2 Ancillary Diagnostic Tests in the Diagnosis of Cutaneous Soft Tissue Neoplasms

Andrew L. Folpe

Abstract

This chapter will cover selected applications of immunohistochemistry (IHC) in the diagnosis of soft tissue tumors of the skin. This section will emphasize applications of IHC to common differential diagnoses in cutaneous soft tissue pathology, including (1) pleomorphic spindle cell tumors; (2) epithelioid malignant neoplasms; (3) monomorphic spindle cell tumors; (4) "fibrohistiocytic" or "histiocytoid" lesions; (5) small, round blue cell tumors; and (6) adipocytic tumors. It is not possible in this relatively brief section to provide a detailed discussion of each antigen or of every rare soft tissue tumor that may occasionally be seen in the skin, and the reader is referred to the relevant chapters in this book for more detailed discussions of specific entities.

It cannot be overemphasized that IHC is an *adjunctive* diagnostic technique to traditional morphologic methods in cutaneous soft tissue pathology, as in any other area of surgical pathology. The diagnosis of some soft tissue tumors does not generally require IHC (e.g., adipocytic tumors), and IHC should in almost all instances not be relied upon to distinguish benign from malignant tumors (e.g., the distinction of nodular fasciitis from leiomyosarcoma). Additionally, specific markers do not exist for certain mesenchymal cell types and their tumors, and a subset of soft tissue tumors defies classification, even with exhaustive IHC and ultrastructural and genetic study. There are also an increasing number of instances where molecular genetic testing may be more useful for selected differential diagnoses than are IHC tests. Table 2.1 summarizes the most widely used IHC markers for the diagnosis of cutaneous soft tissue tumors and their sarcoma diagnosis. Table 2.2 provides an overview of markers expressed by specific common tumor types.

Basic Principles of Diagnostic Immunohistochemistry

Attention to Positive and Negative Internal Controls

It cannot be overemphasized that all IHC slides must be evaluated for appropriate staining of normal tissues for the antigen in question. Because specimen fixation is the single greatest variable in IHC, internal positive and negative controls are much more important than are external controls, whether those external controls are placed on the same glass slide or not. Thankfully, the skin is rich in normal internal positive controls for the vast majority of antigens (see Table 2.1).

A. L. Folpe (✉)
Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA
e-mail: folpe.andrew@mayo.edu

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Table 2.1 Commonly used immunohistochemical markers in sarcoma diagnosis

Antigen	Expressed in
Keratins	Carcinomas, epithelioid sarcoma, synovial sarcoma, some angiosarcomas and leiomyosarcomas, mesothelioma, rhabdoid tumor, myoepithelial tumors
Desmin	Benign and malignant smooth and skeletal muscle tumors
Glial fibrillary acidic protein	Gliomas, some schwannomas, myoepithelial tumors
Smooth muscle actin	Benign and malignant smooth muscle tumors, myofibroblastic tumors and pseudotumors, myoepithelial tumors
Caldesmon	Benign and malignant smooth muscle tumors
Myogenic nuclear regulatory proteins (myogenin, MyoD1)	Rhabdomyosarcoma
S-100 protein	Melanoma, benign and malignant peripheral nerve sheath tumors, cartilaginous tumors, normal adipose tissue, Langerhans cells, myoepithelial tumors
SOX10	Melanoma, benign and malignant peripheral nerve sheath tumors, myoepithelial tumors
Epithelial membrane antigen	Carcinomas, epithelioid sarcoma, synovial sarcoma, perineurioma, meningioma, anaplastic large-cell lymphoma, some low-grade fibromyxoid sarcoma
CD31	Benign and malignant vascular tumors, histiocytes
CD34	Benign and malignant vascular tumors, solitary fibrous tumor, epithelioid sarcoma, dermatofibrosarcoma protuberans
STAT6	Solitary fibrous tumor
CD99 (MIC2 gene product)	Ewing sarcoma/primitive neuroectodermal tumor, some rhabdomyosarcomas, some synovial sarcomas, lymphoblastic lymphoma
CD45 (leukocyte common antigen)	Non-Hodgkin lymphoma
TdT	Lymphoblastic lymphoma
CD30	Anaplastic large-cell lymphoma, embryonal carcinoma
CD68 and CD163	Macrophages, fibrohistiocytic tumors, granular cell tumors, various sarcomas, melanomas, carcinomas
Melanosome-specific antigens (HMB-45, Melan-A, tyrosinase, microphthalmia transcription factor)	Melanoma, PEComa, clear cell sarcoma, melanotic schwannoma
Claudin-1	Perineurioma
Mdm2 and CDK4	Well-differentiated liposarcoma
Glut-1	Perineurioma, infantile hemangioma
SMARCB1 (INI1)	Expression lost in extrarenal rhabdoid tumor, epithelioid sarcoma, epithelioid malignant peripheral nerve sheath tumor, malignant myoepithelioma
TLE1	Synovial sarcoma
WT-1 (carboxy-terminus)	Desmoplastic small round cell tumor

Table 2.2 Commonly evaluated antigens and their normal positive internal controls in the skin

Antigen	Normal positive internal control in the skin
Pan-keratin and low-molecular-weight cytokeratins	Suprabasal keratinocytes and adnexae
High-molecular-weight cytokeratins	Basal keratinocytes
S100 protein	Melanocytes, Langerhans cells, nerves, myoepithelial cells
Melanocytic markers (e.g., HMB-45, Melan-A, MiTF, tyrosinase)	Dermal melanocytes (may be HMB-45 negative)
Smooth muscle actins	Pilar smooth muscle
Desmin	Pilar smooth muscle
Endothelial markers (e.g., CD31, CD34, FLI-1, ERG)	Endothelial cells

Attention to detail is critical here, as is, for example, not uncommon to see instances in which "pan-keratin" antibodies fail to label only basal keratinocytes, a phenomenon which may result in the misdiagnosis of high-molecular-weight keratin-positive sarcomatoid squamous cell carcinoma as "atypical fibroxanthoma" (Fig. 2.1a–c). Evaluation of negative controls simply involves ascertaining that tissues that do not express an antigen are negative. Inappropriate staining of negative controls is most often the result of inappropriate antibody concentration and/or excessive epitope retrieval.

Evaluation of all Pieces of Tissue on the Entire Slide and Careful Discrimination of Neoplastic from Nonneoplastic Cell Populations

A surprising number of cases are referred in consultation because the referring pathologist simply overlooked the most relevant IHC findings, such as a small focus of keratin or HMB-45-positive cells in a sarcomatoid carcinoma or melanoma, respectively. Similarly, it is critical to evaluate IHC studies at close enough magnification in order to determine whether staining is present in the neoplastic cells themselves or in a background population of nonneoplastic cells (e.g., misinterpretation of smooth muscle actin-positive myofibroblasts in desmoplastic melanoma as evidence of a myofibroblastic tumor or misinterpretation of CD31-positive intralesional histiocytes in carcinoma as "epithelioid angiosarcoma").

The Use of a Panel of Immunostains, Including Both Expected Positives and Negatives for All of the Entities in the Histologic Differential Diagnosis

The routine use of a small, carefully selected panel is time- and cost-effective and greatly eliminates the potential for misdiagnosis owing to anomalous expression of antigens (e.g., keratin expression in melanoma or angiosarcoma).

Epithelial Markers

Keratins

IHC to detect keratin expression is the most common way to detect epithelial differentiation, especially in the differential diagnosis of sarcomatoid squamous cell carcinoma, but also in some mesenchymal tumors with epithelioid morphology (e.g., epithelioid sarcoma and myoepitheliomas).

The skin serves as a superb control tissue for the evaluation of keratin expression, as each epithelial cell type present within the skin expresses unique keratins, as detailed in Table 2.3. The keratins (also known as cytokeratins) are a family of 20 intermediate filament proteins, whose expression is largely restricted to epithelial cells (although certain mesenchymal cell types, such as endothelium and smooth muscle, also routinely express keratins). Keratins can be thought of as individual cytokeratins (e.g., K 7, K 20), as acidic and basic pairs (e.g., K 8/18), or as "low"- and "high"-molecular-weight keratins. It is important to realize that the division of the keratins into low- and high-molecular weights is quite arbitrary, and it is more useful to think of the low-molecular-weight keratins as those of simple epithelia, such as simple ductules, and the high-molecular-weight keratins as those of complex epithelia, such as the urothelium or epidermis.

Pathologists most often consider keratins in terms of the antibodies used to identify them. The most widely used pan-keratin antibody is probably still the AE1/AE3 cocktail. AE1 recognizes the acidic keratins 10, 14, 15, 16, and 19, whereas AE3 recognizes the basic keratins 1, 2, 3, 4, 5, 6, and 8. Both antibodies recognize a mixture of high- and low-molecular-weight keratins, and there is no value in running them independently. So-called "wide-spectrum" keratin antibodies, such as the OSCAR mAb and a variety of polyclonal antibodies, potentially have somewhat broader keratin coverage than does the AE1/AE3 cocktail, although this is laboratory dependent. For example, the OSCAR mAb seems in most laboratories to perform as a more sensitive version of CAM 5.2, without coverage of high-molecular-weight keratins. MNF116 is another broad-spectrum keratin antibody that recognizes

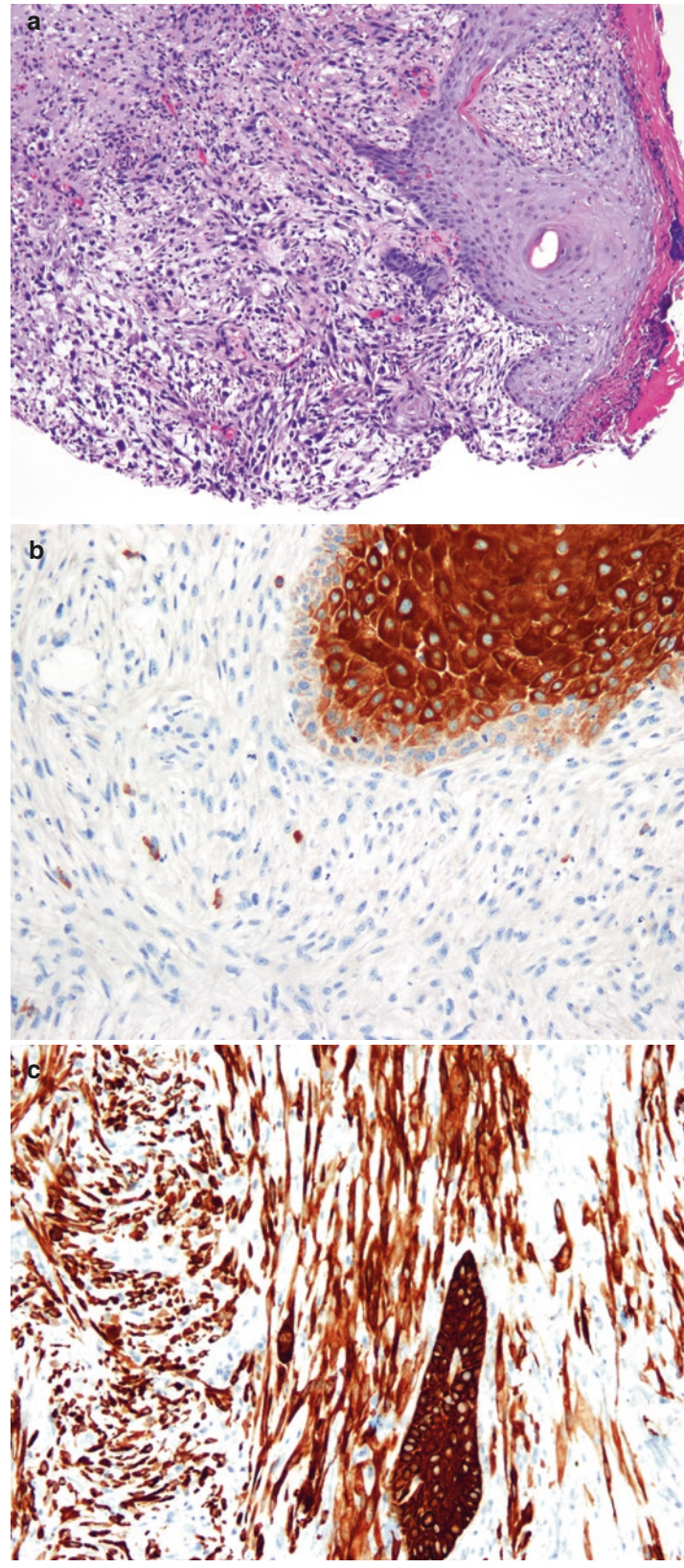


Fig. 2.1 Sarcomatoid squamous cell carcinoma (**a**). This case was referred in consultation with a suggested diagnosis of "atypical fibroxanthoma," because the outside pan-keratin immunostains (AE1/AE3) were negative. Close inspection, however, showed that the normal basal cells showed weak to absent staining (**b**), suggesting a false-negative study. Immunohistochemistry for keratins 5/6 was strongly positive in the tumor, confirming the diagnosis of sarcomatoid squamous cell carcinoma (**c**)

Table 2.3 Cytokeratin expression in normal skin

Cell type	Cytokeratins expressed
Suprabasal keratinocytes	1, 10, (11)
Basal keratinocytes	5 and 14
Hair shaft	5, 6, (14), 15, 16, 17
Eccrine ductular cells	7, 8, 17, 18, 19
Eccrine myoepithelial cells	5, 6, 14

keratins 5, 6, 8, 17, and 19. The most widely used low-molecular-weight keratin antibodies are CAM 5.2 (CK 8, 18, 19) and 35BH11 (CK 8 and 18). Almost all laboratories use the high-molecular-weight keratin antibody 34BE12 (CK 1, 5, 10, 14/15), also known as "cytokeratin 903," and keratin 5/6 antibodies are also widely utilized.

As mentioned previously, pan-keratin antibodies (e.g., AE1/3) frequently do not highlight the higher-molecular-weight keratins as well. As a general rule, IHC for keratins primarily plays a role in the diagnosis of sarcomatoid squamous cell carcinomas and a few mesenchymal tumors, such as epithelioid sarcoma.

Epithelial Membrane Antigen (EMA)

EMA is best thought of as a "second-line" epithelial marker. EMA is neither as specific nor as sensitive as are various keratin antibodies in the diagnosis of sarcomatoid carcinoma, epithelioid sarcoma, and other cutaneous soft tissue tumors with epithelial marker expression, such as myoepithelioma. EMA is, however, still the most useful marker for the diagnosis of perineurial tumors.

p63 and p40

The *p63* gene is a member of the *p53* gene family but does not appear to be involved in tumor suppression. p63 protein expression is routinely present in keratinocytes, as well as myoepithelial cells in the skin. Although p63 expression is a relatively sensitive marker of sarcomatoid carcinoma of various sites, including sarcomatoid squamous cell carcinoma, it can also be seen in soft tissue myoepithelial tumors, cellular neurothekeomas, soft tissue perineuriomas, Ewing sarcoma, diffuse-type giant cell tumor, giant cell tumors of soft tissue and bone, and other soft tissue tumors. p40 is an isoform of p63 whose expression is almost entirely limited to squamous carcinoma. Regrettably, p40 is usually negative in sarcomatoid squamous cell carcinoma.

