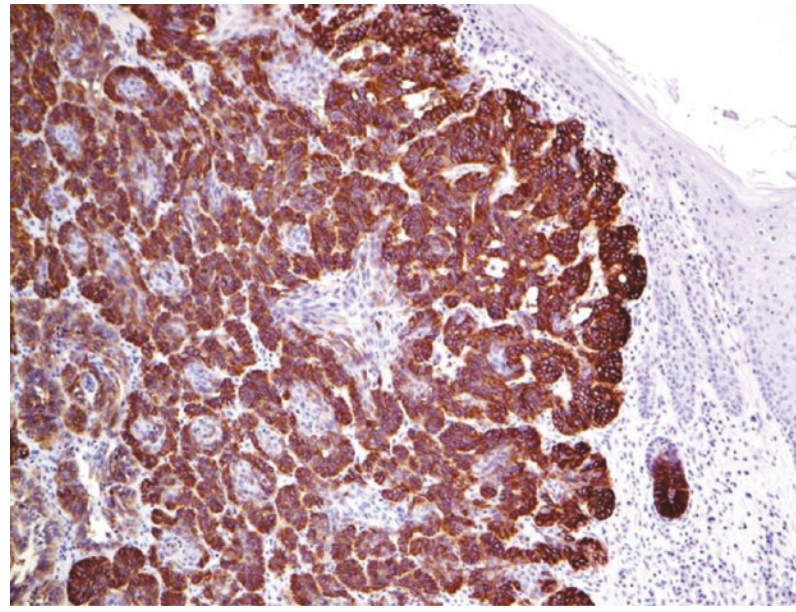

Fig. 2.9 Basal cell carcinoma with strong EPCAM (clone Ber-EP4) expression

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

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

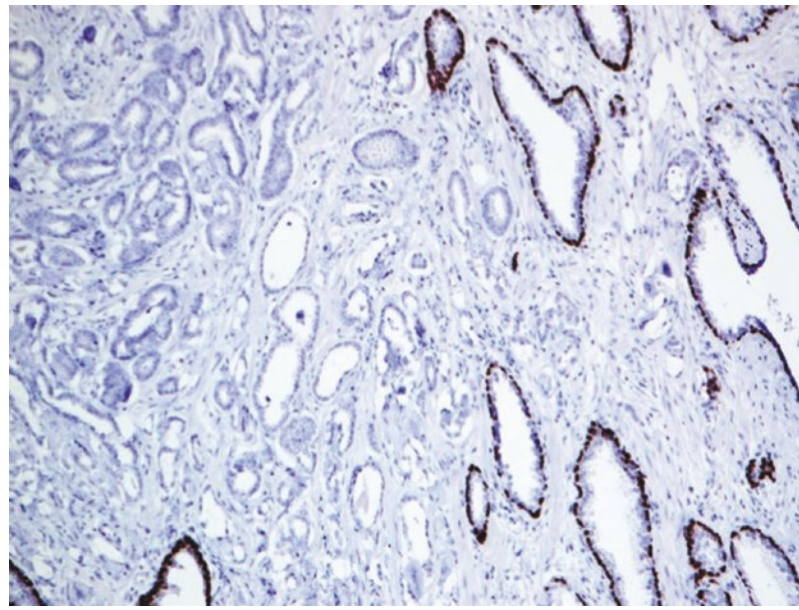

Fig. 2.10 p63 highlighting basal cells in normal prostatic glands; note neoplastic glands lacking the basal cell layer

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

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

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

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

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

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

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