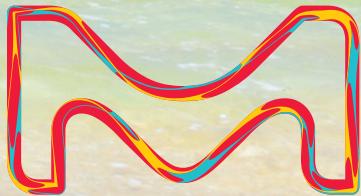


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Immunohistochemical Applications in Dermatopathology

Mike Lacey, M.D.

Dermatopathology



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IMMUNOHISTOCHEMICAL APPLICATIONS IN DERMATOPATHOLOGY

Mike Lacey, M.D.

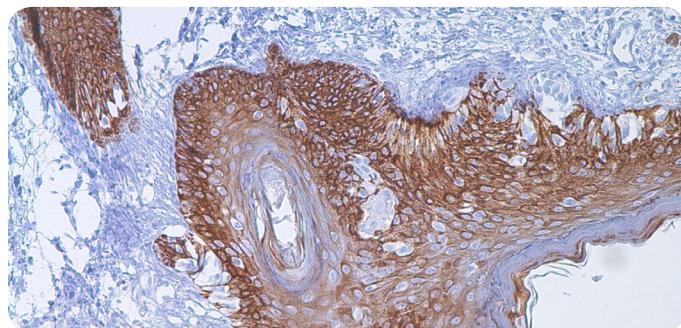
Although immunohistochemistry techniques and applications have made great strides in the past 25 years, diagnostics in the world of dermatopathology is still largely based on H&E stained slides. Despite that fact, there are many ways in which immunohistochemistry (IHC) can assist the pathologist in the detection of problematic cases. Outlined are a few of the ways in which antibodies can be utilized in dermatopathology.

Cutaneous Spindle Cell Tumors

The differential diagnosis of atypical spindle cell neoplasms on sun-damaged skin includes atypical fibroxanthoma, spindle cell or desmoplastic melanoma, spindle cell squamous cell carcinoma, and leiomyosarcoma. This is a not-infrequent challenging differential in skin tumor pathology. Because of potential overlapping reactivity and rare, anomalous expression, a grid of IHC markers should be selected that will support or refute each of those differentials.

Spindle Cell Squamous Cell Carcinoma

Squamous cell carcinomas (SCC), in general, stain with cytokeratin and not with vimentin. However, spindle cell SCCs that may lack an obvious origin in the epidermis or evidence of keratinization are often positive for vimentin and negative or only focally positive with routine cytokeratin stains, including AE1 & AE3. Folpe and Cooper¹ found high molecular weight (HMW) cytokeratins (CKs), such as CK 903 (34betaE12) and CK 5 & 6 to be sensitive markers in this context; however, some spindle cell SCCs do not stain with either.²⁻⁴



Cytokeratin 5 & 6

Atypical Fibroxanthoma

Atypical fibroxanthoma (AFX) is a pleomorphic, superficial spindle cell tumor of a borderline fibrous tumor or low-grade malignancy that must be distinguished histologically from other atypical spindle cell tumors of the dermis. Although there are immunohistochemical markers for many of those other tumors, the detection of the proteins associated with AFX are generally those of exclusion. A variety of markers have been identified with reactivity in AFX, but those markers alone are not specific. Procollagen 1 (PC1), CD10, and S100A6 are typically reactive in AFX, but those markers often stain a variety of other neoplasms.

Procollagen is secreted by fibroblasts and is cleaved to form collagen in the extracellular matrix. Expression of PC1 in most AFXs supports classification of this tumor as fibrohistiocytic. However, PC1 staining should not be used in isolation. Reactivity with keratin, S-100, or desmin should outweigh PC1 positivity because approximately three-fourths of leiomyosarcomas and

one-third of desmoplastic melanomas (DMs) and spindle cell SCCs are also PC1 positive.⁵⁻⁷ As with all IHC markers, careful localization of the antibody staining should be established. PC1+ tumor cells must be distinguished from positive background fibroblasts.

S100A6, also known as calcyclin, is a calcium-binding protein of the S-100 family, which has been isolated from melanocytes, Schwann cells, Langerhans cells, and dermal dendrocytes. Aside from staining nevi and some melanomas, numerous fibrohistiocytic lesions stain positive with S100A6. A small study⁸ revealed S100A6 in 80% (4 of 5) of the AFX cases. However, other neoplasms in the spindle cell differential have also revealed reactivity with S100A6, including a significant proportion of DMs, leiomyosarcomas, and spindle cell SCCs.⁹

Similarly, CD10 is sensitive but not specific for AFX. In one small study,¹⁰ 94% (15 of 16) of the AFXs showed strong expression of CD10, but on the other hand, reactivity was also identified in one-third (3 of 9) of the DMs and one-half (5 of 10) of the spindle cell SCCs.

Focal expression of myogenic markers (calponin, smooth muscle actin (SMA), HHF35), indicative of myofibroblastic differentiation, can be seen in up to one-third of AFXs.^{1,11} Therefore, use of SMA or calponin alone may be insufficient to detect leiomyosarcoma, and an antibody grid should include another muscle marker, such as desmin or h-caldesmon. Caution is required in interpreting S-100 in AFXs because S-100+ dendritic cells colonize the lesion (possibly Langerhans cells),^{12,13} but the neoplastic cells are generally S-100- essentially excluding DM. Multinucleate giant cells of AFX can also express MART-1 (Melan A) and HMB-45.^{14,15}

Historically, other nonspecific markers with lower reactivity used in AFX have included CD68,¹³ antichymotrypsin, antitrypsin,¹⁶ and CD99.¹⁷ Studies have shown that CD163 is more specific for histiocytes than CD68, which is an organelle-specific marker that stains lysosomes. Because CD68 reactivity is seen in some AFXs, it is not surprising that CD163 expression was reported.¹⁸ Caution is required in distinguishing AFX with pseudoangiomatous or hemorrhagic features from angiosarcoma. It can be further complicated by occasional expression of D2-40, FLI-1, and CD31 in AFX.¹⁹⁻²² CD34 and ERG may be useful to differentiate those entities.²¹

Desmoplastic Melanoma

Desmoplastic melanoma (DM) often lacks melanin and, sometimes, junctional involvement. When considering its differential utility, it must be remembered that cytokeratin (2%), SMA, and desmin can be anomalously expressed in DMs. CD68+ melanomas may also be mistaken for AFX. HMB-45 is usually negative, and Melan A is positive in only 7% of DMs.²³

Microphthalmia transcription factor (MiTF) similarly has low sensitivity in DM. S-100 is a sensitive, albeit not specific, marker for spindle/DM. However, many scars contain S-100+ spindle cells complicating

SOX-10 has become an important alternative in the identification of DM, especially in re-excision specimens.

differentiation, particularly of re-excision specimens, but unlike DM, the S-100+ cells are only focal and are predominantly in a horizontal orientation. Nerve growth factor receptor (NGFR) (p75), is a neural crest marker expressed in most desmoplastic and neurotropic melanomas, often more intensely than S-100.^{24,26}

Leiomyosarcomas, AFX, and 81% of spindle cell SCCs are negative for p75, and those spindle cell SCCs that do react show only focal nests of positivity.²⁵ However, many other malignant spindle cell tumors are also positive for p75, including peripheral nerve sheath tumors, dermatofibrosarcoma protuberans (DFSP), rhabdomyosarcoma, synovial sarcoma, and neurotized nevi. Spindle cell melanoma without desmoplasia or neurotropism, many typical epithelioid melanomas, and nevi fail to stain with p75 or do so only focally.²⁶ Scars may reveal p75+ cells, similar to S-100 (possibly myofibroblasts, nerve twigs, or Schwann cells) requiring caution in distinguishing scars from DMs.²⁷

Sex-determining region Y-box 10 (SOX-10) has proven to be as sensitive a marker for DM as S-100 with improved specificity. AFX and spindle cell SCCs fail to show SOX-10 expression, but malignant peripheral nerve sheath tumors show scattered positivity similar to S-100.²⁸ SOX-10 has become an important alternative in the identification of DM, especially in re-excision specimens. Ramos-Herberth et al²⁹ reported only rare, weak SOX-10 expression in spindled fibroblasts of scars, in comparison to greater MiTF and S-100 expression in histiocytes and fibroblasts of scars.

Use of immunohistochemical markers in the setting of atypical dermal spindle cell tumors is required to prevent misdiagnosis; some combination of S-100 and SOX-10 for DM, HMW keratin and p63 for spindle cell SCC, desmin and h-caldesmon for leiomyosarcoma, and if hemorrhagic, ERG or CD34 for angiosarcoma.