Nerve Sheath Markers

S100 Protein

IHC for S100 protein is primarily used in the diagnosis of spindle cell melanoma and nerve sheath tumors of schwannian origin. Although S100 protein is a highly sensitive marker of in these entities, it should be kept in mind that it is by no means specific for melanoma or nerve sheath tumors. Expression may be seen in non-melanoma/non-nerve sheath tumors such as synovial sarcoma, rhabdomyosarcoma, leiomyosarcoma, myoepithelioma, adipocytic tumors, chondrocytic tumors, ossifying fibromyxoid tumor, and chordoma. In contrast to spindled melanomas, which are typically diffusely S100 protein-positive, S-100 protein expression in malignant peripheral nerve sheath tumors tends to be weaker and patchier (Fig. 2.2a and b). Malignant peripheral nerve sheath tumors are exceedingly rare in the skin, and one should be very reluctant to make this particular diagnosis. Regrettably, the distinction of very subtle, hypocellular desmoplastic melanomas from neurofibromas may not be possible by IHC methods alone. It should always be remembered that S100 protein is also present in Langerhans cells; intratumoral Langerhans cells should be rigorously distinguished from positive tumor cells.

Approximately 2–3% of melanomas, particularly "small-cell" melanomas of the sinonasal tract and metastatic melanomas, are negative for S-100 protein (and SOX10). Obviously, in such cases additional immunostaining for a melanosome-specific marker such as HMB-45 or Melan-A is essential for arriving at the correct diagnosis.

SOX10

SOX10 is a transcription factor involved in neural crest development and differentiation of neural crest cells into melanocytic and schwannian lineages. SOX10 is expressed by >85% of melanomas and clear cell sarcomas, essentially all schwannomas and neurofibromas, and 30% of malignant peripheral nerve sheath tumors. SOX10 expression is also common in myoepithelial tumors. In general, other S100 protein-positive tumors are SOX10-negative. The sensitivity of SOX10 for spindle cell melanoma is roughly comparable to that of S100 protein, although there are occasional cases in which one or the other may show more robust immunoreactivity (Fig. 2.2c).

Claudin-1

The claudins are a family of approximately 20 proteins involved in tight junction structure and permeability. Various claudins are differentially expressed in different normal tissue types and tumors. Claudin-1 expression appears to be limited to perineurial cells, and claudin-1 is a useful adjunctive marker of perineuriomas, present in 20–90% of perineuriomas, but not in other tumors in this differential diagnosis, such as neurofibromas, schwannomas, low-grade fibromyxoid sarcomas, etc.

GLUT-1

GLUT-1 is the erythrocyte-type glucose transporter protein. Expression of GLUT-1 protein is a consistent feature of normal perineurial cells and perineurial tumors. However, GLUT-1 expression is by no means specific for perineurial tumors, as it is frequently upregulated in ischemic foci within tumors (i.e., adjacent to necrosis). As with claudin-1, GLUT-1 is best used as an adjunct to EMA immunostaining, rather than as a standalone marker. Among vascular tumors, expression of GLUT-1 protein is seen in essentially all rapidly involuting juvenile capillary hemangiomas, but not in kaposiform hemangioendothelioma.

Melanocyte Specific Markers

HMB-45

Monoclonal antibody HMB-45 identifies the Pmel 17 gene product, gp100. The gp100 antigen is a premelanosomal protein, and as such HMB45 is melanosome-specific, but not melanoma-specific. HMB45 is positive in roughly 85% of conventional melanomas but is almost never positive in spindle cell/desmoplastic melanoma (Fig. 2.2d). HMB45 immunoreactivity is also commonly present in other melanosome-containing tumors, such

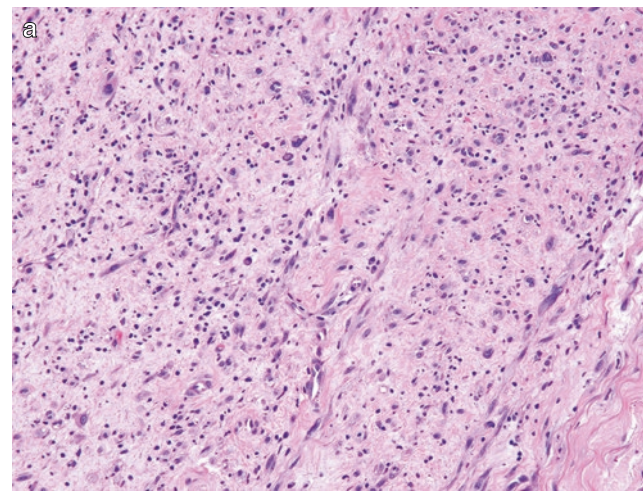
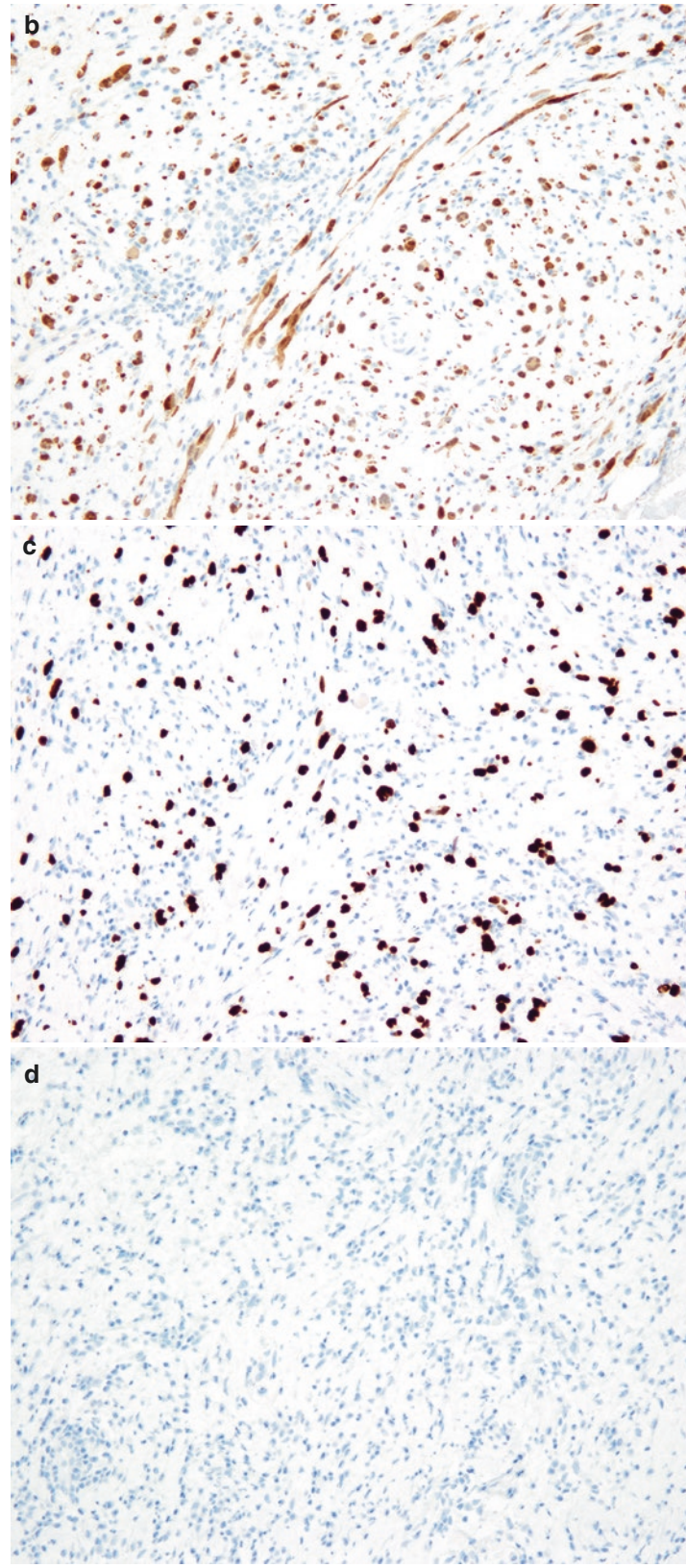


Fig. 2.2 Desmoplastic malignant melanoma (**a**), showing diffuse, strong immunoreactivity for S100 protein (**b**) and SOX10 (**c**). HMB45, Melan-A, tyrosinase, and MiTF were negative in this tumor, as is typically the case (**d**)

Fig. 2.2 (continued)



as perivascular epithelioid cell tumors and malignant melanotic schwannian tumors (Fig. 2.3a–c).

Melan-A

Melan-A, the product of the *MART-1* gene, is a component of the premelanosomal membrane. In general, the sensitivity and specificity of Melan-A are quite similar to those of HMB45. For unknown reasons, some melanomas express HMB45 but not Melan-A and vice versa. Melan-A is more often positive in resting melanocytes and nevi. The widely used A103 clone to Melan-A also shows reproducible cross-reactivity with an unknown epitope present in steroid-producing tumors and is useful in the diagnosis of adrenal cortical tumors, for instance.

Tyrosinase

Tyrosinase is an enzyme involved in the synthesis of melanin. Antibodies to tyrosinase have a sensitivity and specificity that are roughly equivalent to that of HMB-45 and Melan-A.

Microphthalmia Transcription Factor

Microphthalmia transcription factor (MiTF), the product of the microphthalmia (*MITF*) gene, is a transcription factor critical for melanocyte development. MiTF is expressed in essentially all resting melanocytes and nevi. MiTF is expressed in over 90% of epithelioid melanomas; spindle cell melanomas are less often positive (40%), and true desmoplastic melanomas are very infrequently positive (<5%). MiTF is also expressed in nearly all clear cell sarcomas and in PEComas of all types (Fig. 2.3d). However, MiTF expression is not limited to melanocytic tumors and may be seen in cellular neurothekeomas, leiomyosarcomas, atypical fibroxanthomas, atypical lipomatous neoplasms, and very rare carcinomas. MiTF is best used for the confirmation of S100 protein/SOX10-positive, HMB-45/Melan-A/tyrosinase-negative tumors suspected of being melanomas.

Markers of Smooth and Skeletal Muscle

Desmin

Desmin is the intermediate filament protein associated with both smooth and skeletal muscle differentiation. Desmin expression is much less common in myofibroblastic lesions. Desmin is the most sensitive marker of skeletal muscle tumors, present in nearly 100% of rhabdomyosarcomas of all subtypes and rhabdomyomas. Desmin is a considerably less sensitive marker of tumors of smooth muscle, as it is frequently negative in normal vascular smooth muscle and in tumors derived from such cells. Desmin expression is not, however, specific for myogenic tumors and may be seen in Ewing sarcoma, desmoplastic small round cell tumors, mesenchymal chondrosarcomas, mesothelial tumors, tenosynovial giant cell tumors, ossifying fibromyxoid tumors, angiomatoid fibrous histiocytomas, schwannomas, and melanoma.

Smooth Muscle Actin

Smooth muscle actin isoforms (most often identified with the 1A4 clone) are expressed by essentially all benign and malignant smooth muscle tumors, in glomus tumors and other "myopericytic" tumors such as myopericytoma and myofibroma, and in myofibroblastic lesions such as nodular fasciitis (Fig. 2.4). Smooth muscle actins are also often present in tumors showing myoepithelial differentiation, such as mixed tumors and myoepitheliomas. In general, myofibroblasts show expression of smooth muscle actin only at the periphery of their cytoplasm in a so-called "tram-track" pattern; this is in contrast to the uniform cytoplasmic expression in smooth muscle. One pitfall to be aware of with the use of the 1A4 mAb is aberrant nuclear cross-reactivity, a phenomenon sporadically encountered in many laboratories. Nuclear 1A4 immunoreactivity should not be taken as evidence of smooth muscle differentiation in any tumor.

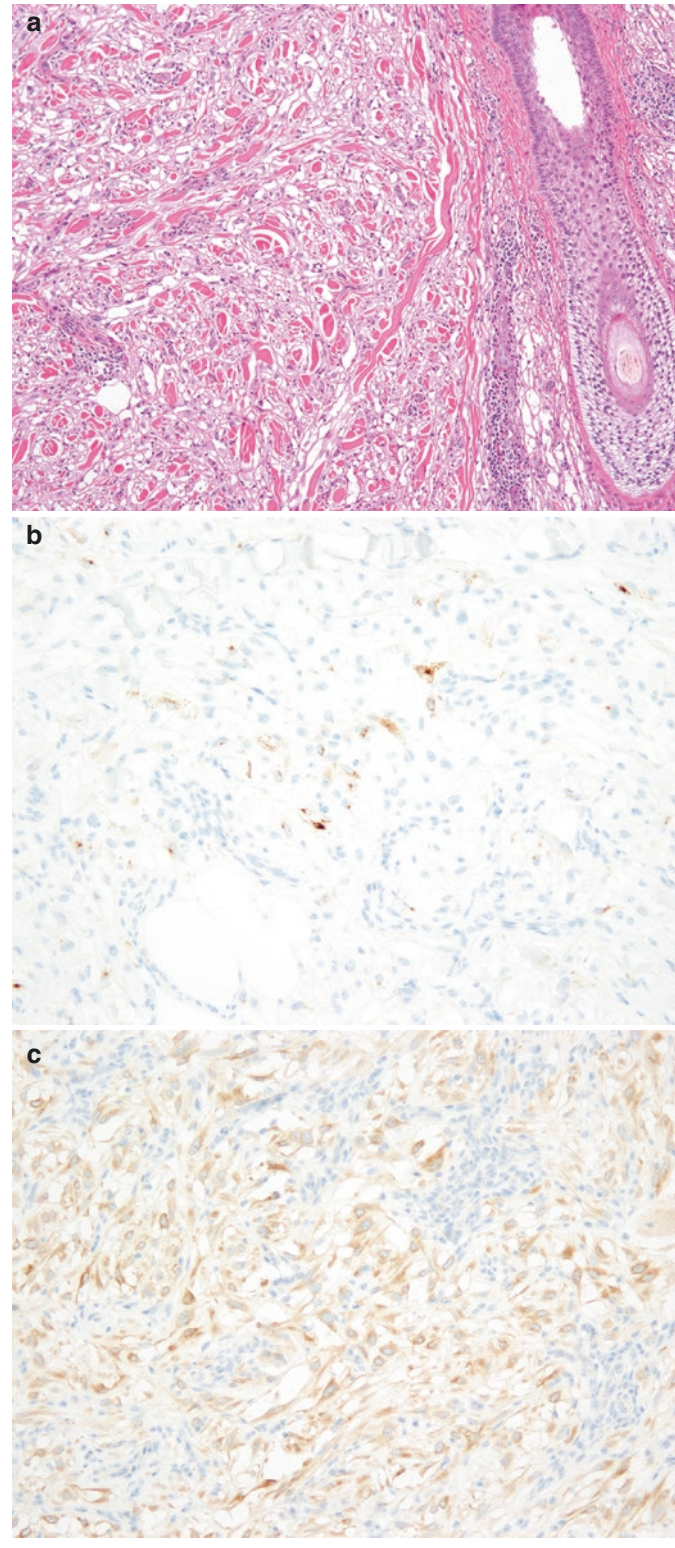
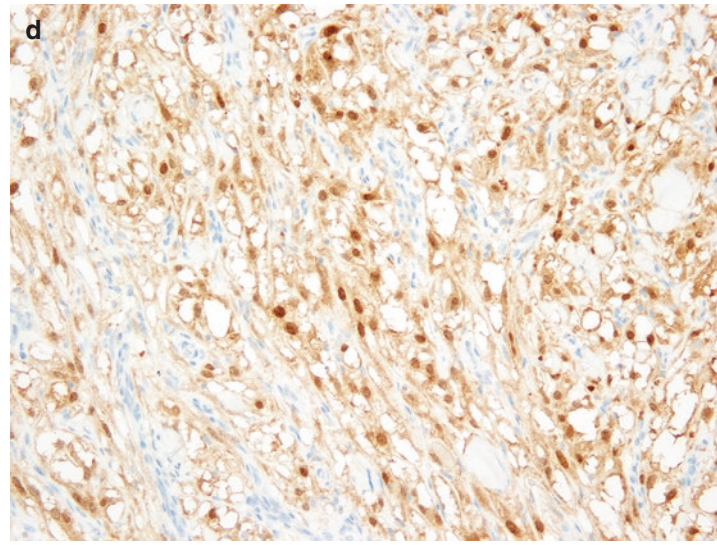


Fig. 2.3 Dermal perivascular epithelioid cell tumor (PEComa) (**a**), showing focal immunoreactivity with HMB45 (**b**) and more extensive expression of smooth muscle actins (**c**) and MiTF (**d**)

Fig. 2.3 (continued)



MyoD1 and Myogenin

MyoD1 and myogenin, members of the basic helix-loop-helix family of DNA-binding myogenic nuclear regulatory proteins, are responsible for activation of genes encoding structural proteins such as desmin during embryonic skeletal muscle differentiation. Both MyoD1 and myogenin are expressed by >95% of rhabdomyosarcomas of all types; alveolar rhabdomyosarcomas tend to express very high levels of myogenin and comparatively less MyoD1, whereas embryonal and spindle cell/sclerosing rhabdomyosarcomas often show the opposite pattern. Expression of MyoD1 and myogenin are highly specific for rhabdomyoblastic differentiation, although it is important to keep in mind that they may be expressed in tumors showing heterologous rhabdomyoblastic differentiation, such as squamous cell carcinoma, melanoma, and Merkel cell carcinoma (Fig. 2.5).

H-Caldesmon

Heavy caldesmon is a calcium-binding protein involved in the regulation of smooth muscle contractility. Although the sensitivity of h-caldesmon for smooth muscle tumors is lower than that of smooth muscle actin, h-caldesmon is not expressed by myofibroblastic tumors. H-Caldesmon expression is frequently present in glomus tumors. H-Caldesmon is generally best used for the distinction of poorly differentiated smooth muscle tumors from myofibroblastic tumors, rather than as a first-line marker of smooth muscle cells.

Endothelial Markers

CD31

CD31 is the single best currently available marker of endothelial differentiation, expressed in >90% of angiosarcomas, hemangioendotheliomas, hemangiomas, and Kaposi sarcoma (Fig. 2.6). Other than exceptional carcinomas, CD31 expression is not seen in almost any non-endothelial tissue or tumor, including melanoma. CD31 is, however, commonly expressed by macrophages and platelets. Large numbers of CD31-positive macrophages are routinely present in various malignant neoplasms, and great care should be taken not to mistake these as evidence of focal expression by the tumor cells themselves. CD31 expression in macrophages is distinctly granular and somewhat weak, as compared with the intense, "linear" membrane staining of the endothelium (Fig. 2.7).

CD34

CD34 is cell adhesion molecule expressed on hematopoietic stem cells, endothelium, the interstitial cells of Cajal, and dendritic cells present in the dermis, around blood vessels, and in the nerve sheath. CD34 is expressed in more than 90% of vascular tumors and is a particularly sensitive marker of Kaposi sarcoma. However, CD34 expression is wholly non-specific for vascular tumors and may be seen in dermatofibrosarcoma protuberans, solitary fibrous tumors, malignant peripheral nerve sheath tumors, spindle cell lipomas, and epithelioid sarcomas, among others. CD34 should not be used as a sole endothelial marker.

Podoplanin (D2-40)

Several different markers have been proposed to more specifically identify lymphatic endothelium, including podoplanin (D2-40) and Prox1. Expression of podoplanin is regulated by the homeobox gene *PROX1* and is normally seen in lymphatic endothelial cells, glomerular

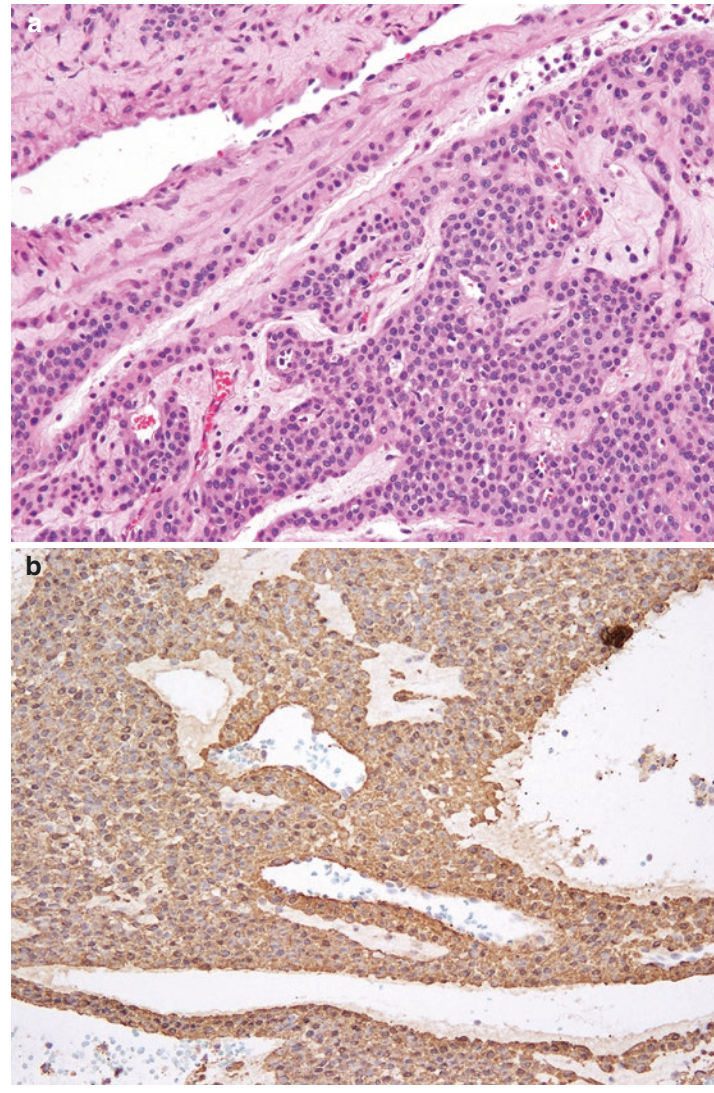
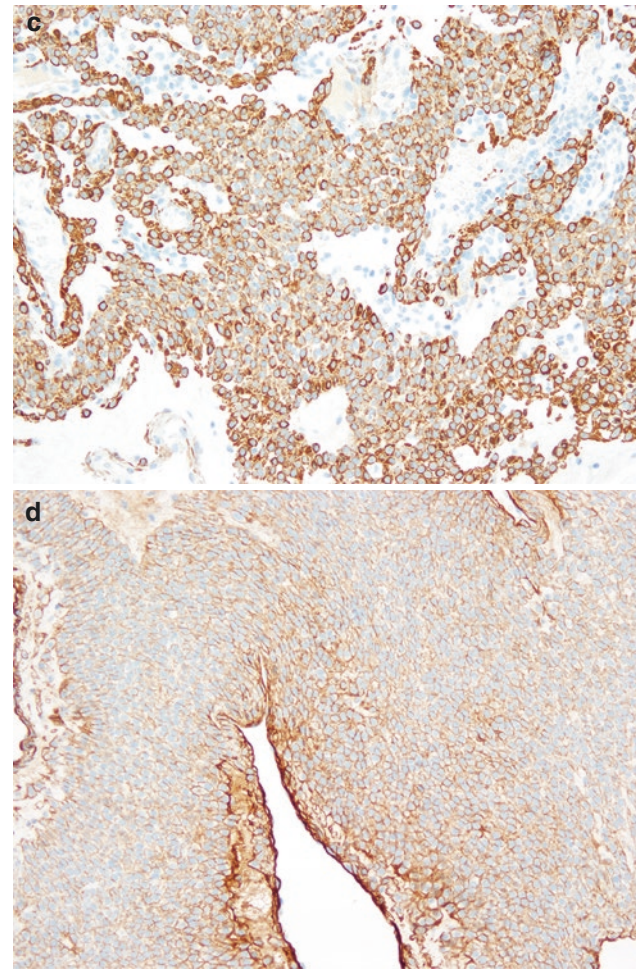


Fig. 2.4 Glomus tumor (**a**) showing diffuse expression of smooth muscle actins (**b**) and caldesmon (**c**). Glomus cells are also typically uniformly invested by collagen IV, as shown here (**d**)

Fig. 2.4 (continued)



podocytes, choroid plexus epithelium, type 1 alveolar cells, osteoblasts, and mesothelial cells. Regrettably, podoplanin expression is present in a wide variety of endothelial tumors, including those without obvious features of lymphatic differentiation, and in many different types of mesenchymal, germ cell, and glial neoplasms. Similarly, Prox1expression does not appear to be confined to lymphatic-type endothelium or even to endothelial tumors. Thus, although antibodies to podoplanin and Prox1 may occasionally be of some value in the identification of certain lymphatic proliferations, they generally play little role in the diagnosis of endothelial tumors more generally. Certainly these markers have no role in the differential diagnosis of cutaneous spindle cell tumors.

FLI1 and ERG

FLI1 and ERG are members of the ETS family of transcription factors and represent the best *nuclear* markers of endothelial differentiation. Both FLI-1 and ERG are positive in >95% of endothelial neoplasms of all types. It is important to recognize that FLI1 and ERG are not specific markers of endothelial differentiation, as they may also be present in Ewing sarcoma, a variety of carcinomas, and rare melanomas, mesotheliomas, and hematolymphoid neoplasms. FLI1 and ERG have not been reported in spindle cell melanomas, sarcomatoid squamous carcinomas or AFX/SUDPS, although relatively few cases have been studied. FLI-1 and ERG are helpful in the differential diagnosis of epithelioid forms of angiosarcoma

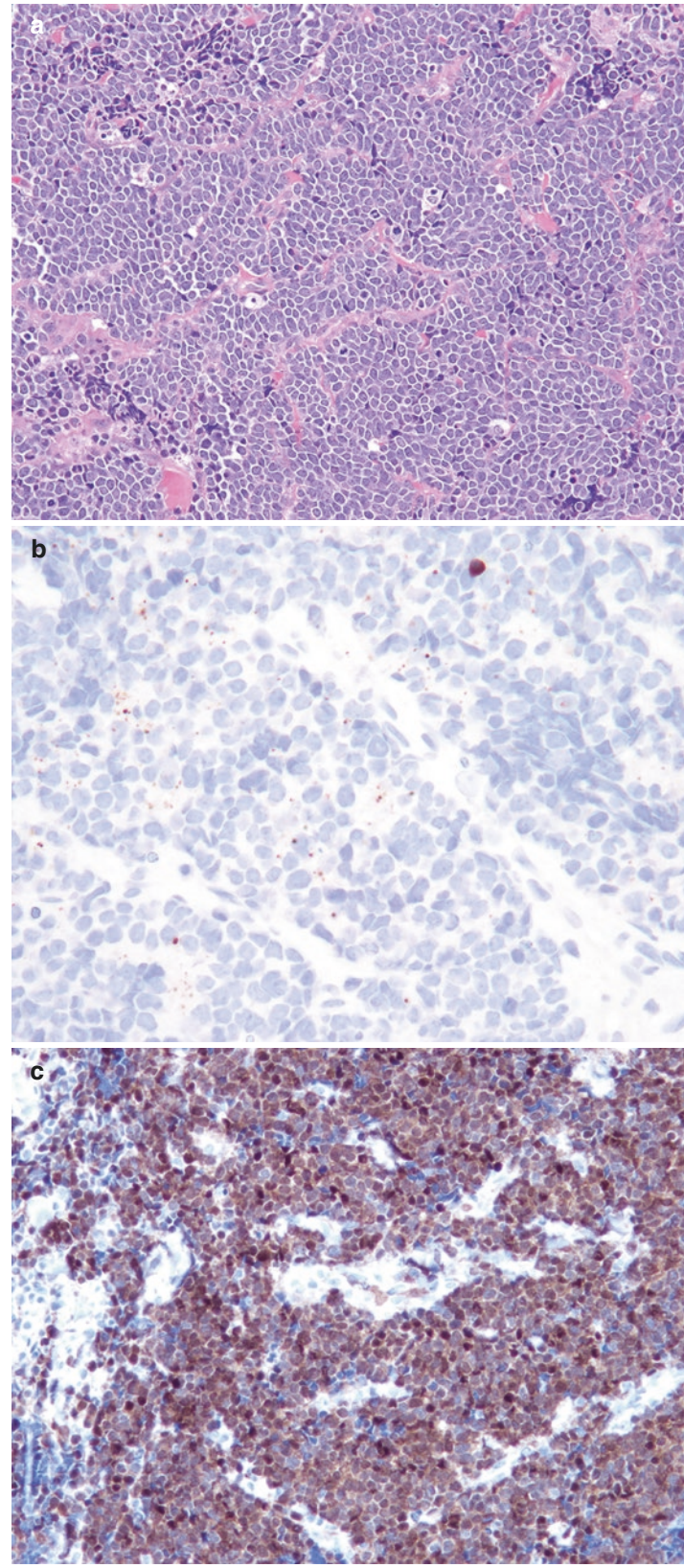
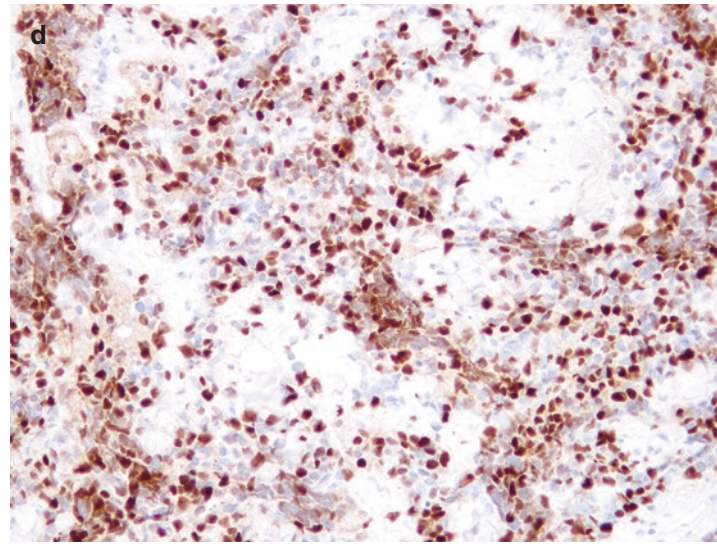


Fig. 2.5 Merkel cell carcinoma, (**a**), showing "dot-like" expression of keratin 20 (**b**) and diffuse expression of Merkel cell polyomavirus large T antigen (**c**). This case also showed aberrant expression of skeletal muscle markers, including desmin (not shown) and myogenin (**d**)

Fig. 2.5 (continued)



from epithelioid sarcoma, which is usually (but not always) negative for both markers. Although FLI1 and ERG are very useful markers, they are best used in combination with CD31 and a panel of other relevant markers and should not be used as one's sole endothelial marker.

vWF (Factor VIII)

The von Willebrand factor (vWF) was the first endothelium-specific marker employed in diagnostic immunohistochemical studies. vWF is the least sensitive of the vascular markers, positive in only 50–75% of vascular tumors. Although vWF expression is, in theory, absolutely specific for vascular tumors, technical problems limit its usefulness. vWF is not only produced by endothelial cells but circulates in the serum; and it therefore can be found often in zones of tumor necrosis and hemorrhage. In general, CD31, FLI1, and ERG are superior endothelial markers.