Cutaneous Lesion

	CK Cocktail	HMB-45	MART-1	S-100	SOX-10
Conventional Melanoma	-	+	+	+	+
Desmoplastic Melanoma	-	-	-	+/-	+
Squamous Cell Carcinoma	+	-	-	-/+	-
Basal Cell Carcinoma	+	-	-	-	-
Merkel Cell Carcinoma	+	-	-	-/+	-

Cutaneous Adnexal Neoplasms

Sebaceous Neoplasms

Differentiation of sebaceous carcinoma and SCC can be challenging. Ansai et al³⁰ found that androgen receptor and adipophilin expression supports the detection of sebaceous carcinoma, rather than SCC. Sebaceous epitheliomas (sebaceomas) are mostly composed of basaloid cells with a small proportion of mature sebocytes. Thus, there is histologic overlap between the basaloid cells of sebaceomas and those of basal cell carcinoma (BCC). Although IHC is not commonly

necessary in differentiation, epithelial membrane antigen (EMA) highlights most mature sebocytes in sebaceoma, whereas its expression is uncommon in BCC.^{31,32} Reactivity for adipophilin in sebaceous tumors can also distinguish them from BCC.³¹ The utility of Ber-EP4 in this setting is controversial; although BCCs stain positive for Ber-EP4, there is varying literature on its reactivity in sebaceous lesions.^{31,33}

Adipophilin is a protein on the surface of intracellular lipid droplets and thus shows expression in sebaceous lesions, particularly well-differentiated lesions. Caution is necessary when using adipophilin to distinguish sebaceous neoplasms from other cutaneous lesions with clear cell histology. Metastatic renal cell carcinoma and eccrine-apocrine carcinomas have shown adipophilin expression.^{34,35} Xanthomatous lesions, including xanthelasma and xanthogranuloma, reportedly show adipophilin reactivity, but in contrast to the membranous vesicular pattern in sebaceous neoplasms, xanthomatous lesions show a more granular pattern.³⁴ This granular pattern has also been reported in clear cell BCCs and SCCs potentially causing confusion when differentiating those neoplasms from sebaceous carcinoma; however, a granular pattern and decreased intensity of expression favor the former tumors.^{35,36} The potentially aggressive course of sebaceous carcinoma necessitates accurate differentiation from benign sebaceous neoplasms. Cabral et al³⁷ sought to find an immunohistochemical method that could aid morphologic differentiation, especially in small, partial biopsies. The p53 protein is a tumor suppressor. Mutations lead to an abnormally stable but inactive p53 protein that can be detected immunohistochemically (nuclear staining). Sebaceous carcinomas are associated with increased, presumably defective, p53. Sebaceous carcinomas have significantly increased nuclear staining with p53 and Ki-67 (MIB-1) and reduced levels of BCL2 and p21, in comparison to sebaceous adenomas and sebaceous epitheliomas.³⁷

In some cases, IHC can serve as surrogate protein markers for underlying genetic events. Sebaceous gland neoplasms are relatively rare, and that diagnosis should raise the possibility of Muir-Torre syndrome (MTS). The dermatopathologist may be the first to consider the possibility of this syndrome because, in some patients, only a single sebaceous tumor is the first indication. MTS is an autosomal-dominant disease associated with multiple keratoacanthomas and visceral malignancies, especially genitourinary and gastrointestinal. Less common associations include tumors of the breast and lung. MTS is most frequently caused by mutational

inactivation of mismatch repair genes MSH2 and MLH1 with resultant microsatellite instability. Other mismatch repair proteins can be involved, such as MSH6 and PMS2. It is reasonable to check this panel (MSH2, MLH1, MSH6, with or without PMS2) in sebaceous neoplasms (other than sebaceous hyperplasia), especially in patients younger than 50 years, when multiple lesions are present, neoplasms involve nonfacial sites, or when cystic or keratoacanthoma-like architecture is present.^{38,39} Although some authors are conservative and recommend screening only in patients with a personal or family history of colorectal cancer,⁴⁰ others^{41,42} recommend screening regardless of age or other clinical characteristics.

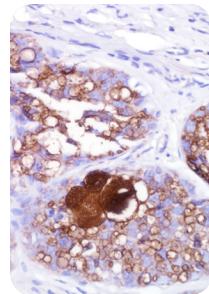
Trichilemmomas

Solitary trichilemmomas are typically sporadic but can be associated with Cowden syndrome, which is an autosomal dominant disorder characterized by multisystem hamartomatous growths and carcinomas. This syndrome is linked to a germline mutation in the tumor suppressor phosphatase and tensin homolog (PTEN), located on band 10q23.3. Complete loss of immunoreactivity with PTEN was reported in 5 of 6 patients (83%) with Cowden syndrome-associated trichilemmomas, but in only 1 of 33 patients (3%) with sporadic lesions.⁴³ Shon et al⁴⁴ reported similar results with 13 Cowden syndrome-associated lesions and 19 sporadic trichilemmomas. This suggests a potential role for immunohistochemical screening similar to its use in MTS. Partial biopsies of desmoplastic tricholemmoma may require distinction from BCC. CD34 immunodetection in the epithelial cells of the tricholemmoma can help in the differentiation.⁴⁵

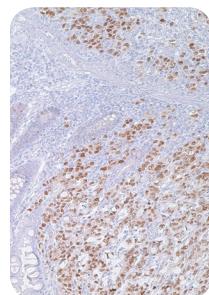
Primary Cutaneous Adnexal Neoplasms From Metastatic Adenocarcinoma

Differentiation of metastatic adenocarcinomas from primary cutaneous adnexal neoplasms (PCAN), especially malignant ones, can be difficult. Qureshi et al⁴⁶ found that most PCANs with sweat gland differentiation were positive for p63 and HMW CK 5 & 6, whereas expression was rare in metastatic adenocarcinomas. Differential p63 reactivity has also been supported in other studies.⁴⁷⁻⁴⁹

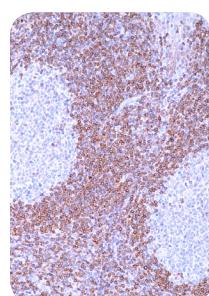
Plumb et al²⁴ similarly found CK 5 & 6 expression in more than 95% of PCANs, albeit most tumors studied were benign. In general, metastatic adenocarcinomas expressed CK 5 & 6 in only 33% (9 of 27) of the cases, predominantly with weak intensity. However, metastatic breast carcinoma was reactive for CK 5 & 6 in almost half of their cases.⁵⁰



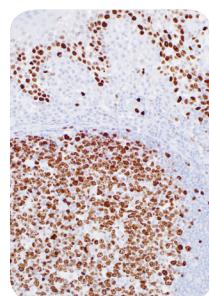
Adipophilin



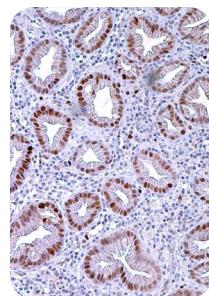
p53



BCL2



Ki-67



p21^{WAF1}

Ivan et al⁵¹ extended the analysis of p63 to include metastases from adnexal carcinomas and found that 91% of PCANs were strongly marked with p63 and, excluding apocrine and mucinous carcinoma, their metastases labeled similarly.

Podoplanin (D2-40) expression is also a useful adjunct in differentiating adenocarcinomas that have metastasized to the skin from PCAN. Reactivity with D2-40 is seen in primary cutaneous carcinomas and skin adnexal tumors but is absent in metastatic adenocarcinomas to the skin.^{47,52} A group of immunohistochemical stains, as suggested by Mahalingam et al,⁴⁸ may provide the best sensitivity and specificity in distinction of metastatic adenocarcinomas and PCANs. Their recommended grouping included p63, D2-40, and CK 15. Positive staining with all three markers helps identify PCAN, rather than that of metastatic adenocarcinoma.

Adenocarcinomas do metastasize to the skin, and rarely, that can be the first manifestation of the primary diagnosis. IHC has been useful in a number of cases. Differential cytokeratin staining (CK 7 and CK 20) may be helpful in this setting but is not specific. Of the most common metastases to skin, breast and lung adenocarcinomas are CK 7+/CK 20-, and colorectal carcinoma is CK 7-/CK 20+.⁵³ Other potentially useful immunohistochemical stains include CDX-2 and TTF-1. CDX-2, a nuclear marker, is expressed in almost all colorectal carcinomas but is also expressed in a few other adenocarcinomas, including gastric, biliopancreatic, and mucinous ovarian adenocarcinomas.^{54,55} TTF-1 is a relatively specific marker for tumors of lung or thyroid origin. Around two thirds of lung adenocarcinomas express TTF-1. However, expression is typically absent in SCC of the lung and lung mucinous adenocarcinomas/bronchioloalveolar carcinomas.^{54,56} CDX-2 and TTF-1 are reasonable additions that aims to identify the origin of a metastatic adenocarcinoma of unknown primary. Park et al⁵⁷ proposed and evaluated a panel of antibodies to improve prediction of the primary site. In addition to CK 7, CK 20, TTF-1, and CDX-2, the grid included carcinoembryonic antigen (CEA), epithelial mucin genes MUC2 and MUC5AC, Estrogen Receptor (ER), and gross cystic disease fluid protein 15 (GCDFP-15). GCDFP-15 is a relatively specific marker for breast carcinoma with reactivity ranging from 43% to 77%. The numbers indicate limited sensitivity. Mucin expression varies between carcinomas arising from different organs. MUC2 and MUC5AC are expressed predominantly in colon adenocarcinoma and mucinous ovarian adenocarcinoma, respectively; however, there are overlapping patterns. The combined phenotypes correctly predicted the primary site in 75% of cases.⁵⁷ Cutaneous metastases occur in up to 11% of patients with renal cell carcinoma (RCC) and may be the presenting sign of disease.⁵⁸

CD10 is a metallomembrane endopeptidase first identified in acute lymphoblastic leukemia and also expressed in follicular lymphoma, Burkitt lymphoma, hepatocellular carcinoma, urothelial carcinoma, and prostatic carcinoma.⁵⁹ CD10 may be used to confirm

the detection of renal carcinomas, especially clear cell carcinomas and less so papillary or chromophobe RCCs.⁶⁰ The histologic differential diagnosis of metastatic RCCs includes primary cutaneous adnexal tumors. CD10 was evaluated in adnexal tumors by Bahrami et al⁵⁸ to determine its utility in the distinction. Six percent of eccrine and apocrine neoplasms and 40% of sebaceous neoplasms demonstrated CD10 reactivity. RCC metastatic to the skin can also simulate other clear cell lesions. Perna et al⁶¹ evaluated CD10 in clear cell mimickers, including xanthomas, xanthelasma, and xanthogranulomas.