Histiocytic Markers (CD68, CD163, CD11c, CD4)

Antibodies to CD68 continue to be used by many pathologists as a marker of histiocytic differentiation; CD68 represents an entirely non-specific lysosomal antigen. Although macrophages contain large numbers of lysosomes (accounting for their CD68 expression), lysosomal accumulation may also be seen in a very large number of non-histiocytic tumors, including (but not limited to) granular cell tumors and granular cell variants of melanoma, carcinoma, angiosarcoma, and undifferentiated pleomorphic sarcoma. Thus, CD68 is an exceptionally non-specific marker. In particular, CD68 expression in a spindle cell neoplasm does not imply "fibrohistiocytic" differentiation. CD163, CD11c, and CD4 represent much more lineage-restricted markers of histiocytes and their tumors, including solitary (juvenile) xanthogranuloma (Fig. 2.8).

ALK

Expression of ALK is characteristic of epithelioid fibrous histiocytoma and is not seen in other variants of fibrous histiocytoma or in various epithelioid neoplasms that may mimic EFH (Fig. 2.9).

Markers that Are Generally of Little Value

Vimentin

Vimentin, an intermediate filament protein, is expressed in all mesenchymal cells and in virtually all mesenchymal tumors and is of minimal

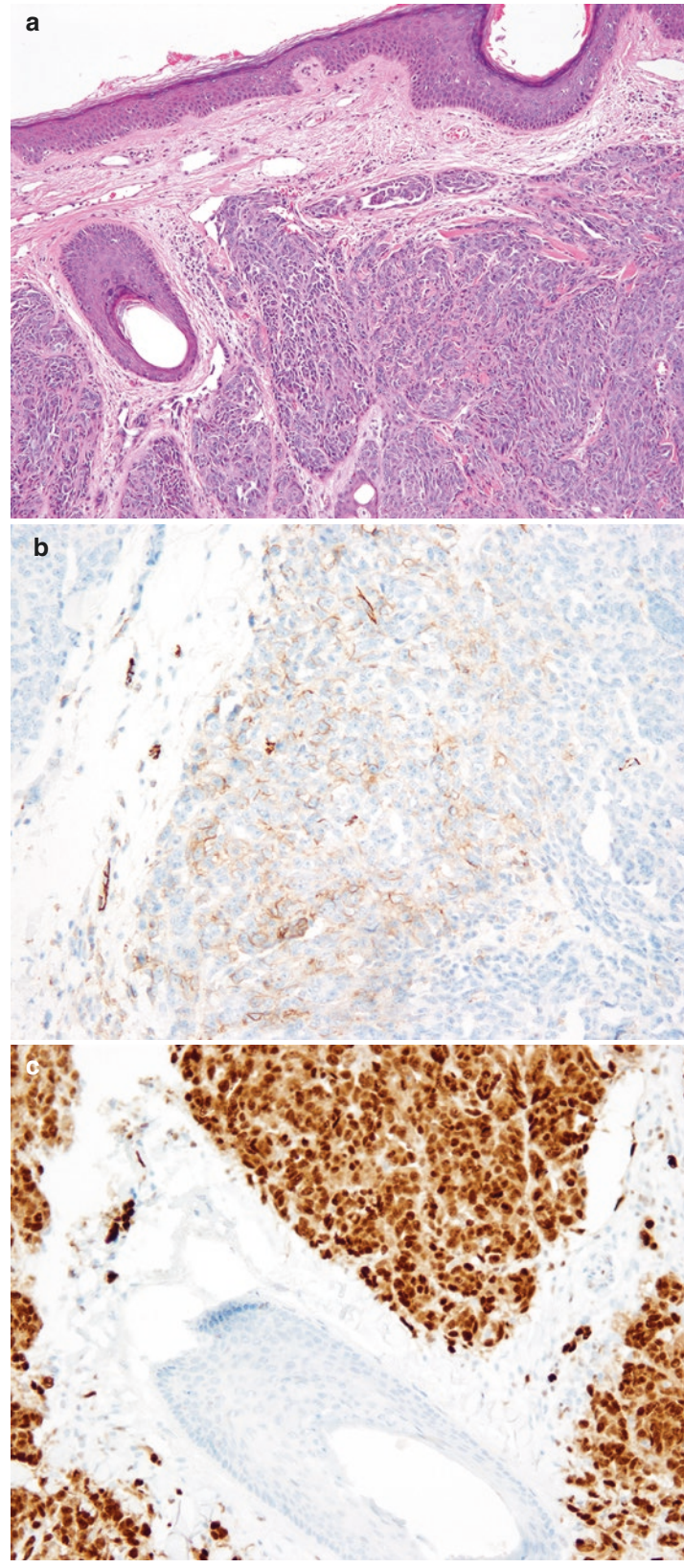
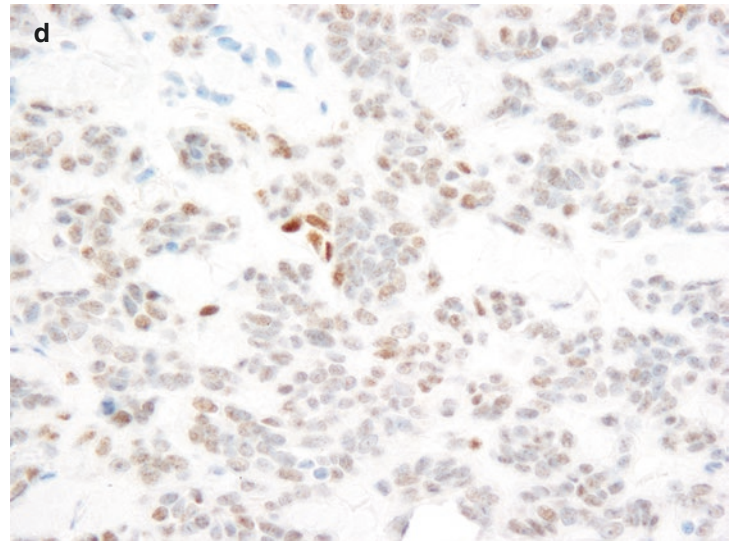


Fig. 2.6 Poorly differentiated angiosarcoma (**a**) with membranous expression of CD31 (**b**), intense nuclear immunoreactivity for ERG protein (**c**), and more limited expression of FLI1 protein (**d**)

Fig. 2.6 (continued)



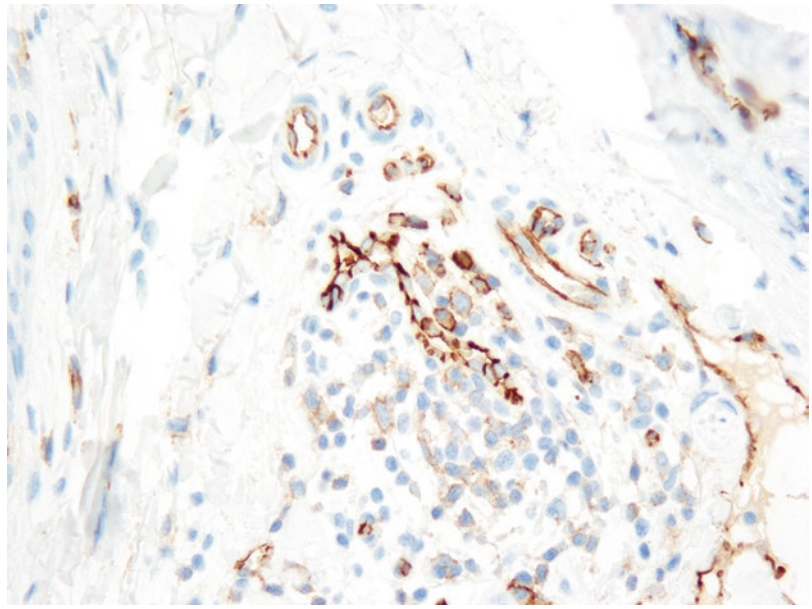


Fig. 2.7 CD31 expression is not confined to endothelial cells, and is also routinely present in macrophages, as shown here. CD31 expression in macrophages tends to be weaker and more granular than expression in endothelial cells. It is important not to mistake CD31-positive macrophages for tumor cells in a non-endothelial neoplasm

value in identifying particular tumors. Vimentin expression is also of little value in the immunohistochemical distinction of carcinomas from sarcomas. Vimentin immunoreactivity has been touted as a good marker of tissue preservation. However, vimentin expression, similar to that of all the intermediate filaments, is rather hardy and may remain present in tissues in which all other immunoreactivity has been lost. In general, there is no value in performing vimentin immunostains on any spindle cell neoplasm.

PGP9.5

PGP9.5 is an ubiquitin carboxyl terminal hydrolase originally identified in neurons and initially thought to be neuron-specific. However,

PGP9.5 expression is in fact essentially "ubiquitous" among tumors. Despite claims to the contrary, there is really no role for PGP9.5 immunohistochemistry in the diagnosis of dermal soft tissue tumors, in particular in the differential diagnosis of nerve sheath tumors and neurothekeoma.

Putative Markers of Atypical Fibroxanthoma and/or Superficial Undifferentiated Pleomorphic Sarcoma (CD99, CD10, Procollagen, CD34)

The dermatopathology literature is replete with studies examining the relative value of markers such as CD99, CD10, procollagen, and CD34 in the diagnosis of atypical fibroxanthoma (AFX) and in its distinction from superficial undifferentiated pleomorphic sarcoma (SUPS). Despite their widespread usage, none of these markers is useful in this differential diagnosis or in the distinction of AFX/SUPS from other pleomorphic spindle cell malignancies. Expression of all of these markers can be seen in both AFX and in SUPS and in many other spindle cell malignancies that may mimic these tumors. In the appropriate morphological context, however, expression of CD10 in combination with MiTF is characteristic of cellular neurothekeoma (Fig. 2.10).

Applications of Immunohistochemistry and Fluorescence In Situ Hybridization in Various Differential Diagnoses in Cutaneous Soft Tissue Pathology

IHC in the Evaluation of Cutaneous Pleomorphic Spindle Cell Tumors

This is one of the most common differential diagnoses in cutaneous soft tissue pathology and in most instances relates to the distinction of non-mesenchymal tumors (e.g., sarcomatoid squamous cell carcinoma and spindle cell melanoma) from atypical fibroxanthoma/superficial undifferentiated pleomorphic sarcoma (AFX/SUPS). Particularly in the setting of significant sun damage, this differential diagnosis may also include spindled forms of angiosarcoma. Rarely, other pleomorphic spindle cell tumors, such as leio-

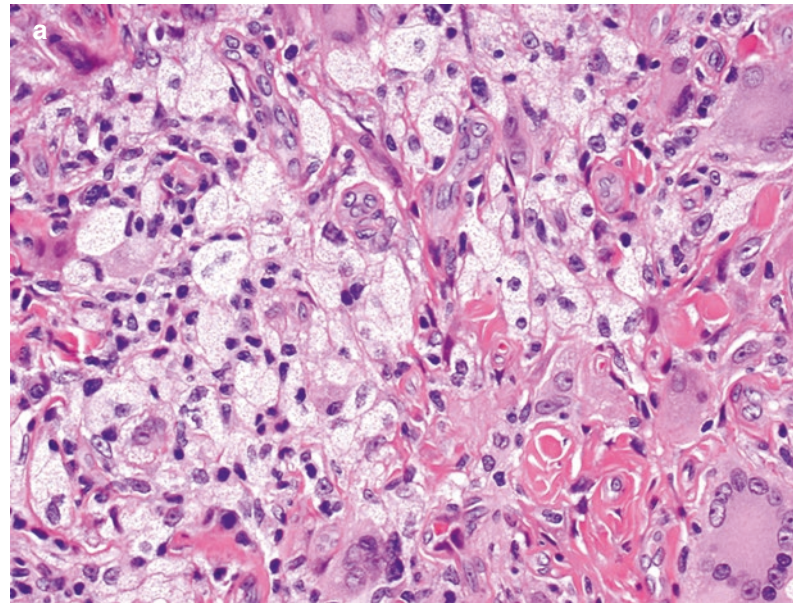
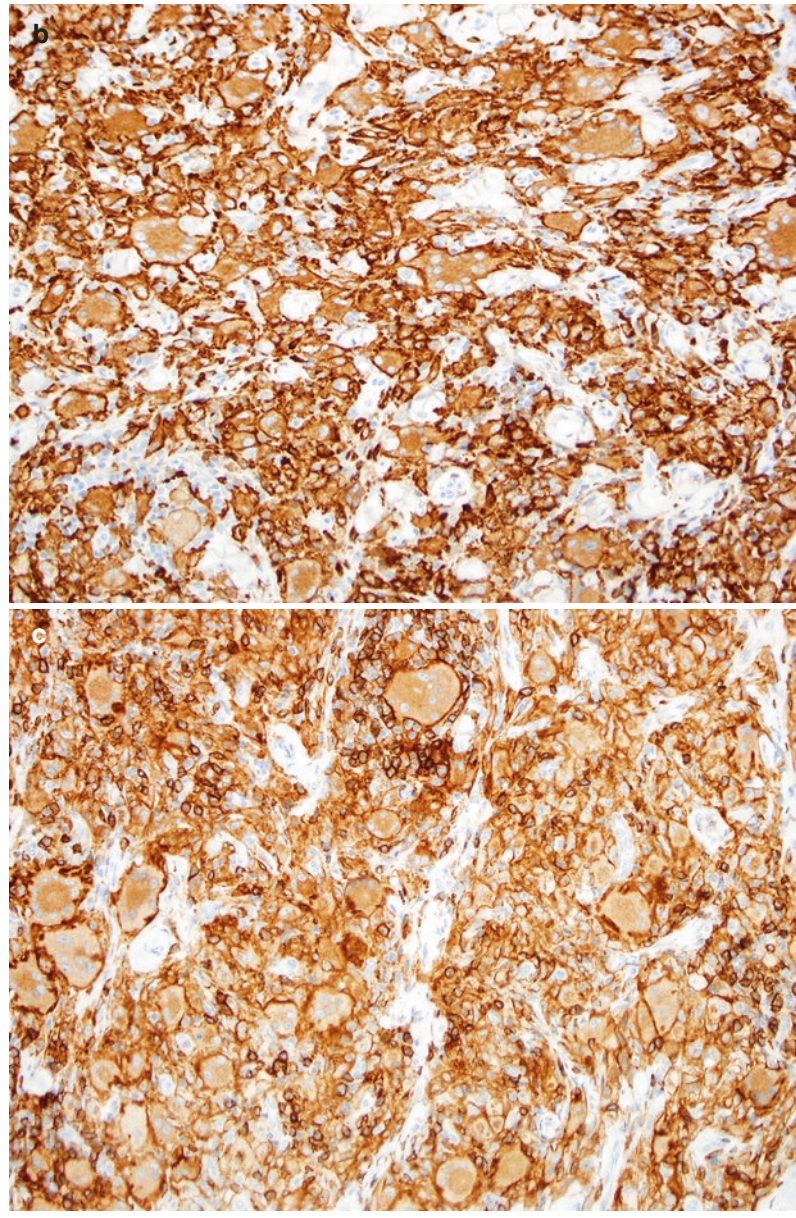


Fig. 2.8 Lipidized solitary xanthogranuloma (**a**), showing diffuse expression of the histiocyte-restricted markers CD163 (**b**) and C11c (**c**)

Fig. 2.8 (continued)



myosarcoma, rhabdomyosarcoma, pleomorphic liposarcoma, and osteosarcoma, may enter this differential diagnosis.