CD10 stained most of those lesions; however, that was predominantly in a membranous pattern compared with the cytoplasmic pattern of RCC. CD10 was also expressed in 25% and 33% of balloon cell nevi and clear cell hidradenomas, respectively, but expression was limited to less than 10% of the clear cells. That overlap of reactivity between metastatic RCC and other cutaneous clear cell lesions is not present with the renal cell carcinoma marker (RCC-Ma). Perna et al⁶² found RCC-Ma expression in 62.5% of cutaneous RCC metastases and no reactivity in clear cell mimics, including xanthomas, xanthogranulomas, balloon cell nevi, clear cell hidradenoma, and sebaceous neoplasms. PAX-8 is also a renal tumor marker with greater sensitivity than RCC-Ma. PAX-8 also stains thyroid, gynecologic, and neuroendocrine tumors, including Merkel cell carcinoma.^{63,64}

Melanocytic Neoplasms

The melanocytic origin of a tumor may not always be apparent. In addition to morphologic clues, IHC can assist in distinguishing melanocytic from nonmelanocytic lesions. Staining with S-100 was one of the first and most enduring markers for melanocytic lesions. Although most melanomas are S-100+, the marker lacks specificity and stains neural tissue, Langerhans cells, Rosai-Dorfman disease, and other tumors. Thus, other antibodies may be necessary to confirm the melanocytic nature of S-100+ neoplasms. Both the MART-1 (melanoma antigen recognized by T cells) and Melan A antibodies recognize the same gene product and are expressed by normal melanocytes, nevi, and melanoma, but less frequently by DM.⁶⁶ HMB-45, also known as anti-gp100, a premelanosome marker, has been shown to mark the intraepidermal and superficial dermal components of melanocytic nevi, with the exception of diffuse dermal staining in blue nevi.⁶⁷ When compared with MART-1, HMB-45 has been found to have weaker and more-focal staining in both primary and metastatic melanomas. Therefore, HMB-45 is relatively specific, staining only rare other tumors (eg, perivascular epithelioid cells tumors [PEComas]) but is not very sensitive for melanoma.^{68,69} MiTF is responsible for the normal embryonic development of melanocytes, mast cells, cells of the retinal pigment epithelium, and osteoclasts.⁷⁰ Melanocytes express nuclear MiTF. Most melanomas retain MiTF reactivity; however, a large proportion of desmoplastic and spindle cell melanomas fail to stain.^{70,71} Positivity with MiTF has been reported in 88%

Positivity with MiTF has been reported in 88% (235 of 266) of the conventional metastatic melanomas.

(235 of 266) of the conventional metastatic melanomas.⁷¹ However, MiTF is not specific, and staining has also been reported in neurofibromas, dermatofibromas, AFX, leiomyosarcomas, schwannomas, malignant peripheral nerve sheath tumors, solitary fibrous tumor, giant cell tumors of the tendon sheath, dermal scars, and granular cell tumors.^{68,72}

SOX-10, a nuclear transcription factor expressed in neural crest cells, is crucial for differentiation of Schwann cells and melanocytes. Nuclear staining is noted in normal melanocytes, Schwann cells, secretory cells of the eccrine coil, myoepithelial cells, and predominantly cytoplasmic staining in mast cells.^{73,74} Expression has been shown in all types of nevi (blue, neurotized, dysplastic, Spitz, capsular) and melanoma (epithelioid, spindled, desmoplastic, metastatic).^{75,76} The specificity is also high, with expression in only a few other tumors, including granular cell tumor, schwannoma, neurofibroma, myoepithelioma, clear cell sarcoma, and some ductal breast carcinomas. It is notably absent in cellular neurothekeoma, Langerhans cell histiocytosis, perineurioma, MCC, spindle cell SCC, AFX, and leiomyosarcoma.^{72-75,77,78}

SOX-10 is particularly useful in the setting of junctional melanocytic proliferations on sun-damaged skin, DM, and in evaluation of sentinel lymph node biopsies.

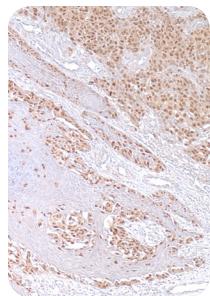
Several other melanocytic markers have been studied, including NKI-C3, TRP-1, and TRP-2 (antibodies to tyrosinase), SM5-1, PNL2, and KBA.62.⁷⁹⁻⁸⁴ Multiple myeloma 1 (MUM1), a known hematolymphoid marker, shows positive immunostaining in nevi; in more than 90% of primary melanomas, with the exception of DM; and in 80% of metastatic melanomas.^{85,86} To date, none of these have shown any significant benefit over

those previously discussed.

Some melanomas exhibit aberrant immunohistochemical expression and may express desmin, SMA, CD68, CEA, and EMA.⁸⁷ Korabiowska et al⁸⁸ found cytokeratin expression in 6% of malignant melanomas, with staining tending to be focal, and only 3% or more of the cells staining.

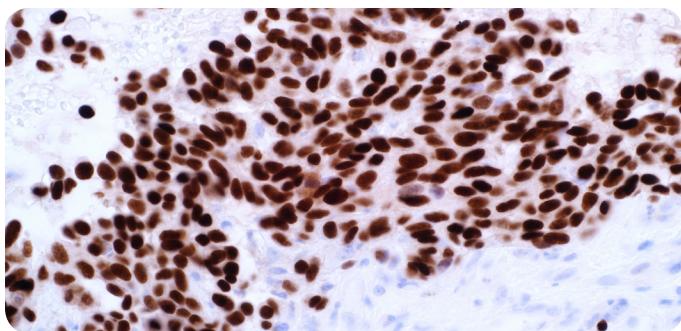
PEComas have immunophenotypic features of smooth muscle and melanocytes. Although many are retroperitoneal or visceral, a subset arises in the skin. Those lesions can mimic melanocytic neoplasms on routine sections and immunohistochemically. HMB-45 is the most-sensitive marker, but Melan A and MiTF are also expressed in many cases. PEComas differ from melanocytic lesions in absence of S-100 expression.⁸⁹

Heavily pigmented melanocytic neoplasms are difficult to assess on routine hematoxylin-eosin (H&E)-stained slides because pigmented melanocytes can be confused with pigmented keratinocytes and melanophages. Immunostaining using diaminobenzidine, which forms a brown product, as the chromogen is challenging to distinguish from melanin. Alternatives include use of a red chromogen or use of melanin bleach; however, the bleaching may result in loss of antigenicity, incomplete melanin removal, or loss of cytologic detail.



MiTF

Junctional Melanocytic Proliferations



SOX-10

It can be difficult to reliably identify intraepidermal melanocytes in H&E-stained sections, especially on sun-damaged skin. Even close inspection may not unequivocally discriminate pigmented keratinocytes from melanocytes or clearly delineate the melanocyte density. Thus, many pathologists employ IHC to differentiate melanoma *in situ* from its mimics. S-100 also highlights intraepidermal Langerhans cells making it less favored. MART-1/Melan A has historically been valued in this setting, but studies⁹⁰⁻⁹² have shown that those markers may artificially increase the perceived number of melanocytes because of the labeling of cytoplasmic dendrites encircling neighboring keratinocytes. Available nuclear melanocytic markers are sensitive, specific, and circumvent the problem of cytoplasmic dendrites.⁹¹ SOX-10 is such a nuclear marker, with the same or better sensitivity as S-100, and it does not stain additional distracting cells, such as Langerhans cells of the epidermis.⁹¹ Nuclear expression of MiTF has similar benefits to SOX-10 but is much less sensitive in identifying subtle underlying DM. Caution is required with initial use of nuclear melanocytic markers and should be performed in parallel with cytoplasmic markers until one is comfortable with the altered staining pattern. Confluence, as seen with cytoplasmic markers, may not be as obvious at the outset because nuclear markers lack reactivity in the juxtaposed melanocytic cytoplasm.

Pagetoid Tumors

Intraepidermal lesions with a pagetoid distribution can be diagnostically difficult. CK 7 positivity reportedly differentiates Paget disease of the breast and extramammary Paget disease (EMPD) from pagetoid Bowen disease and melanoma *in situ*; however, the marker is not 100% sensitive, and occasional cases of CK 7+ pagetoid Bowen disease have been described.^{93,94} Similarly, CAM 5.2 expression favors Paget disease or EMPD, rather than SCC *in situ*, but reactivity has been reported in both.^{94,95} Sellheyer and Krah⁹⁶ proposed the addition of Ber-EP4 to the panel used to evaluate pagetoid cutaneous tumors. In their study,³⁰ Ber-EP4 labeled all cases of EMPD but failed to label Bowen disease and melanoma *in situ*. Other authors⁹⁷ have identified strong nuclear p63 in

pagetoid SCC *in situ*, in contrast to no reactivity in EMPD. Several studies have attempted to use IHC to differentiate primary from secondary EMPD, but the immunoprofiles tend to overlap, including occasional CK 20 and prostate specific antigen (PSA) expression in primary EMPD.⁹⁸ Thus far, CDX-2 reactivity has only been reported in EMPD secondary to anorectal adenocarcinoma.