Table 2.4 lists a small panel of immunostains that may be helpful in this differential diagnosis, with their expected patterns of immunoreactivity in common and less common pleomorphic spindle cell tumors of the skin. In general, the differential diagnosis of these tumors is fairly straightforward, with expression of keratins in sarcomatoid squamous cell carcinoma, expression of S100 protein and SOX10 in spindle cell melanoma, expression of endothelial markers such as CD31/FLI1/ERG in spindled angiosarcoma, and absent expression of all relevant markers in AFX/SUPS. Essentially all spindle cell tumors in any anatomical location are vimentin-positive, and vimentin plays no role in this differential diagnosis.

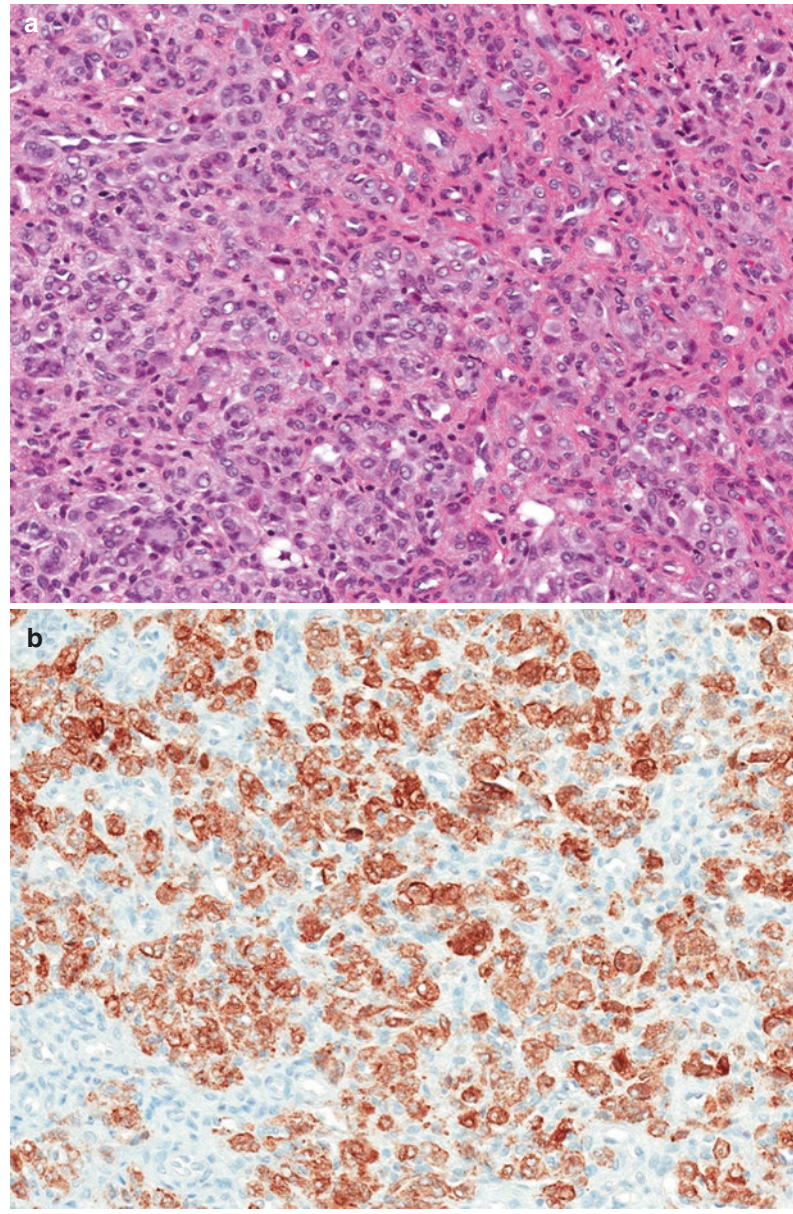


Fig. 2.9 Epithelioid fibrous histiocytoma (**a**) showing diffuse expression of ALK protein (**b**)

Leiomyosarcoma

It is important to keep in mind that the overwhelming majority of primary cutaneous leiomyosarcomas (also referred to as "atypical intradermal smooth muscle tumors" because of their superb prognosis) are very well-differentiated, non-pleomorphic lesions. Thus, leiomyosarcoma does not generally enter into the differential diagnosis of pleomorphic spindle cell tumors of the skin, except when a metastatic lesion from a soft tissue, visceral, or gynecologic primary tumor is a consideration. Antibodies to smooth muscle actin isoforms (e.g., the 1A4 mAb) represent the most sensitive markers of smooth muscle differentiation, although they are also routinely expressed by a variety of other cutaneous tumors, including myofibroblastic proliferations and neoplasm, cellular fibrous histiocytomas, glomus tumors, myoepithelial tumors, and cellular neurothekeomas. Antibodies to caldesmon and myosin heavy

chain represent more specific markers of smooth muscle differentiation than do smooth muscle actins, although they are expressed by a smaller percentage of leiomyosarcomas, in particular poorly differentiated tumors. Glomus tumors are also routinely caldesmon-positive, and myosin heavy chain may be expressed by myoepithelial tumors. Although antibodies to desmin are used in many laboratories as "screening" markers for leiomyosarcoma, desmin expression is actually frequently absent in normal vascular smooth muscle and in tumors derived from it. Desmin is a much better screening marker for tumors of skeletal muscle differentiation.

Atypical Fibroxanthoma/Superficial Undifferentiated Pleomorphic Sarcoma (AFX/SUPS)

AFX typically presents as a rapidly growing mass in a sun-exposed region of an older adult. The diagnosis of AFX should be reserved for small (<1–1.5 cm) lesions that are confined to the dermis and which are completely visualized. The prognosis for larger tumors and tumors showing involvement of the subcutis is considerably worse, and such lesions should be labeled SUPS or pleomorphic dermal sarcoma. Histologically, most AFX are undifferentiated, pleomorphic spindle cell tumors, although relatively monomorphic variants do exist. In younger patients, and in non-sun-exposed skin, AFX/SUPS should be rigorously distinguished from atypical fibrous histiocytomas.

AFX/SUPS is a histologic and immunohistochemical diagnosis of exclusion. There are no markers or combinations of markers that establish the diagnosis of AFX/SUPS. The lesional cells of AFX must be negative for keratins (using more than one keratin antibody), S100 protein, and SOX10 (Fig. 2.11). Limited expression of smooth muscle actins, indicative of myofibroblastic differentiation, is acceptable, but caldesmon/desmin expression is not seen. AFX commonly contains S100 protein-positive Langerhans cells, CD31-positive endothelial cells and macrophages and Factor XIIIa-positive dendritic cells, and these must be carefully distinguished from the neoplastic cells. Despite their historical labeling as "fibrohistiocytic" neoplasms, AFX/SUPS have no relationship to true histiocytic tumors, and IHC for markers such as CD68 and CD163 plays no role in their differential diagnosis. CD68, in

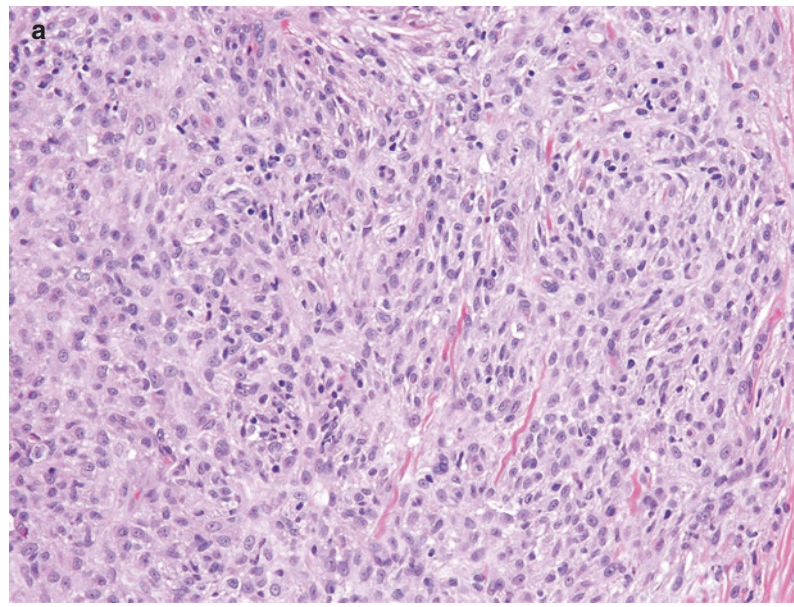


Fig. 2.10 Cellular neurothekeoma (**a**). Although there are no specific markers of cellular neurothekeoma, co-expression of CD10 (**b**) and MiTF (**c**) is a characteristic in the appropriate morphological context

Fig. 2.10 (continued)

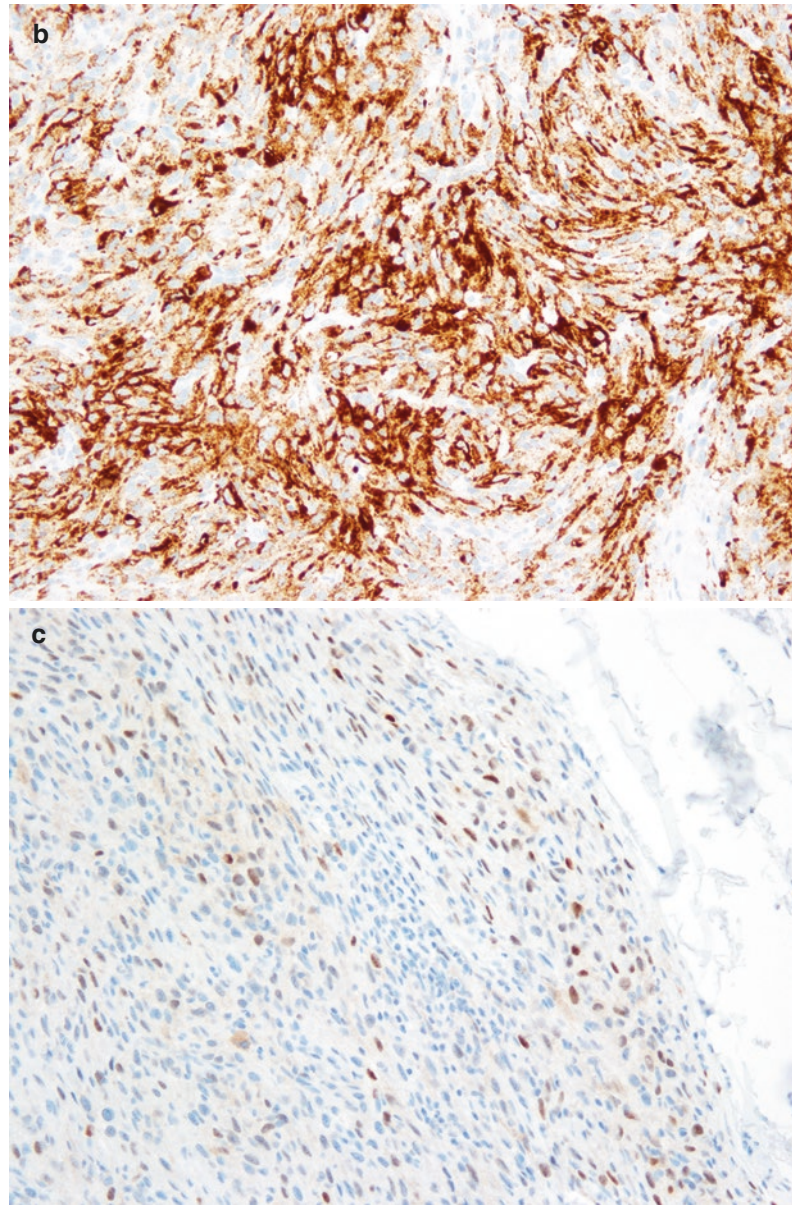


Table 2.4 IHC panel for the evaluation of pleomorphic spindle cell neoplasms

Antigen	CA	Melanoma	AS	LMS	RMS	AFX/SUDPS
Keratin	Positive	Variable	Variable	Variable	Negative	Negative
S-100 protein and/or SOX10	Negative	Positive	Negative	Negative	Negative	Negative
Smooth muscle actin	Negative	Negative	Negative	Positive	Negative	Variable
Desmin	Negative	Variable	Negative	Variable	Positive	Negative
CD31	Negative	Negative	Positive	Negative	Negative	Negative

AFX/SUDPS atypical fibroxanthoma/superficial undifferentiated pleomorphic sarcoma, *AS* angiosarcoma, *CA* carcinoma, *LMS* leiomyosarcoma, *RMS* rhabdomyosarcoma

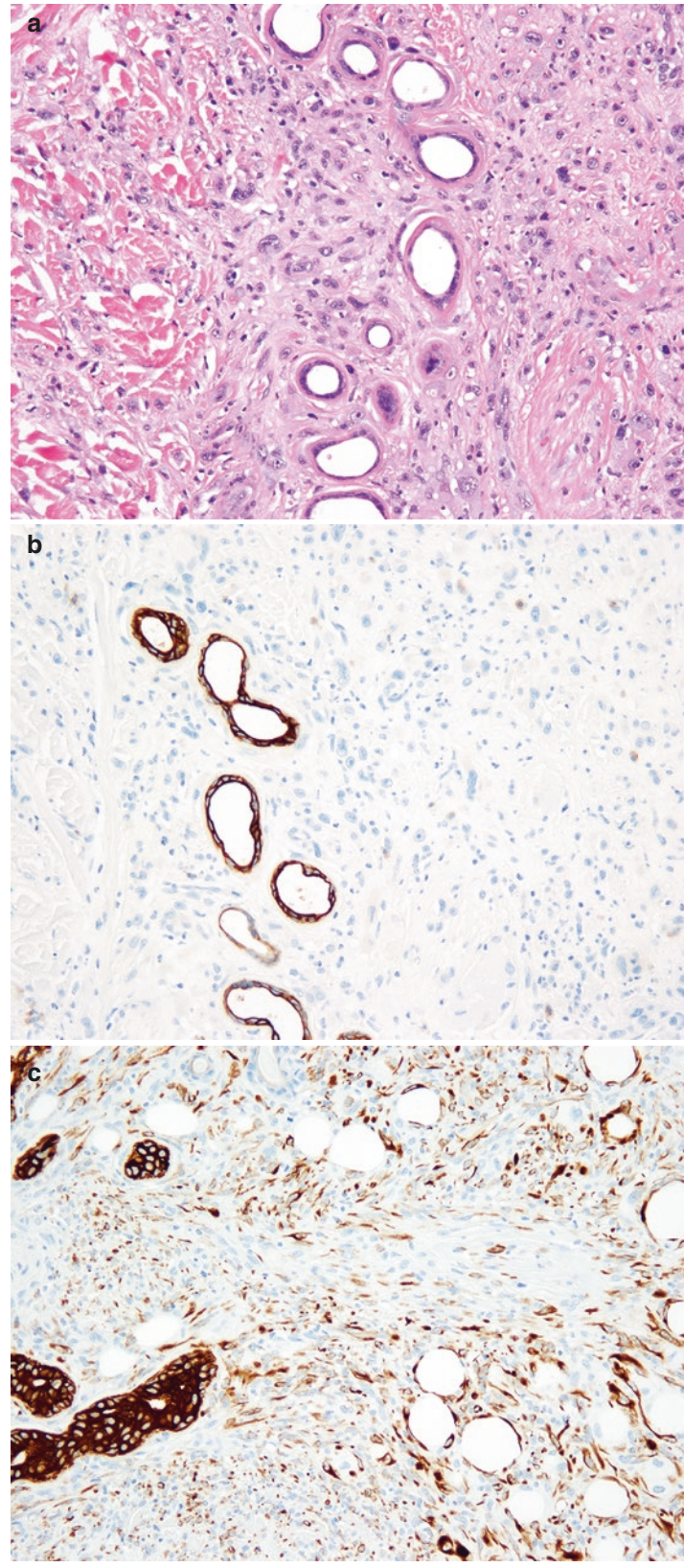


Fig. 2.11 The diagnosis of atypical fibroxanthoma must be one of exclusion, rendered only after a comprehensive panel of immunostains has excluded other possibilities. This malignant spindle cell tumor (**a**) was submitted in consultation as "atypical fibroxanthoma," with negative immunostains for melanoma-associated markers, markers of muscle differentiation, and keratins (using the AE1/AE3 antibodies) (**b**). However, it was strongly positive with the OSCAR keratin antibody (**c**) and represents a sarcomatoid squamous cell carcinoma

particular, is a non-specific marker of lysosomes and may be expressed by essentially any tumor type. There is no role for CD99, CD10, procollagen, or CD34 in this differential diagnosis.

Other Rare Pleomorphic Spindle Cell Sarcomas that May Involve the Skin

Essentially any pleomorphic sarcoma may occasionally present as a tumor involving the skin. For many of these tumors, IHC is not of value, as diagnosis depends on the identification of specific histologic features (e.g., pleomorphic lipoblasts in pleomorphic liposarcoma, osteoid in extraskeletal osteosarcoma). Exceptionally rare pleomorphic rhabdomyosarcomas involving the skin show expression of the specific markers of skeletal muscle differentiation, myogenin, and MyoD1, in addition to desmin.

IHC in the Differential Diagnosis of "Monomorphic" Spindle Cell Cutaneous Tumors

For practical purposes, the differential diagnosis of relatively monomorphic spindle cell tumors involving the skin centers around the distinction of dermatofibrosarcoma protuberans (DFSP) from cellular forms of benign cellular fibrous histiocytoma (CFH) (dermatofibroma). Other tumors that can be included in this differential diagnosis include predominantly spindled forms of solitary (juvenile) xanthogranuloma (SXG), dermatomyofibroma (DMF), low-grade fibromyxoid sarcoma (LGFMS), and perineurioma. Table 2.5 illustrates a panel of immunostains that may be helpful in some of these diagnoses.

DFSP, CFH, and Related Lesions

Despite the massive literature that exists on the immunohistochemical distinction of DFSP from CFH, this seems to us a fairly straightforward distinction. DFSP consists of a rather monomorphic proliferation of slender, lightly eosinophilic spindled cells with darkly staining nuclei, which grow in a diffusely infiltrative fashion into the subcutaneous fat and in almost all instances show intense, diffuse expression of CD34. Although pigmented forms of DFSP (Bednar tumor) may contain intermixed S100 protein and Melan-A-positive melanocytes, the great majority of DFSP lack other intermixed cell types showing expression of markers such as actins, CD163, etc. In contrast, CFH tends to consist of more variable, plumper spindled cells, occasionally with admixed siderophages and foamy macrophages. Involvement of the subcutaneous fat is typically quite limited in CFH and is often accompanied by lymphoid aggregates. By IHC, the lesional cells of CFH are negative for CD34, although nonneoplastic CD34-positive cells are often found adjacent to the tumor cells, sometimes forming a rim around the deep border of the tumor (Fig. 2.12). Although many pathologists believe that CFH should be Factor 13A-positive, this is in fact not the case. Large series of CFH have shown absent Factor 13A expression in up to 70% of CFH, and our own experience suggests that this figure may be much higher, if only Factor 13A expression by the lesional cells themselves is considered. The most characteristic feature of CFH, immunohistochemically, is the presence of multiple cell populations, including Factor 13A, CD34, S100 protein, and CD68-positive cells. Many CFH show limited myofibroblastic differentiation in the form of smooth muscle actin expression in a "tram-track" pattern; this should not be misinterpreted as evidence of "leiomyosarcoma," particularly as CFH may show mitotic figures and foci of necrosis. In exceptional instances, demonstration of the DFSP-associated molecular events, rearrangements, and amplification of *PDGFB* may be helpful in the definitive diagnosis of DFSP.

The morphological features of CFH also overlap to a degree with those of SXG. IHC may be quite helpful in the diagnosis of SXG, as these lesions typically display diffuse, strong immunoreactivity for Factor 13A in the neoplastic cells themselves, in addition to co-expression of histiocytic markers, including CD163, CD45, CD11c,

Table 2.5 Screening panel for selected monomorphic spindle cell tumors of the skin

Antigen	DFSP	BFH	SXG	PN	LGFMS
CD34	Positive	Variable[a]	Negative	Variable	Rare
Factor XIIIa	Negative	Variable[a]	Positive	Negative	Negative
CD163	Negative	Variable[a]	Positive	Negative	Negative
EMA	Negative	Negative	Negative	Positive	Variable
MUC4	Negative	Negative	Negative	Negative	Positive

BFH benign fibrous histiocytoma (dermatofibroma), *DFSP* dermatofibrosarcoma protuberans, *LGFMS* low-grade fibromyxoid sarcoma, *PN* perineurioma, *SXG* solitary (juvenile) xanthogranuloma

[a]BFH most often shows an admixture of CD34, Factor 13A, and CD163-positive cells, but close inspection often shows the lesional cells to be negative for all tested markers

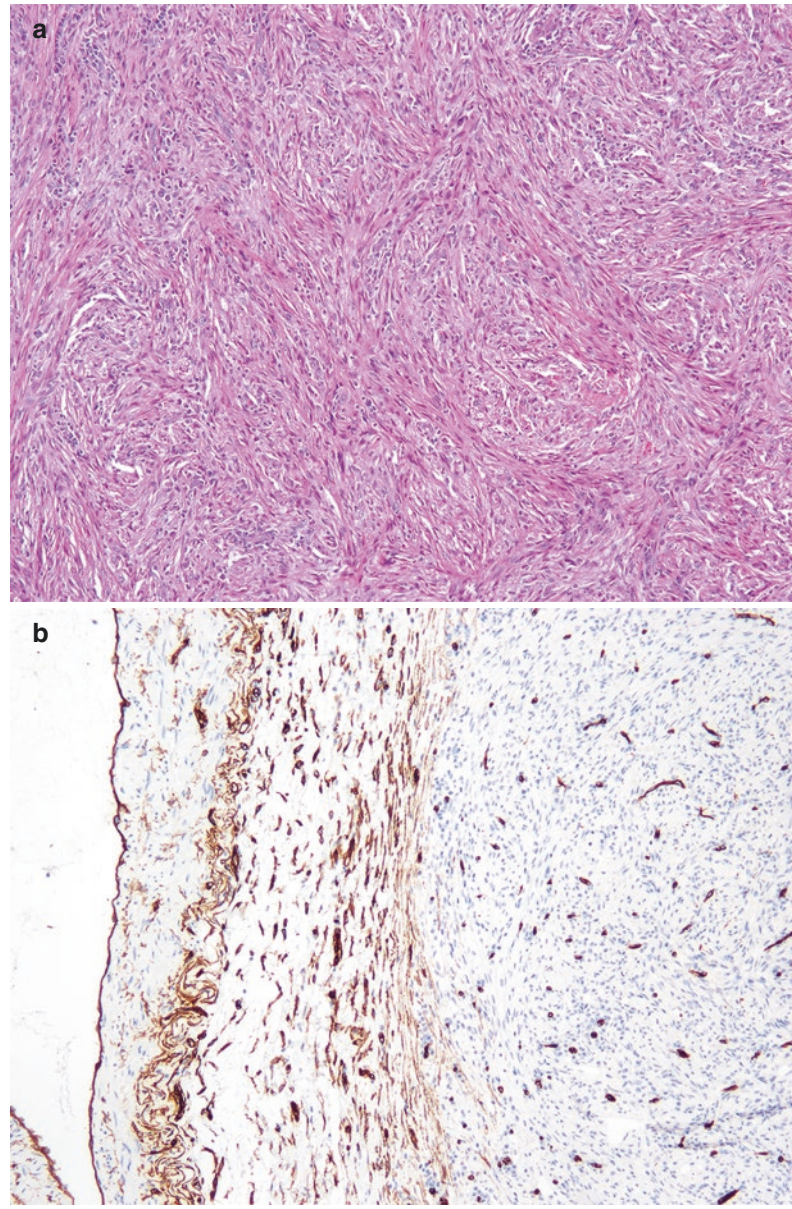


Fig. 2.12 Cellular benign fibrous histiocytoma (**a**). Although the lesional cells themselves are negative for CD34, a surrounding rim of CD34-positive spindled cells is often present (**b**). This should not be interpreted as evidence of "progression" to dermatofibrosarcoma protuberans

CD4, and CD31 (Fig. 2.13). Again, although CFH often contain cell populations that express Factor 13A or histiocytic markers, the lesional cells tend to be negative for both.

DMF may also resemble CFH, although the tumor cells tend to grow in fascicles oriented parallel to the overlying epidermis, and generally lack the cytologic variability of CFH. Despite their name, fewer than 30% of DMF express smooth muscle actins, and IHC generally does not play a large role in this diagnosis. DMF most often show a purely fibroblastic phenotype, with expression only of vimentin.

LGFMS and Perineurioma

Although LGFMS is typically thought of as a deeply situated soft tissue sarcoma of adults, it may occur in superficial locations including the skin, particularly in children. The morphological features of LGFMS in the skin are similar to those of deeply seated tumor, with markedly hypocellular, heavily collagenized zones, juxtaposed to more cellular myxoid nodules, often showing a whorling growth pattern and a well-developed, arborizing vasculature. Until very recently, IHC played little role in the diagnosis of LGFMS. However, it is now understood that nearly 100% of LGFMS are MUC4-positive, a finding that correlates extremely well with the presence of the LGFMS-specific *FUS-CREB3L2/CREB3L1* gene fusions (Fig. 2.14). IHC for MUC4 may thus serve as an excellent surrogate to molecular genetic studies (*FUS* FISH) in the diagnosis of LGFMS.

Approximately 40% of LGFMS also express epithelial membrane antigen (EMA), a finding that may cause confusion with perineurioma, especially as the morphological features of these two tumors overlap to a degree. In addition to EMA, perineuriomas typically show some combination of CD34, claudin-1, and GLUT1 expression, markers not expressed by LGFMS (Fig. 2.15). Expression of claudin-1, a tight junction-related protein, and GLUT1, a glucose transported molecule, in perineuriomas likely reflects the normal role of the perineurial cell in the establishment of the blood-nerve barrier. Expression of these markers, and absent expression of S100 protein and SOX10, also helps to distinguish perineuriomas from other nerve sheath tumors, such as schwannoma, solitary circumscribed neuroma, and neurofibroma.

IHC in the Differential Diagnosis of Epithelioid Malignant Cutaneous Tumors

The differential diagnosis of epithelioid malignancies in the skin is relatively broad and includes various types of carcinoma, melanoma, hematolymphoid tumors, and sarcomas, including epithelioid angiosarcoma, epithelioid sarcoma, malignant myoepithelioma (myoepithelial carcinoma), epithelioid malignant peripheral nerve sheath tumor, and other very rare sarcomas. Discussions of the immunophenotypes of the many different types of carcinoma that may involve the skin are beyond the scope of this chapter, as is a discussion of hematolymphoid tumors. Table 2.6 details a relatively small panel of IHC markers that may be of value in the differential diagnosis of malignant epithelioid tumors involving the skin.