Sclerosing Epithelial Neoplasms

Differentiation of sclerosing epithelial neoplasms is not only of academic interest but also paramount to clinical management, and diagnosis is often requested on small, superficial biopsies. Various immunohistochemical markers have been studied to help distinguish desmoplastic trichoepitheliomas (DTE), infiltrating or morphaform BCCs, and microcystic adnexal carcinomas, including CD23, CD5, CD10, CD34, CK 20, CK 15, stromelysin-3, BCL2, androgen receptor (AR), pleckstrin homology-like domain, family A, member 1 protein (PHLDA1), p75 neurotrophin receptor (p75^{NTR}), fibroblast activation protein (FAP), Ber-EP4, p63, and others.⁹⁹⁻¹⁰³ Much of the available data are based on a few cases reported by a single group of authors and require additional validation. Conflicting results were found with the same marker in other studies. A few deserve further mention, but the histopathologic criteria and clinical data remain the current gold standard.

Testing AR and CK 20 together has proven useful in distinguishing DTE and morphaform BCC.^{100,104} CK 20+ Merkel cells are identified as colonizing DTEs but not BCCs and microcystic adnexal carcinomas.¹⁰⁵ However, the Merkel cells identified in DTE may be few, requiring serial sections, and care is needed to avoid misinterpretation of Merkel cells in preexisting vellus follicles. Nuclear AR expression is focally present in most morphaform BCCs and is absent in most DTEs.^{100,104} AR reactivity has also been noted in classic or conventional trichoepitheliomas.¹⁰⁶

Several studies have attempted to identify immunomarkers to differentiate conventional trichoepithelioma or trichoblastoma from nonaggressive forms of BCC. Although there are supporting data for use of CD34 and BCL2 in this setting, minimal research has been conducted evaluating those markers in the setting of sclerosing epithelial neoplasms. As with conventional trichoepitheliomas, Kirchmann et al¹⁰⁷ found the stroma of DTEs was positive for CD34, differentiating it from the negative staining stroma of morphaform BCC and microcystic adnexal carcinoma; however, Costache et al¹⁰⁴ failed to identify stromal staining in any of their 19 DTEs. BCL2 tends to show expression only at the periphery of the tumor islands in conventional trichoepitheliomas but is present throughout the tumor island in BCC, yet the small islands of DTE and morphaform BCC make that distinction nebulous, as seen in the study by Costache et al.¹⁰⁴

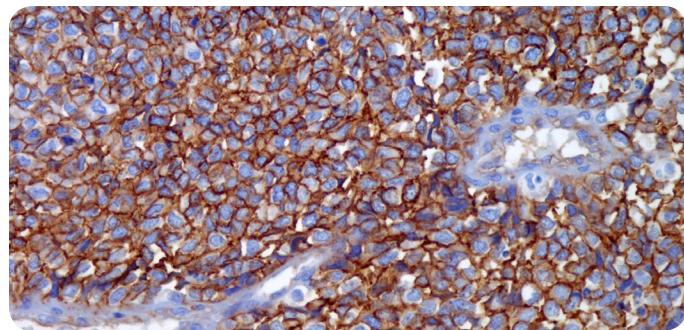
Recent studies have evaluated the utility of p75^{NTR} (CD271) and PHLDA1. Morphaform BCCs tend to

lack expression of p75 in the tumor cells or only show focal or weak expression, whereas DTEs are strongly and diffusely positive.^{108,109} p75 is typically expressed in the outer root sheath of follicles; therefore, reactivity in DTEs supports their classification as a follicular hamartoma mimicking that portion of the follicle. Microcystic adnexal carcinomas cannot be reliably distinguished with p75 because nearly one-half are strongly positive.¹⁰⁸ PHLDA1 is a hair-follicle stem-cell marker in the bulge area of the follicle. Reactivity for that marker in more than 50% of tumor cells characterizes DTEs versus the lack or minimal expression seen in infiltrating or morpheaform BCCs.^{102,103,110} Colonizing melanocytes and tumor cells in proximity to an ulcer can be positive requiring caution in interpretation.¹⁰² As noted above with CD34, some studies have concentrated on tumoral stroma when attempting to differentiate these tumors.

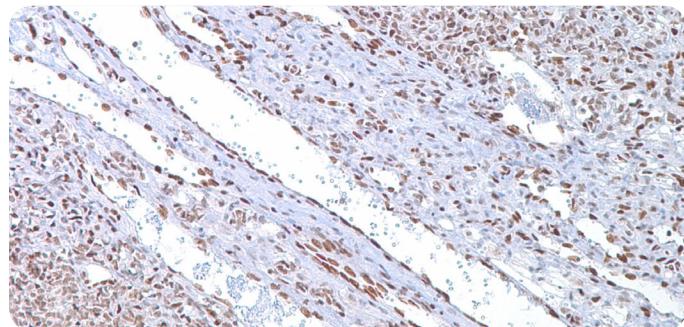
Malignant Small Blue Cell Tumors

The small blue cell appearance of Merkel cell carcinoma (MCC) elicits a differential diagnosis including Ewing sarcoma/primitive neuroectodermal tumor (EWS/PNET), metastatic small cell carcinoma of the lung, neuroblastoma, lymphoma, and melanoma. Small cell melanoma can be distinguished immunohistochemically because MCC does not typically express S-100 but does express neuroendocrine markers, such as chromogranin, neuron-specific enolase (NSE), and synaptophysin, and displays paranuclear dot staining with neurofilament protein and CK 20. Although metastatic small cell carcinoma of the lung is typically negative for CK 20 and neurofilament protein, it is classically positive for CK 7 and thyroid transcription factor 1 (TTF-1), a finding not usually seen in MCC.^{118,119} CK 20 is present in most MCCs (87%), whereas CK 7 is rarely identified.¹¹⁹ Despite the classic CK 20+/CK 7-pattern, CK 20-/CK 7+ MCCs have been described.^{119,120} It is important to be aware of the staining pattern of the primary tumor when evaluating sentinel lymph nodes if IHC is needed. The absence of CD45 (LCA) in MCC is typically sufficient to exclude lymphoma, but some markers used in evaluation of lymphoma can be detected in MCC, leading to erroneous results, including ALK1 (depending on the clone used) as in anaplastic large cell lymphoma (ALCL), BCL2 as in B-cell lymphomas, TdT and PAX-5 as in B-lymphoblastic lymphoma, and CD56 as in natural killer T-cell lymphoma.^{119,121-124}

As in MCC, EWS/PNET also has the potential to express NSE, chromogranin, and synaptophysin. CD99 is a marker used for EWS/PNET, but it is not specific and has been seen in lymphoblastic lymphoma, selected rhabdomyosarcomas, small cell carcinomas, carcinoid tumors, melanomas, and even a few MCCs.¹²⁵⁻¹²⁸ The FLI-1 antibody is also a nuclear marker for EWS/PNET, as well as for vascular tumors. However, it is also expressed in subsets of lymphomas, MCCs, and melanomas.^{127,129-132} Pan-keratin reactivity is usually absent in EWS/PNET, and CK 20 expression has not been reported, unlike MCCs. The surest way to distinguish EWS/PNET from MCC is cytogenetic analysis



CD99

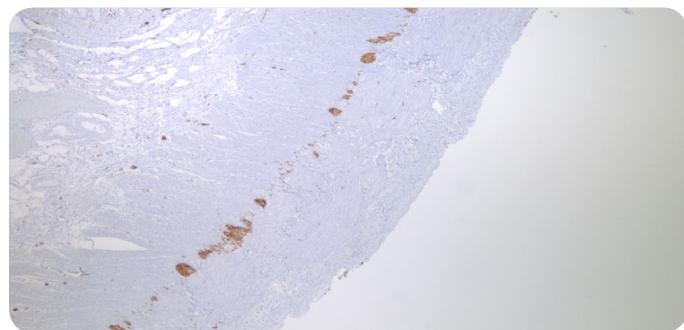


FLI-1

revealing t(11;22), usually involving EWS and FLI-1 genes, in EWS/PNET.^{128,133} The c-kit proto-oncogene codes for a transmembrane receptor tyrosine kinase (KIT receptor/CD117). CD117 is expressed in a variety of tumors, including acute myeloid leukemia, mast cell disease, melanoma, small cell lung cancer, and gastrointestinal stromal tumors. CD117 is expressed by most MCCs.^{134,135} Although KIT expression is present in MCC, response to imatinib mesylate has been poor and anecdotal.¹³⁵⁻¹³⁷ Rarely, MCC can mimic BCC, and both can and often do express Ber-EP4, BCL2, and NCAM/CD56. In addition, BCC, on occasion, can express chromogranin and synaptophysin.