Epithelioid Sarcoma and Epithelioid Endothelial Tumors

The distinction of epithelioid sarcoma from primary cutaneous carcinomas of various types, in particular squamous cell carcinoma, may be challenging and is of great clinical significance. Like squamous cell carcinoma, epithelioid sarcoma typically expresses keratins of both low- and high-molecular-weight types. Expression of keratin 5/6 is not, however, generally seen in epithelioid sarcoma. Squamous carcinomas may also express vimentin, particularly when they have at least some spindled morphology, and the presence of vimentin co-expression is not a specific feature of epithelioid sarcoma. In contrast, expression of CD34 is not a feature of carcinomas and is present in roughly 60% of epithelioid sarcomas. The most

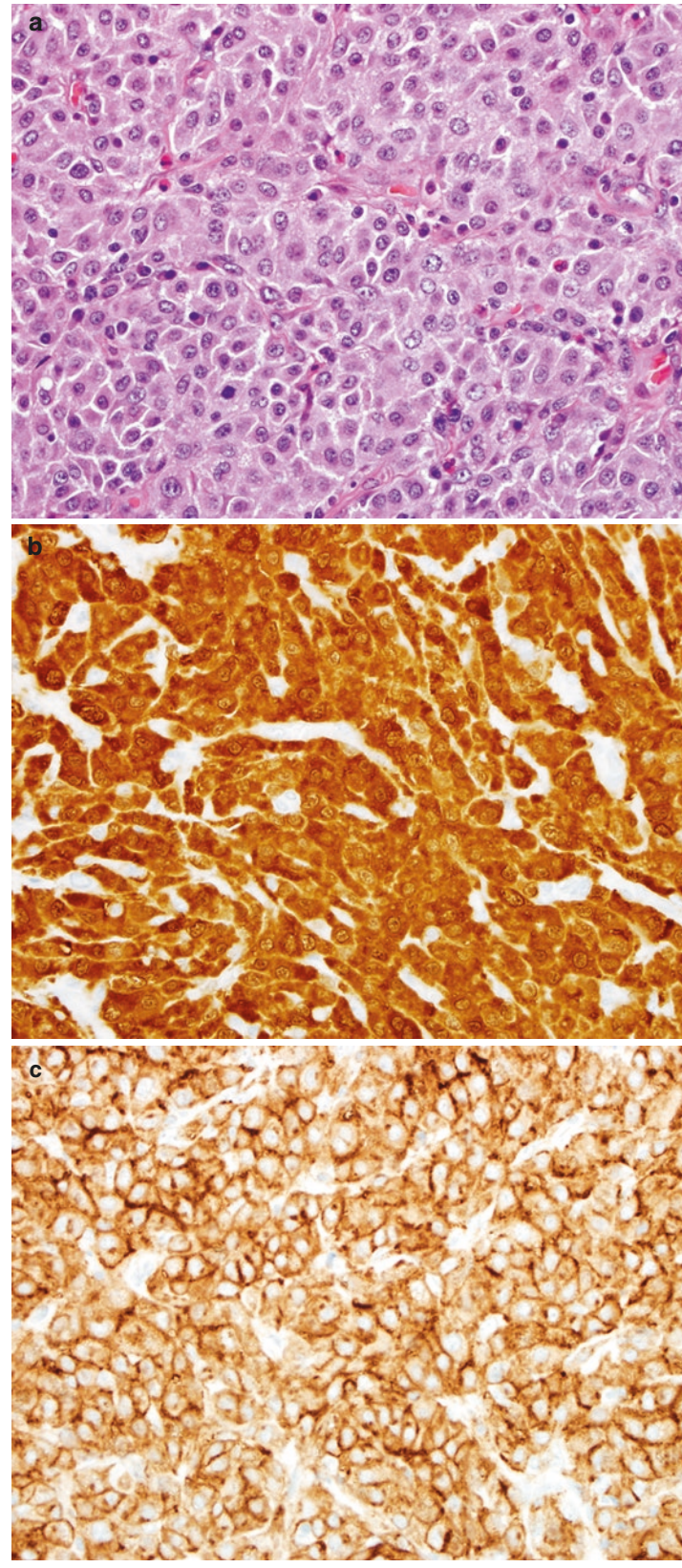


Fig. 2.13 Non-lipidized solitary xanthogranuloma (**a**), with intense immunoreactivity for Factor 13A in the tumor cells (**b**). Histiocytic markers, such as CD4, were also positive (**c**)

useful IHC marker for the distinction of epithelioid sarcoma from carcinoma is loss of expression of the SMARCB1 (INI1) tumor suppressor gene product. Loss of SMARCB1 is seen in approximately 90% of epithelioid sarcomas and is not a feature of the squamous cell carcinoma or of the overwhelming majority of other common human epithelial neoplasms (Fig. 2.16). Loss of SMARCB1 expression in epithelioid sarcoma typically is the result of *SMARCB1* gene deletion.

Loss of expression of SMARCB1 is also useful in the distinction of epithelioid sarcoma (especially those showing "pseudovascular" changes) from epithelioid endothelial tumors, in particular angiosarcoma. Epithelioid endothelial tumors that enter the differential diagnosis of epithelioid sarcoma include epithelioid angiosarcoma, epithelioid hemangioma, epithelioid hemangioendothelioma, and epithelioid sarcoma-like/pseudomyogenic hemangioendothelioma. Although all of these lesions may show keratin expression (and sometimes CD34), they all show normal (retained) expression of SMARCB1. Epithelioid hemangiomas and epithelioid sarcoma-like/pseudomyogenic hemangioendotheliomas frequently express FOSB protein, reflecting the *FOS* gene rearrangements and *SERPINE1-FOSB* fusions seen in these entities, respectively (Fig. 2.17). Epithelioid hemangioendotheliomas typically express CAMTA1 protein, resulting from the *WWTR1-CAMTA1* fusions that define this entity (Fig. 2.18).

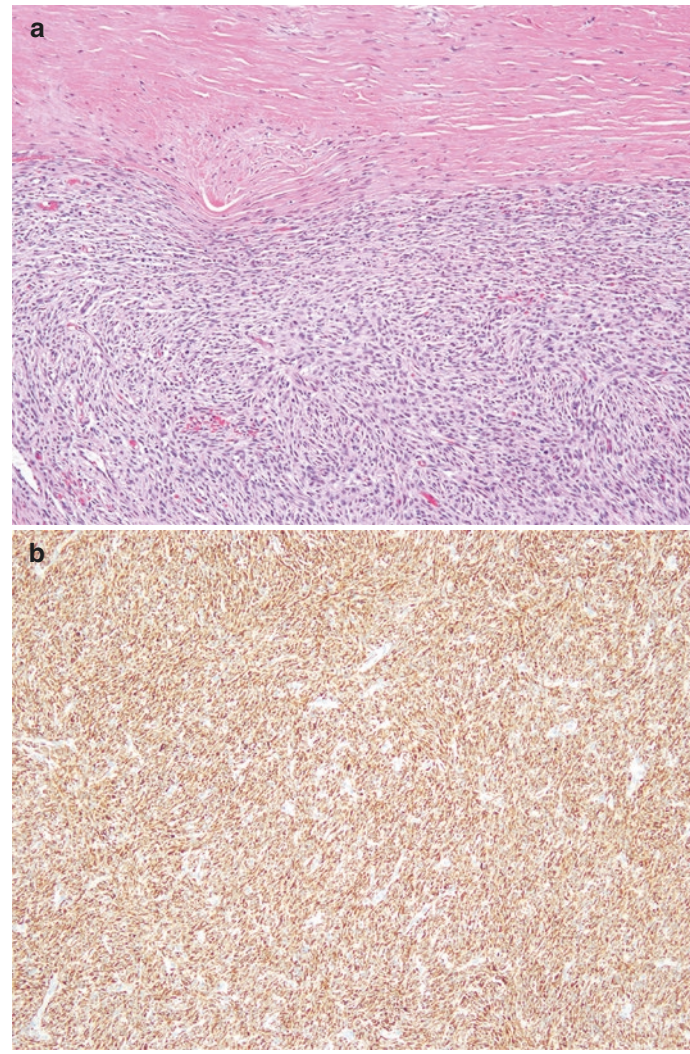


Fig. 2.14 Low-grade fibromyxoid sarcoma (**a**) with diffuse expression of MUC4 (**b**)

Melanoma

As noted above, almost all melanomas show strong expression of S100 protein and SOX10. Expression of S100 protein can be seen in some carcinomas, but usually not squamous cell carcinoma, whereas SOX10 expression seems to be limited to carcinomas showing some myoepithelial differentiation. The specific markers of melanocytic differentiation (HMB45, Melan-A, tyrosinase, MiTF) are positive in roughly 85% of epithelioid melanomas; individual cases may be positive for one or more than one of these markers.

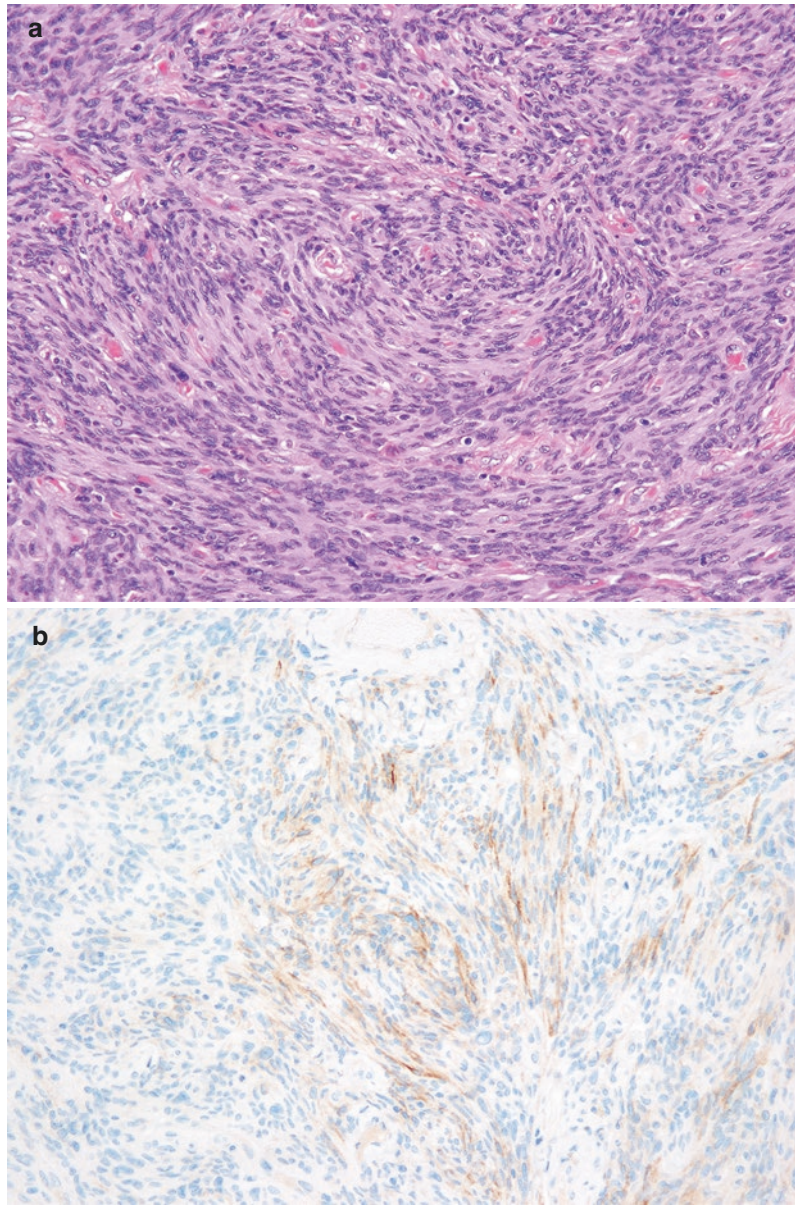


Fig. 2.15 Perineurioma (**a**) showing focal expression of epithelial membrane antigen (**b**). EMA expression may be limited in perineuriomas, and confirmation of this diagnosis may require immunostains for other perineurial markers, such as claudin-1 (**c**) or GLUT1

Fig. 2.15 (continued)

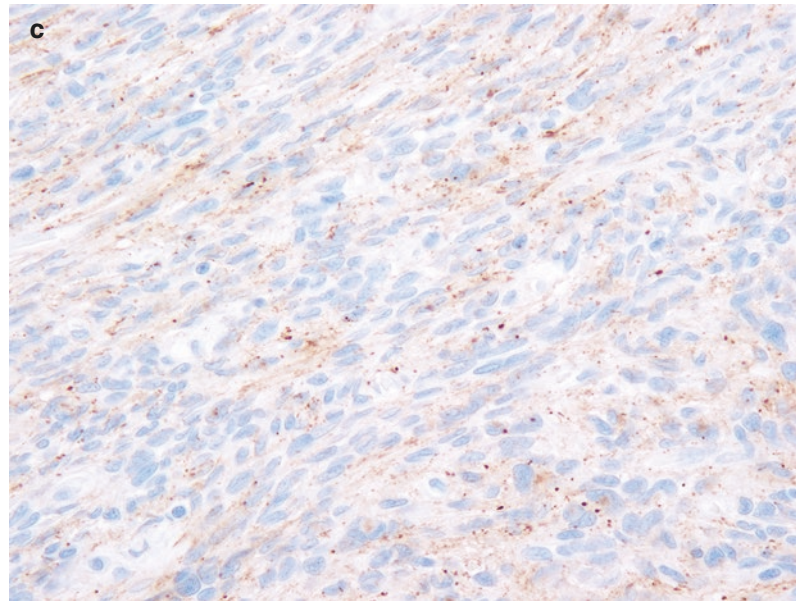


Table 2.6 Screening panel for epithelioid neoplasms

Antigen	CA	Melanoma/E-MPNST	Lymphoma	Myoepithelioma	ES	EAS
Keratin	Positive	Variable in melanoma, negative in E-MPNST	Negative	Negative	Positive	Variable
S-100 protein/ SOX10	Negative	Positive	Negative	Variable	Negative	Negative
CD45	Negative	Negative	Positive in conventional B and T cell lymphomas, negative in most ALCL	Negative	Negative	Negative
CD30	Negative	Negative	Negative in most conventional B and T cell lymphomas, positive in ALCL	Positive	Negative	Negative
CD31	Negative	Negative	Negative	Negative	Negative	Positive
SMARCB1 (INI1)	Normal	Normal in melanoma; lost in 50% of EMPNST	Normal	Lost in 50%	Lost in 90%	Normal

ALCL anaplastic large cell lymphoma, *CA* carcinoma, *EAS* epithelioid angiosarcoma, *E-MPNST* epithelioid malignant peripheral nerve sheath tumor, *ES* epithelioid sarcoma

Aberrant expression of intermediate filament proteins in epithelioid melanoma represents a significant diagnostic pitfall, in particular keratin expression (Fig. 2.19). Aberrant keratin expression may be seen in up to 40% of otherwise-typical epithelioid melanomas, in particular with the AE1/AE3 keratin antibodies. Melanomas may also show aberrant expression of desmin, neurofilament protein, and GFAP in smaller percentages of cases. Aberrant expression of neuro-