Fibrohistiocytic Lesions

Cellular Neurothekeoma



PGP 9.5

Nerve sheath myxomas, believed to be of peripheral nerve sheath origin, were redesignated as neurothekeoma by Gallager and Helwig¹³⁸ in 1980. In

1986, the more-cellular form was designated cellular neurothekeoma (CNT) by Rosati et al.¹³⁹ Currently, there are 3 recognized subtypes of neurothekeoma: myxoid, cellular, and mixed. Controversy exists about the lineage of CNT, with arguments for myofibroblastic, nerve sheath, melanocytic, and leiomyomatous differentiation. Immunoreactive markers span a spectrum of cell types, including focal staining with SMA, CD68, and CD10.^{140,141} Protein gene product 9.5 (PGP 9.5, also known as ubiquitin carboxyl-terminal hydroxylase-1) reactivity can be helpful in the identification of neurothekeoma but also suffers from low specificity.¹⁴² PGP 9.5 is positive in nerve sheath tumors, including granular cell tumors; fibroblastic tumors, including dermatofibromas, vascular tumors, and other tumors, such as leiomyomas.^{143,144} D2-40, as discussed previously, is expressed in lymphatics and PCANs, but reactivity has also been reported in the 15 CNTs thus far studied.¹⁴⁵ Cellular neurothekeomas and fibrous histiocytomas can share morphologic features, and both have shown expression of D2-40, further complicating differentiation.

Although myxoid neurothekeomas are S-100+, CNTs are not, arguing strongly against a peripheral nerve sheath or melanocytic histogenesis. However, CNTs are positive for S100A6 protein (calcyclin).^{142,146} Reactivity with that protein is not equivalent to a positive identification of CNT because it is also expressed in melanocytes and dermal dendrocytes.¹⁴² Cellular neurothekeomas also can be confused with melanocytic lesions, histologically. When considering this differential, S100A6 reactivity, with negative S-100 staining, essentially eliminates a melanocytic lesion. S-100 expression of colonizing antigen-presenting cells can be seen and should not be confused with lesional cell reactivity. Although the typical melanocytic

markers, S-100, HMB-45, and Melan A are absent in CNT, other less-specific melanocytic markers, such as S100A6 have been identified.^{141,142} Many CNTs express NKI-C3 (CD63), but that marker lacks specificity and has been identified in nevi, melanomas, granular cell tumors, and some fibrohistiocytic lesions.^{142,147} Expression of KBA.62, an antimelanoma monoclonal antibody, has recently been reported¹⁴⁸ in 18 of 18 CNTs (100%). MiTF is also a melanocytic marker that highlights most CNTs.

Dermatofibroma and Dermatofibrosarcoma Protuberans

Most dermatofibromas (DFs) can be easily distinguished from dermatofibrosarcoma protuberans (DFSPs); however, morphologic features alone do not always allow reliable distinction between deep or cellular DF and DFSP. Commonly, CD34 and factor XIIIa have been used in differentiation. Typically, DFSP is CD34+ and factor XIIIa-, and DF is CD34- and factor XIIIa+; however, that is not absolute.¹⁴⁹⁻¹⁵¹ Some DFs exhibit focal staining with CD34, especially at the periphery of cellular and deep DFs, and occasional DFSPs are negative for CD34.¹⁵² Caution is required in interpretation of DFSP margins with CD34 because CD34 disappears from scars but proliferates in pericicatricial tissue.¹⁵³ CD34 positivity is not exclusive to DFSP. It is also an endothelial marker and stains solitary fibrous tumor, spindle cell lipoma, superficial acral fibromyxoma, sclerotic fibroma, Kaposi sarcoma, neurofibroma, tricholemmoma, scleromyxedema, and nephrogenic systemic fibrosis, among others.¹⁵⁴⁻¹⁵⁶ Factor XIIIa is typically weak and only identified at the periphery of DF or more diffusely in spindle cell-dominant cellular DFs.¹⁵⁰ Many alternative markers have been explored but mostly in isolated studies with few cases. Those markers include HMGA1, HMGA2, CD163, ApoD, tenascin, S100A6, MMP-2 and -11, IGFBP7, cathepsin K, and D2-40.

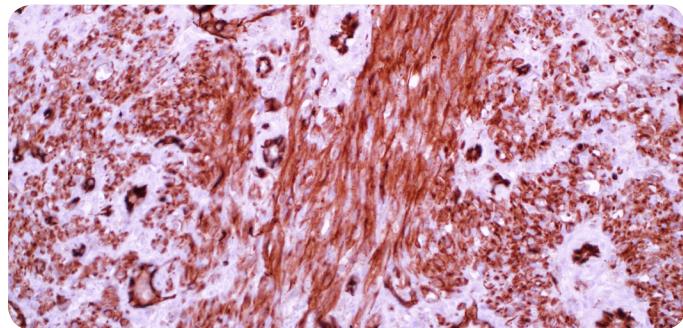
ERG has proven a sensitive and specific marker for vascular lesions, showing expression only in a subset of prostate carcinomas.

Merkel Cell Carcinoma vs. Cutaneous Small Cell Tumors

	CD45	CD117	Chromogranin A	CK, pan	CK 5&6	CK 20	HMB-45	Neurofilament	S-100	Synaptophysin	TTF-1	Vimentin
Merkel Cell Carcinoma	-	+	+	+	-	+	-	+	-	+	-	-
Small Cell Carcinoma	-	+/-	+	+	-	-	-	-	-	+	+	-
Lymphoma	+	-	-	-	-	-	-	-	-	-	-	+/-
Small Cell Melanoma	-	+	-	-	-	-	+	-	+	-	-	+
Squamous Cell Carcinoma	-	-	-	+	+	-	-	-	-	-	-	-

Nestin, a neuroectodermal and mesenchymal stem cell marker, was evaluated in a larger cohort of nearly 200 cases of DFSP and DF in 4 different studies,¹⁵⁷⁻¹⁶⁰ which showed similar results. Strong nestin expression was noted in DFSP, and no or only rare focal expression was seen in DFs. Unlike CD34, which can be absent or decreased in fibrosarcomatous areas of DFSP, nestin remains unaltered.¹⁶⁰ Immunohistochemistry for stromelysin 3, a member of the matrix metalloproteinase family, MMP-11, has also been studied in at least 5 separate studies^{151,161-164} with similar outcomes. Stromelysin 3 has a role in tissue remodeling during wound healing and tumor invasion, and there is diffuse cytoplasmic staining of the spindle cells in most DFs, whereas it has rarely been noted in DFSPs.

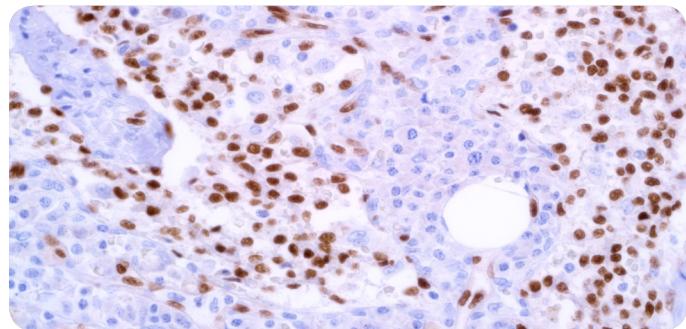
Vascular Neoplasms



CD31

Several endothelial markers are available including: von Willebrand factor (factor VIII-related antigen), CD34, CD31 (platelet-endothelial cell adhesion molecule type 1), FLI-1, and ERG. Factor VIII-related antigen is specific for endothelial cells but has a low sensitivity, and because it circulates in the serum, it can be seen in zones of necrosis and hemorrhage. CD34 is sensitive but is also expressed in several nonvascular tumors, including DFSP and epithelioid sarcoma. CD34 has been considered a sensitive marker for Kaposi sarcoma, but Kaposi sarcoma-associated herpes virus 8 latent nuclear antigen (HHV-8) has supplanted use of CD34 in that context.^{1,165} CD31 has been considered the most-sensitive marker for vascular endothelium and vascular tumors. It is expressed in endothelial cells but is not specific and has been reported in a subset of carcinomas, lymphomas, AFXs, epithelioid sarcoma, EWS/PNET, macrophages, platelets, and plasma

cells.^{21,166} More recently, FLI-1 and ERG were reported as nuclear markers of endothelial differentiation. Folpe et al¹⁶⁷ found FLI-1 expression in 94% of benign and malignant vascular tumors, but FLI-1 is also a marker for EWS/PNET, with expression in approximately 90% of cases, and it shows expression in some melanomas, leiomyosarcomas, SCCs, and AFXs.⁹¹ Thus far, ERG has proven a sensitive and specific marker for vascular lesions, showing expression only in a subset of prostate carcinomas, EWS/PNET, epithelioid sarcomas, and myeloid sarcoma.¹⁶⁸⁻¹⁷⁰ Rao et al¹⁶⁶ evaluated 34 angiosarcomas in a tissue microarray and found ERG and FLI-1 to have higher sensitivity than other immunomarkers. Angiosarcomas often show loss of expression of one or more endothelial markers, especially epithelioid angiosarcomas, which can also show keratin expression, suggesting that multiple antibodies be used for testing.



ERG

Markers that distinguish blood vessel endothelial cells from lymphatic endothelial cells have also been investigated. Prox1, a transcription factor important in the regulation and maintenance of the lymphatic endothelial phenotype, is expressed in lymphangiomas, Kaposi sarcoma, tufted angiomas, and Kaposiform hemangioendothelioma.¹⁷¹ The monoclonal antibody D2-40 binds to podoplanin, a glycoprotein highly expressed in lymphatic endothelium. D2-40 has been identified as a sensitive marker for lymphatic endothelium in normal tissue and in a subset of vascular lesions, including Kaposi sarcoma, Dabska tumor, lymphangioma, hobnail hemangioma (targetoid hemosiderotic hemangioma) and in a subset of angiosarcomas, especially those with epithelioid endothelial cells. In general, classic hemangiomas do not express D2-40.^{172,173} D2-40 is variably expressed in a wide variety of lesions, including sebaceous

neoplasm, epithelioid sarcoma, AFX, SCCs, CNTs, DFs, and PCANs. Expression of lymphatic markers, including Prox1 and D2-40, in angiosarcoma suggests that it may show a mixed endothelial phenotype.^{22,166,171} Infantile hemangiomas become apparent postnatally, enlarge rapidly during the first year of life, and spontaneously involute in the first decade. The characteristic histopathologic features diminish during that evolution, and differentiating them from vascular malformations can be difficult. Endothelial immunoreactivity for the erythrocyte-type glucose transporter protein (GLUT1), normally restricted to endothelia with blood-tissue barrier function, as in the brain and the placenta, is identified in all phases of infantile hemangiomas, whereas reactivity is absent in vascular malformations, pyogenic granulomas, and granulation tissue.¹⁷⁴⁻¹⁷⁶ Normal reactivity in erythrocytes must be discounted when interpreting the expression. Expression of GLUT1 has also been demonstrated in normal perineural cells and perineuriomas.¹⁷⁷ These S-100-, benign peripheral nerve sheath tumors are also positive with EMA but often in a focal and weak pattern because of their thin and widely separated cytoplasmic processes. Claudin-1, a component of tight junctions, and GLUT1 are stronger and stain more diffusely in perineurioma than does EMA.¹⁷⁸ Infantile hemangiomas and vascular malformations have distinct clinical courses, and the distinction is important for management considerations.

Wilms tumor 1 (WT1) cytoplasmic endothelial expression has been reported in vascular tumors, including infantile hemangiomas, noninvoluting congenital hemangiomas, rapidly involuting congenital hemangiomas, tufted angiomas, pyogenic granulomas, microvenular hemangiomas, and cherry hemangiomas, but is lacking in lymphatic and venous vascular malformations.¹⁷⁹⁻¹⁸¹ WT1 positivity was, however, reported in the 5 stage 2 arteriovenous malformations studied,¹⁸⁰ possibly because of active proliferation and clinical enlargement during that stage. Current data are limited, but WT1 results are conflicting for verrucous hemangioma and hobnail hemangioma, further drawing into question the appropriate classification of those lesions as neoplasms or malformations.¹⁸¹⁻¹⁸³ Another important distinction includes distinguishing rapidly involuting congenital hemangioma and noninvoluting congenital hemangioma from infantile hemangioma. Those lesions behave differently, as the names suggest, but have histologic features that overlap with infantile hemangioma. However, neither rapidly involuting congenital hemangioma nor noninvoluting congenital hemangioma expresses GLUT1.^{176,184}

Hematopoietic And Histiocytic Tumors

Cutaneous B-Cell Lymphomas

CD20 is a B-cell-specific marker expressed in 98% of B-cell lymphomas, but it may be lost in the lymphomas treated with the anti-CD20 immunotherapy (rituximab). CD79a is expressed in the precursor B-cell stage and disappears later than CD20 in B-lymphocyte differentiation, explaining why plasma cells are positive for CD79a but not for CD20. CD79a, therefore, stains

most B-cell lesions, even rituximab-treated B-cell lymphomas. PAX-5 is a nuclear marker expressed in early B-cell development and is, therefore, negative in plasma cells, but it can stain recurrent B-cell lymphoma following rituximab therapy.^{185,186} CD38 and CD138 (syndecan-1) are expressed in plasma cells.

Aside from lymphocytoma cutis, a significant B-cell infiltrate in the skin is rarely reactive and should suggest possible B-cell lymphoma. Architecturally, the infiltrate may be nodular or diffuse. The diffuse pattern includes follicle center cell lymphoma, diffuse large B-cell lymphoma (DLBCL) in both the leg type and the otherwise-not-specified type. The nodular pattern is seen in lymphocytoma cutis, marginal zone lymphoma, and a small proportion of follicle center cell lymphomas. Normal germinal centers are BCL6+, BCL2-, and CD10+ with a CD21+ and/or CD23+ follicular dendritic cell network and high proliferative index. Benign lymphoid follicles can be seen in lymphocytoma cutis and marginal zone lymphoma. The interfollicular component of marginal zone lymphoma is neoplastic, and immunohistochemically is BCL2+, whereas BCL6 and CD10 are negative. When there is plasmacytoid differentiation, light-chain restriction can be evaluated immunohistochemically; 2:1 is the normal K:λ ratio, but above 5:1 or a 3:1 λ:K ratio supports clonality. However, reproducibility for this ratio is low.¹⁸⁷

Cutaneous follicle center cell lymphoma is of germinal center cell origin and is thus BCL6+ and BCL2-. If BCL2 expression is present, it suggests spread of the disease to the skin from the lymph nodes.¹⁸⁵ T cells are BCL2+ and comprise a proportion of the lymphoid cells in a follicle center cell lymphoma. BCL2 expression must be interpreted in the neoplastic cells alone and must not be confused with those colonizing T cells. The leg-type DLBCL is also CD20+ and BCL6+, but unlike follicle center cell lymphoma, it expresses BCL2, MUM1, and FOXP1.¹⁸⁸ MUM1 is also expressed in melanoma and anaplastic large cell lymphoma (ALCL).

Cutaneous T-Cell Lymphomas

Cutaneous lymphoproliferative disorders can be one of the most vexing problems in dermatopathology. Mycosis fungoides-type cutaneous T-cell lymphoma (MF) must be differentiated from reactive T-cell infiltrates. Polymerase chain reaction analysis of T-cell receptors for clonality has become helpful in this context; however, many cases of early MF are polyclonal by PCR, and monoclonality has been identified in several cases of benign dermatoses.¹⁸⁹ Like benign inflammatory infiltrates, most cases of MF have a CD4+ T helper cell phenotype but, in contrast, may show loss of expression of other T-cell markers in the epidermal component. The most frequent down-regulated antigen is CD7, closely followed by CD5.¹⁸⁷ However, that down regulation is not absolute, and some benign inflammatory infiltrates, especially acute ones, show loss of CD7.¹⁹⁰ Therefore, interpretation of CD7 should be made in the context of the clinical and histologic features. The relative absence of a pan-T-cell marker CD3, CD5, or CD43, or the concordant expression or loss of both CD4 and CD8, are more

If BCL2 expression is present, it suggests spread of the disease to the skin from the lymph nodes.

likely to be seen in MF than in benign conditions. Some studies have suggested that a CD4:CD8 epidermal ratio greater than 2 supports MF, rather than an inflammatory process.¹⁹¹ However, there is significant overlap, with inflammatory conditions showing a ratio up to 6.¹⁹² Ortonne and colleagues¹⁹³ attempted to improve detection of early MF and found an epidermal CD8:CD3 ratio of less than 25% to be suggestive, but not specific, for MF. Use of CD3, rather than CD4, obviates concern about misinterpretation of CD4+ Langerhans cells. In contrast, there is a subset of CD8+ MF, which is most common in children and hypopigmented cases.¹⁸⁷ The International Society for Cutaneous Lymphoma proposed an algorithm for detection of early MF, based on a scoring system involving clinical, histopathologic, biomolecular, and immunopathologic criteria. The immunohistologic features included in the score are CD2, CD3, or CD5 positivity in less than 50% of T-cells, CD7 positivity in less than 10% of T-cells, and epidermal/dermal discordance in expression of CD2, CD3, CD5, or CD7 expression.¹⁹⁴

Subcutaneous panniculitis-like T-cell lymphoma is a CD8+ cytotoxic T-cell neoplasm, by definition, of the alpha-beta receptor class and is, therefore, β-F1+, distinguishing it from cutaneous gamma-delta lymphoma. Subcutaneous panniculitis-like T-cell lymphoma is typically positive for TIA-1, granzyme B, and perforin. The adipocyte rimming by T cells, particularly when they are Ki-67+, is a useful feature in detection.¹⁸⁷

Infectious Agents

IHC currently provides a rapid morphologic differential of infections in tissue samples from patients, thus facilitating clinical decisions in patient care. In infectious disease detection, IHC has been shown to be useful in identifying microorganisms that are (1) difficult to detect by routine or special stains, (2) stain poorly, (3) present in low numbers, or (4) noncultivable. Although several antibodies have been developed to aid in the identification using IHC for many infectious diseases, only a small subset of these antibodies is useful in dermatopathology for multiple reasons.¹⁹⁵⁻¹⁹⁷ The use of IHC in the detection of cutaneous infections has been applied

to identify specific viral and bacterial infections that may be difficult to detect with certainty using routine microscopy alone.

IHC and Viral Pathogens

IHC can be quite useful in differentiating changes attributed to herpes simplex virus (HSV) from possible mimics, in the case of atypical cutaneous manifestations. For example, IHC was able to identify HSV infection in 5 bedridden geriatric patients (type I in 3 and type II in 2) with genital ulcers, when histology was suggestive of HSV infection in only 2 of the 5 patients.^{197,198} One study showed that the sensitivity and specificity of IHC was comparable with ISH in identifying HSV.¹⁹⁹ Despite the high sensitivity of the polyclonal antibodies used, they do not differentiate between the antigenically similar HSV I and HSV II.⁷ Similarly, IHC has also been shown to have higher specificity and sensitivity than standard microscopic assessments in detecting varicella zoster virus (VZV) infection (varicella and herpes zoster) through detection of VZVORF63 encoded protein (IE63) and VZV late protein gE on both smears and formalin-fixed paraffin-embedded skin sections.²⁰⁰ This can be of special significance in allowing early detection of VZV infection in immunocompromised patients and thus early treatment.

In addition to its presence in various benign (inflammatory pseudotumor) and malignant (non-Hodgkin lymphoma, Hodgkin disease, nasopharyngeal carcinoma, gastric carcinoma) lesions, Epstein-Barr virus (EBV) causes oral hairy leukoplakia and infectious mononucleosis.²⁰¹ IHC has long been used in the detection of EBV infections through identification of EBV-expressed proteins, which also allow distinction of latent from replicative infection based on expression profiles. Actually, LMP1 immunostain (cytoplasmic and membranous localization) has been shown to be nearly as effective as EBV-encoded RNA ISH in identifying EBV in lymph nodes of patients with infectious mononucleosis. Detection of other EBV proteins such as BZLF1, EBNA1, EBNA2, and LMP2A can be achieved by IHC.²⁰¹

Cytomegalovirus (CMV) infection is especially common in immune-compromised patients (such as HIV-infected patients), and the cutaneous manifestations

can be variable including ulcers, vesicles, papules, purpuric macules, verrucous lesions, prurigo nodularis-like lesions, and digital infarcts. Although the sensitivity of light microscopy is high in the detection of CMV inclusions mostly within endothelial cells and, occasionally, within keratinocytes, sweat gland epithelial cells, macrophages, and fibroblasts, immunohistochemical analysis may be needed to confirm the diagnosis in certain cases such as the presence of intense inflammation or in cases where only 1 or 2 cells are infected.²⁰² IHC allows for rapid results and its sensitivity is better than light microscopy and is comparable with culture and ISH.

Human herpesvirus 8 (HHV-8) is a major cause of Kaposi sarcoma (KS). Through positive nuclear immunostaining for HHV-8 latent nuclear antigen-1 in spindle cells and cells lining the primitive and thin-walled vascular channels, immunohistochemical detection of the protein expressed by HHV-8 in paraffin-embedded sections has been shown to be highly sensitive and specific for KS and thus allowing its differentiation from its mimickers including angiosarcoma, kaposiform hemangioendothelioma, spindle-cell hemangioma, among others.^{203,204} Immunolocalization in the nuclei was considered positive.

IHC and Bacterial Pathogens

During routine evaluation, the use of Steiner or Warthin-Starry silver stains to confirm suspected syphilis is of low sensitivity (range from 33% to 71%), as it is usually hampered by significant background staining.²⁰⁴⁻²⁰⁷ Several studies have shown that immunohistochemical staining provides better sensitivity than silver stains for the detection of *Treponema pallidum* (*T. pallidum*).²⁰⁵⁻²⁰⁷ Using a monoclonal antibody, Hoang et al²⁰⁵ showed positive staining in 12 of 17 (71%) biopsy specimens taken from patients with secondary syphilis compared with 41% detection using silver stain. In another study in which Buffet et al²⁰⁶ used a polyclonal antibody to *T. pallidum*, the spirochetes were detected in 11 of 12 (91%) skin biopsy specimens taken from patients with secondary syphilis. Recently, Martín-Ezquerre et al²⁰⁷ studied the sensitivity of silver staining and immunohistochemical *T. pallidum* polyclonal antibody expression in 34 biopsy specimens of 8 patients with primary syphilis and 26 with secondary syphilis. Immunohistochemical analysis showed greater sensitivity than Warthin-Starry staining, as spirochetes were identified in 29 of 34 (85%) cases (8 of 8 primary and 21 of 26 secondary syphilis) using IHC, although Warthin-Starry staining disclosed spirochetes in 17 of 34 (50%) biopsy specimens (4 of 8 primary and 13 of 26 secondary syphilis). In this study, the authors, using IHC, were also able to identify different distribution patterns of *T. pallidum* in primary and secondary syphilis. Although primary syphilis showed both an epitheliotropic (abundant spirochetes in an intercellular distribution in the lower mucosa/epidermis) and vasculotrophic (treponemes surrounding the vascular walls) patterns, secondary syphilis only showed an

epitheliotropic pattern. The authors concluded that the immunohistochemical distribution pattern of *T. pallidum* may permit differentiation of primary from secondary syphilis.²⁰⁷

References

1. Folpe AL, Cooper K. Best practices in diagnostic immunohistochemistry: pleomorphic cutaneous spindle cell tumors. *Arch Pathol Lab Med*. 2007;131:1517-1524.
2. Ko CJ, McNiff JM, Glusac EJ. Squamous cell carcinoma with single cell infiltration: a potential diagnostic pitfall and the utility of MNF116 and p63. *J Cutan Pathol*. 2008;35:353-357.
3. Morgan MB, Purohit C, Anglin TR. Immunohistochemical distinction of cutaneous spindle cell carcinoma. *Am J Dermatopathol*. 2008;30:228.
4. Sigel JE, Skacel M, Bergfeld WF, House NS, Rabkin MS, Goldblum JR. The utility of cytokeratin 5/6 in the recognition of cutaneous spindle cell squamous cell carcinoma. *J Cutan Pathol*. 2001;28:520-524.
5. Jensen K, Wilkinson B, Wines N, Kossard S. Procollagen 1 expression in atypical fibroxanthoma and other tumors. *J Cutan Pathol*. 2004;31:57-61.
6. Krstrup D, Rossen K, Thomsen HK. Procollagen 1—a marker of fibroblastic and fibrohistiocytic skin tumors. *J Cutan Pathol*. 2006;33:614-618.
7. Leinweber B, Hofmann-Wellenhof R, Kaddu S, McCalmon TH. Procollagen 1 and Melan-A expression in desmoplastic melanomas. *Am J Dermatopathol*. 2009;31:173.
8. Fullen DR, Reed JA, Finnerty B, McNutt NS. S100A6 expression in fibrohistiocytic lesions. *J Cutan Pathol*. 2001;28:229-234.
9. Fullen DR, Garrisi AJ, Sanders D, Thomas D. Expression of S100A6 protein in a broad spectrum of cutaneous tumors using tissue microarrays. *J Cutan Pathol*. 2008;35(suppl s2):28-34.
10. Hultgren TL, DiMaio DJ. Immunohistochemical staining of CD10 in atypical fibroxanthomas. *J Cutan Pathol*. 2007;34:415-419.
11. Sakamoto A, Oda Y, Yamamoto H, et al. Calponin and h-caldesmon expression in atypical fibroxanthoma and superficial leiomyosarcoma. *Virchows Archiv*. 2002;440:404-409.
12. Beer TW, Haig D. CD117 is not a useful marker for diagnosing atypical fibroxanthoma. *Am J Dermatopathol*. 2009;31:649-652.
13. Longacre TA, Smoller BR, Rouse RV. Atypical fibroxanthoma. Multiple immunohistologic profiles. *Am J Surg Pathol*. 1993;17:1199-1209.
14. Smith-Zagone MJ, Prieto VG, Hayes RA, Timperman WW Jr, Diwan AH. HMB-45 (gp103) and MART-1 expression within giant cells in an atypical fibroxanthoma: a case report. *J Cutan Pathol*. 2004;31:284-286.
15. Thum C, Hollowood K, Birch J, Goodlad JR, Brenn T. Aberrant Melan-A expression in atypical fibroxanthoma and undifferentiated pleomorphic sarcoma of the skin. *J Cutan Pathol*. 2011;38:954.
16. Ma CK, Zarbo RJ, Gown AM. Immunohistochemical characterization of atypical fibroxanthoma and dermatofibrosarcoma protuberans. *Am J Clin Pathol*. 1992;97:478-483.
17. Montegudo C, Calduch L, Navarro S, Joan-Figueroa A, Llombart-Bosch A. CD99 immunoreactivity in atypical fibroxanthoma: a common feature of diagnostic value. *Am J Clin Pathol*. 2002;117:126-131.
18. Pouryazdanparast P, Yu L, Cutlan JE, Olsen SH, Fullen DR, Ma L. Diagnostic value of CD163 in cutaneous spindle cell lesions. *J Cutan Pathol*. 2009;36:859.
19. Buonaccorsi JN, Plaza JA. Role of CD10, wide-spectrum keratin, p63, and podoplanin in the distinction of epithelioid and spindle cell tumors of the skin. *Am J Dermatopathol*. 2012;34:404.
20. Bull C, Mirzabeigi M, Laskin W, et al. Diagnostic utility of low-affinity nerve growth factor receptor (P 75) immunostaining in atypical fibroxanthoma. *J Cutan Pathol*. 2011;38:631.

21. Thum C, Husain EA, Mulholland K, Hornick JL, Brenn T. Atypical fibroxanthoma with pseudoangiomatous features: a histological and immunohistochemical mimic of cutaneous angiosarcoma. *Ann Diagn Pathol.* 2013;17:502–507.
22. Cuda J, Mirzamani N, Kantipudi R, Robbins J, Welsch MJ, Sundram UN. Diagnostic utility of FLI-1 and D2-40 in distinguishing atypical fibroxanthoma from angiosarcoma. *Am J Dermatopathol.* 2013;35:316–318.23.
23. Kucher C, Zhang PJ, Pasha T, et al. Expression of Melan-A and Ki-67 in desmoplastic melanoma and desmoplastic nevi. *Am J Dermatopathol.* 2004;26:452–457.
24. Radfar A, Stefanato CM, Ghosn S, Bhawan J. NGFR-positive desmoplastic melanomas with focal or absent S-100 staining: further evidence supporting the use of both NGFR and S-100 as a primary immunohistochemical panel for the diagnosis of desmoplastic melanomas. *Am J Dermatopathol.* 2006;28:162–167.
25. Sigal AC, Keenan M, Lazova R. P75 nerve growth factor receptor as a useful marker to distinguish spindle cell melanoma from other spindle cell neoplasms of sun-damaged skin. *Am J Dermatopathol.* 2012;34:145–150.
26. Kanik AB, Yaar M, Bhawan J. p75 nerve growth factor receptor staining helps identify desmoplastic and neurotropic melanoma. *J Cutan Pathol.* 1996;23:205–210.
27. Otaibi S, Jukic DM, Drogowski L, Bhawan J, Radfar A. NGFR (p75) expression in cutaneous scars; further evidence for a potential pitfall in evaluation of re-excision scars of cutaneous neoplasms, in particular desmoplastic melanoma. *Am J Dermatopathol.* 2011;33:65–71.
28. Palla B, Su A, Binder S, Dry S. SOX10 expression distinguishes desmoplastic melanoma from its histologic mimics. *Am J Dermatopathol.* 2013;35(5):576–581.
29. Ramos-Herberth FI, Karamchandani J, Kim J, Dadras SS. SOX10 immunostaining distinguishes desmoplastic melanoma from excision scar. *J Cutan Pathol.* 2010;37:944–952.
30. Ansai S, Takeichi H, Arase S, Kawana S, Kimura T. Sebaceous carcinoma: an immunohistochemical reappraisal. *Am J Dermatopathol.* 2011;33:579–587.
31. Dasgeb B, Mohammadi TM, Mehregan DR. Use of ber-EP4 and epithelial specific antigen to differentiate clinical simulators of basal cell carcinoma. *Biomark Cancer.* 2013;5:7–11.
32. Fan YS, Carr RA, Sanders DS, Smith AP, Lazar AJ, Calonje E. Characteristic ber-EP4 and EMA expression in sebaceousoma is immunohistochemically distinct from basal cell carcinoma. *Histopathology.* 2007;51:80–86.
33. Sramek B, Lisle A, Loy T. Immunohistochemistry in ocular carcinomas. *J Cutan Pathol.* 2008;35:641–646.
34. Ostler DA, Prieto VG, Reed JA, Deavers MT, Lazar AJ, Ivan D. Adipophilin expression in sebaceous tumors and other cutaneous lesions with clear cell histology: an immunohistochemical study of 117 cases. *Mod Pathol.* 2010;23:567–573.
35. Boussahmain C, Mochel MC, Hoang MP. Perilipin and adipophilin expression in sebaceous carcinoma and mimics. *Hum Pathol.* 2013;44:1811–1816.
36. Milman T, Schear MJ, Eagle RC. Diagnostic utility of adipophilin immunostain in periocular carcinomas. *Ophthalmology.* 2014;121:964–971.
37. Cabral ES, Auerbach A, Killian JK, Barrett TL, Cassarino DS. Distinction of benign sebaceous proliferations from sebaceous carcinomas by immunohistochemistry. *Am J Dermatopathol.* 2006;28:465–471.
38. Abbas O, Mahalingam M. Cutaneous sebaceous neoplasms as markers of Muir-Torre syndrome: a diagnostic algorithm. *J Cutan Pathol.* 2009;36:613–619.
39. Singh RS, Grayson W, Redston M, et al. Site and tumor type predicts DNA mismatch repair status in cutaneous sebaceous neoplasia. *Am J Surg Pathol.* 2008;32:936–942.
40. Roberts ME, Riegert-Johnson DL, Thomas BC, et al. Screening for Muir-Torre syndrome using mismatch repair protein immunohistochemistry of sebaceous neoplasms. *J Genet Couns.* 2013;22:393–405.
41. Orta L, Klimstra DS, Qin J, et al. Towards identification of hereditary DNA mismatch repair deficiency: sebaceous neoplasm warrants routine immunohistochemical screening regardless of patient's age or other clinical characteristics. *Am J Surg Pathol.* 2009;33:934–944.
42. Fernandez-Flores A. Considerations on the performance of immunohistochemistry for mismatch repair gene proteins in cases of sebaceous neoplasms and keratoacanthomas with reference to Muir-Torre syndrome. *Am J Dermatopathol.* 2012;34:416.
43. Al-Zaid T, Ditelberg JS, Prieto VG, et al. Trichilemmomas show loss of PTEN in Cowden syndrome but only rarely in sporadic tumors. *J Cutan Pathol.* 1998;25:493–499.
44. Shon W, Wolz M, Sukov W, Wieland C, Gibson L, Peters M. PTEN status in sporadic and Cowden syndrome-associated trichilemmomas: evaluation of immunohistochemistry and fluorescence in situ hybridization [published online ahead of print January 9, 2014]. *Br J Dermatol.* doi: 10.1111/bjd.12827.
45. Illueca C, Monteagudo C, Revert A, Llombart-Bosch A. Diagnostic value of CD34 immunostaining in desmoplastic trichilemmoma. *J Cutan Pathol.* 1998;25:435–439.
46. Qureshi HS, Ormsby AH, Lee MW, Zarbo RJ, Ma CK. The diagnostic utility of p63, CK5/6, CK 7, and CK 20 in distinguishing primary cutaneous adnexal neoplasms from metastatic carcinomas. *J Cutan Pathol.* 2004;31:145–152.
47. Plaza JA, Ortega PF, Stockman DL, Suster S. Value of p63 and podoplanin (D2-40) immunoreactivity in the distinction between primary cutaneous tumors and adenocarcinomas metastatic to the skin: a clinicopathologic and immunohistochemical study of 79 cases. *J Cutan Pathol.* 2010;37:403–410.
48. Mahalingam M, Nguyen LP, Richards JE, Muzikansky A, Hoang MP. The diagnostic utility of immunohistochemistry in distinguishing primary skin adnexal carcinomas from metastatic adenocarcinoma to skin: an immunohistochemical reappraisal using cytokeratin 15, nestin, p63, D2-40, and calretinin. *Mod Pathol.* 2010;23:713–719.
49. Rollins-Raval M, Chivukula M, Tseng GC, Jukic D, Dabbs DJ. An immunohistochemical panel to differentiate metastatic breast carcinoma to skin from primary sweat gland carcinomas with a review of the literature. *Arch Pathol Lab Med.* 2011;135:975–983.
50. Plumb SJ, Argenzi ZB, Stone MS, De Young BR. Cytokeratin 5/6 immunostaining in cutaneous adnexal neoplasms and metastatic adenocarcinoma. *Am J Dermatopathol.* 2004;26:447–451.
51. Ivan D, Nash JW, Prieto VG, et al. Use of p63 expression in distinguishing primary and metastatic cutaneous adnexal neoplasms from metastatic adenocarcinoma to skin. *J Cutan Pathol.* 2007;34:474–480.
52. Liang H, Wu H, Giorgadze TA, et al. Podoplanin is a highly sensitive and specific marker to distinguish primary skin adnexal carcinomas from adenocarcinomas metastatic to skin. *Am J Surg Pathol.* 2007;31:304–310.
53. Alcaraz I, Cerroni L, Rüttgen A, Kutzner H, Requena L. Cutaneous metastases from internal malignancies: a clinicopathologic and immunohistochemical review. *Am J Dermatopathol.* 2012;34:347–393.
54. Barbareschi M, Murer B, Colby TV, et al. CDX-2 homeobox gene expression is a reliable marker of colorectal adenocarcinoma metastases to the lungs. *Am J Surg Pathol.* 2003;2:141–149.
55. Tanaka S, Saito K, Ito T, et al. CDX2 as a useful marker of colorectal adenocarcinoma metastases to lung in pre-operative biopsy specimens. *Oncol Rep.* 2007;18:87–92.
56. Ordóñez NG. Thyroid transcription factor-1 is not expressed in squamous cell carcinoma of the lung: an immunohistochemical study with review of the literature. *Appl Immunohistochem Mol Morphol.* 2012;20:525–530.
57. Park SY, Kim BH, Kim JH, Lee S, Kang GH. Panels of immunohistochemical markers help determine primary sites of metastatic adenocarcinoma. *Arch Pathol Lab Med.* 2007;131:1561–1567.
58. Bahrami S, Malone JC, Lear S, Martin AW. CD10 expression in cutaneous adnexal neoplasms and a potential role for differentiating cutaneous metastatic renal cell carcinoma. *Arch Pathol Lab Med.* 2006;130:103–107.

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