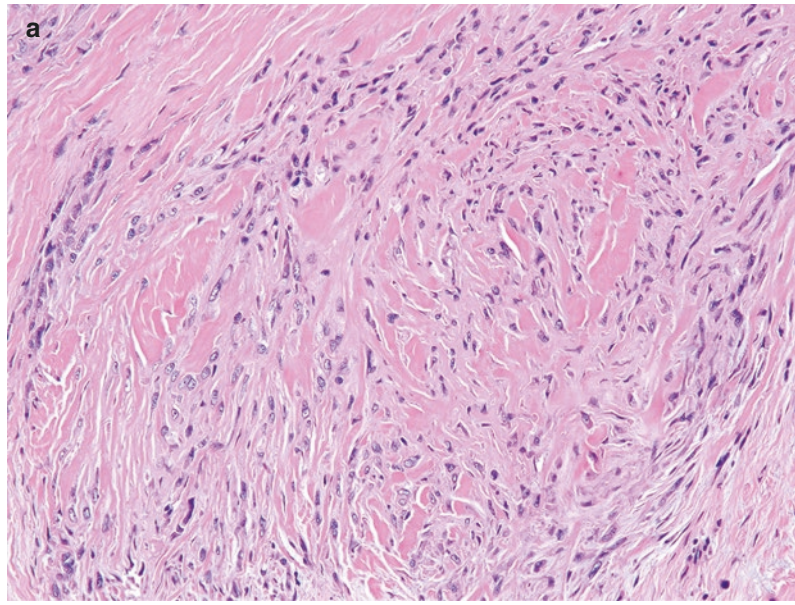


Fig. 2.16 Epithelioid sarcoma (**a**), positive for keratins (**b**). Although keratin expression in epithelioid sarcoma may cause confusion with carcinoma, loss of expression of SMARCB1 (INI1) is seen in >90% of epithelioid sarcomas (**c**) and is not a feature of the overwhelming majority of carcinomas

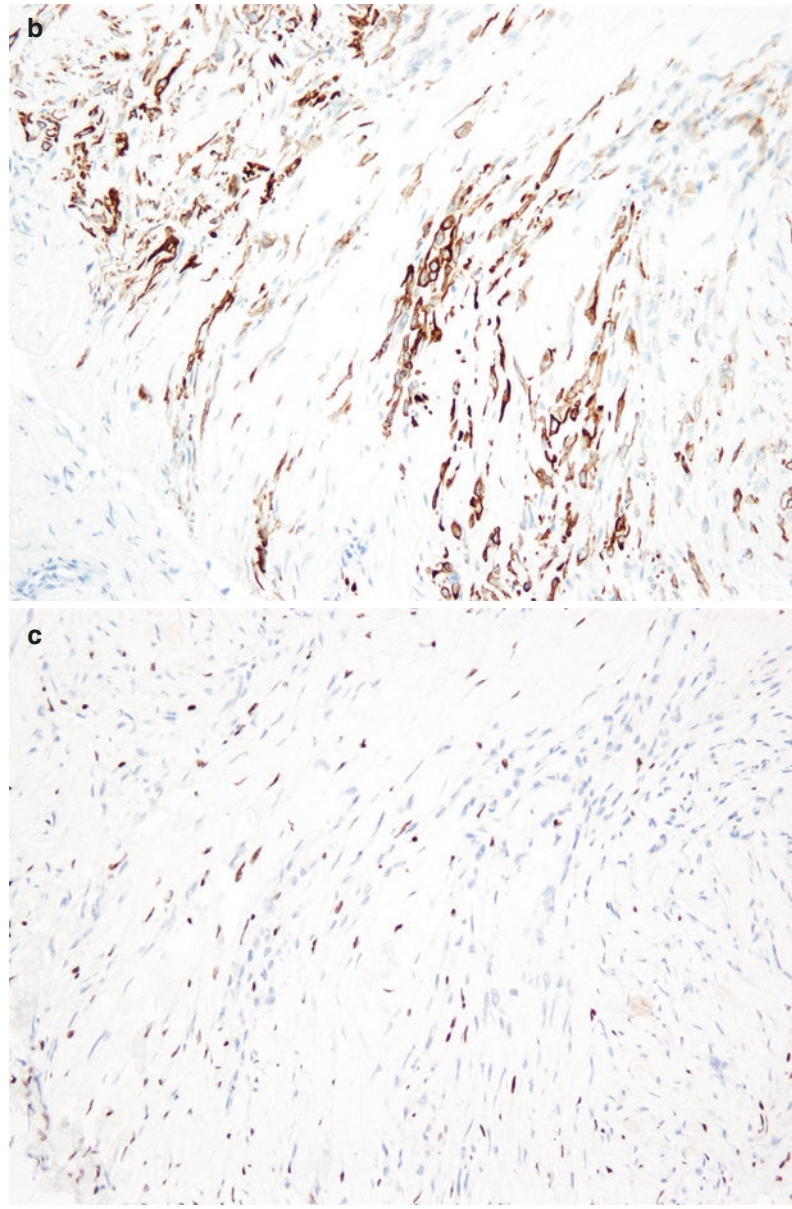
endocrine markers, such as synaptophysin, may also be seen potentially resulting in confusion with various neuroendocrine tumors. Application of a panel of IHC markers, to include S100 protein, SOX10, and specific melanoma markers, is the key to the recognition of melanomas with aberrant intermediate filament protein and/or neuroendocrine marker expression.

Epithelioid melanomas may also be confused with epithelioid malignant peripheral nerve sheath tumors (EMPNST) and with clear cell sarcoma (CCS). Although both EMPNST and CCS typically present as more deeply situated soft tissue masses, they may involve the skin on occasion or rarely present as primary dermal neoplasms. EMPNST are almost always strongly and diffusely positive for S100 protein, in contrast to other types of MPNST, which typically show only patchy and weak expression (Fig. 2.20). EMPNST characteristically show abundant collagen IV expression surrounding nests of cells, a feature not generally seen in melanoma. Unlike melanomas and CCS, which also show diffuse S100 protein expression, EMPNST are negative for melanocytic markers such as HMB45, Melan-A, and tyrosinase. Approximately 50% of EMPNST show loss of SMARCB1 expression, a finding that may be of value in their differential diagnosis with melanoma and CCS, both of which show retained expression of this protein. There are no IHC markers that distinguish melanoma and CCS; definitive diagnosis of CCS in the skin requires demonstration of the CCS-associated *EWSR1-ATF1/CREB1* gene fusions (Fig. 2.21).

Myoepithelial Tumors

The diagnosis of myoepithelial tumors in the skin and soft tissues is challenging, as these lesions may show a broad morphological spectrum, ranging from predominantly myxoid and reticulated tumors, mimicking extraskeletal myxoid chondrosarcoma, to solid, epithelioid, and rhabdoid lesions, closely resembling carcinoma, proximal-type epithelioid sarcoma, or rhabdoid tumor. Morphologically benign soft tissue myoepithelial tumors typically co-express epithelial

Fig. 2.16 (continued)



markers (e.g., keratins, EMA) and S100 protein/SOX10 and express other myoepithelial markers (e.g., muscle actins, calponin, glial fibrillary acidic protein, and p63) in up to 50% of cases (Fig. 2.22). IHC is less helpful in the diagnosis of myoepithelial carcinomas, as epithelial marker and S100 protein expression may be extremely limited (or even absent). It is generally (but not universally) accepted that expression of muscle actins *alone* in the appropriate morphological setting is sufficient for the diagnosis of myoepithelial carcinoma. SMARCB1 loss by IHC is seen in approximately 50% of myoepithelial carcinomas. Genetically, soft tissue myoepitheliomas are characterized in approximately 45% of cases by *EWSR1* gene rearrangements with a variety of different fusion partners, findings not seen in carcinoma or epithelioid sarcomas.

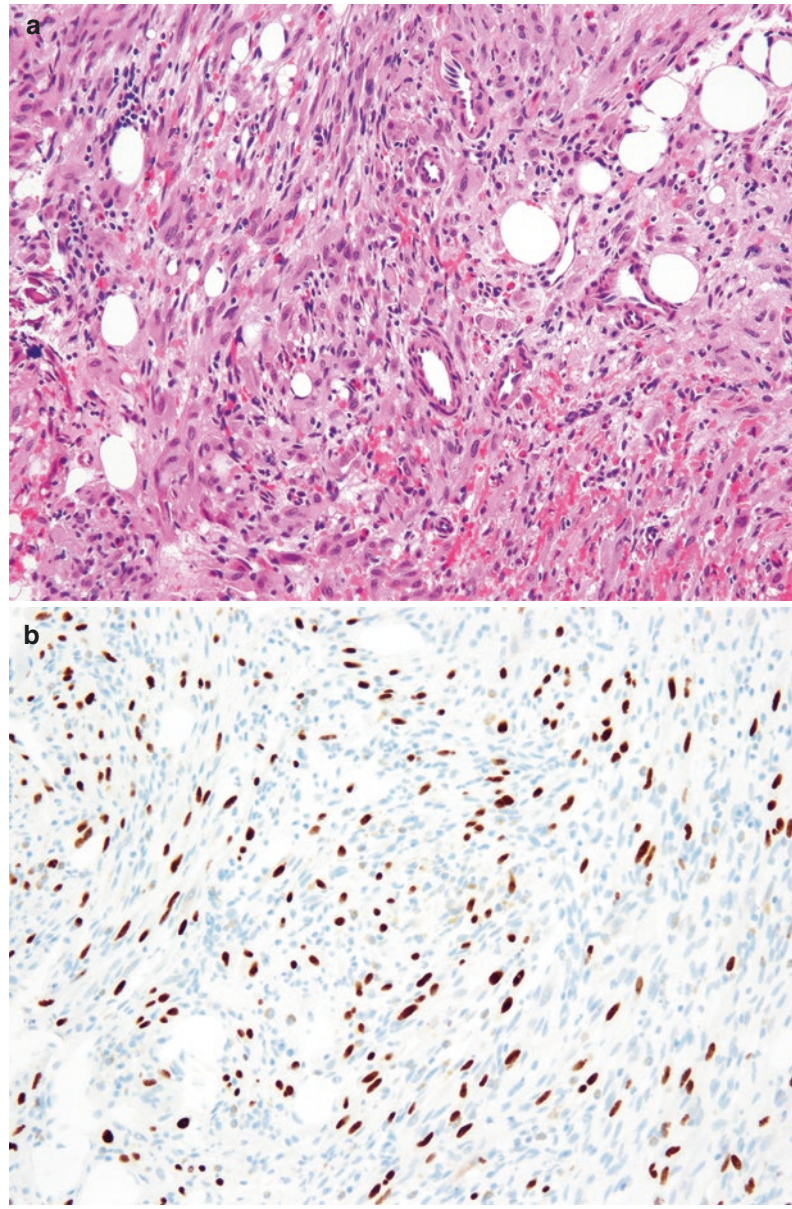


Fig. 2.17 Epithelioid sarcoma-like (pseudomyogenic) hemangioendothelioma (**a**) showing nuclear immunoreactivity for FOSB protein (**b**), indicative of the entity-defining *SERPINE1-FOSB* fusion

IHC in the Differential Diagnosis of Tumors Composed of Small, Round Cells

The differential diagnosis for tumors composed chiefly of small, round cells in the skin is quite broad, and different than in other soft tissue locations. Whereas in non-cutaneous soft tissue locations this differential diagnosis tends to center on lesions such as Ewing sarcoma, alveolar rhabdomyosarcoma, poorly differentiated synovial sarcoma, desmoplastic round cell tumor, mesenchymal chondrosarcoma, neuroblastoma, and the "Ewing-like" sarcomas defined by *CIC* or *BCOR* rearrangements, these tumors are quite rare in the skin. In the skin, tumors of small, round cells much more often represent carcinoma (e.g., Merkel cell carcinoma), small-cell melanoma, hematolymphoid tumors, and sometimes glomus tumors (includ-

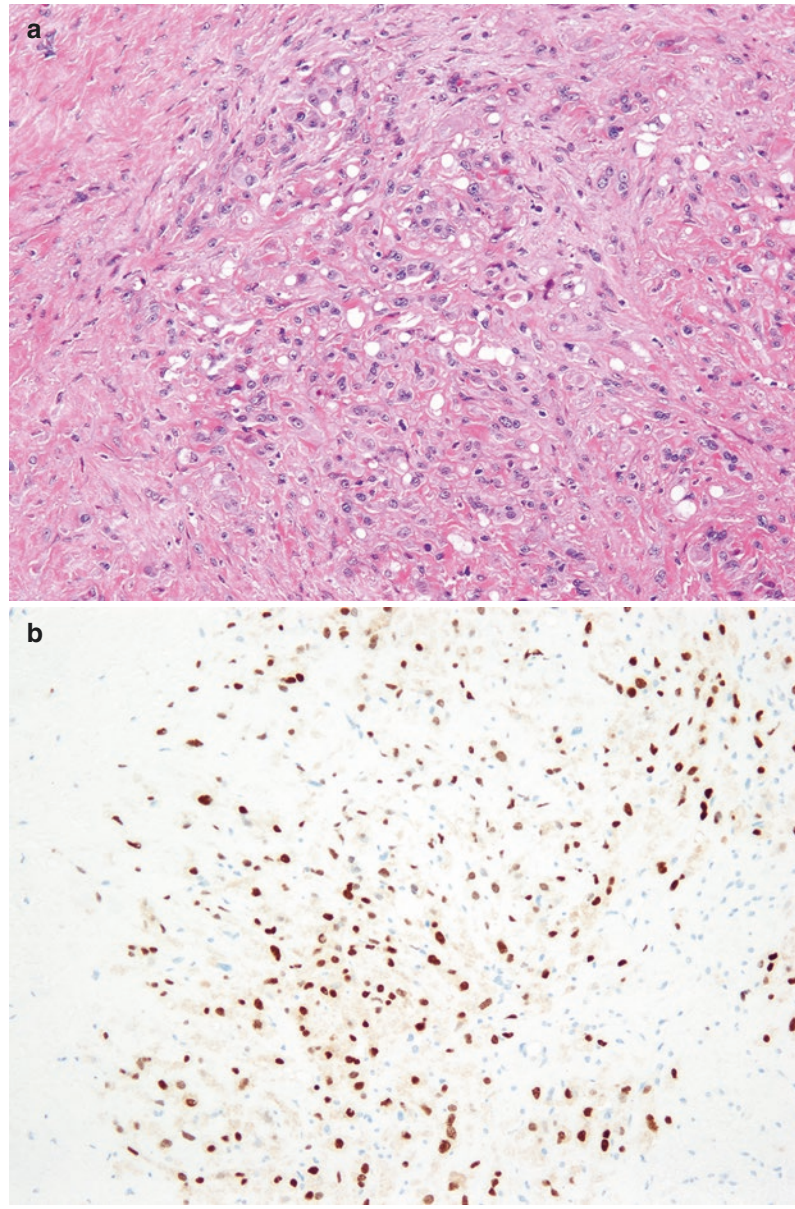


Fig. 2.18 Epithelioid hemangioendothelioma (**a**), with nuclear expression of CAMTA1 protein (**b**). CAMTA1 immunohistochemistry serves as a useful surrogate for molecular genetic studies to identify the EHE-specific *WWTR1-CAMTA1* gene fusion

ing malignant glomus tumors). Applications of IHC to the differential diagnosis of hematolymphoid tumors in the skin are outside of the focus of this chapter. Table 2.7 presents a screening panel of antibodies and the expected results for these tumors. The results of this panel dictate what additional studies are needed to confirm a specific diagnosis. In some instances, the best additional tests may be molecular genetic studies, rather than IHC.

Comments on Specific Round Cell Tumors

Merkel cell carcinomas will typically label for keratin 20 in addition to low molecular weight keratins, often in a distinctive "dot-like" pattern. Synaptophysin, chromogranin A, and CD56 are almost always positive as well. Antibodies to Merkel cell polyomavirus may also be helpful in confirming this diagnosis.

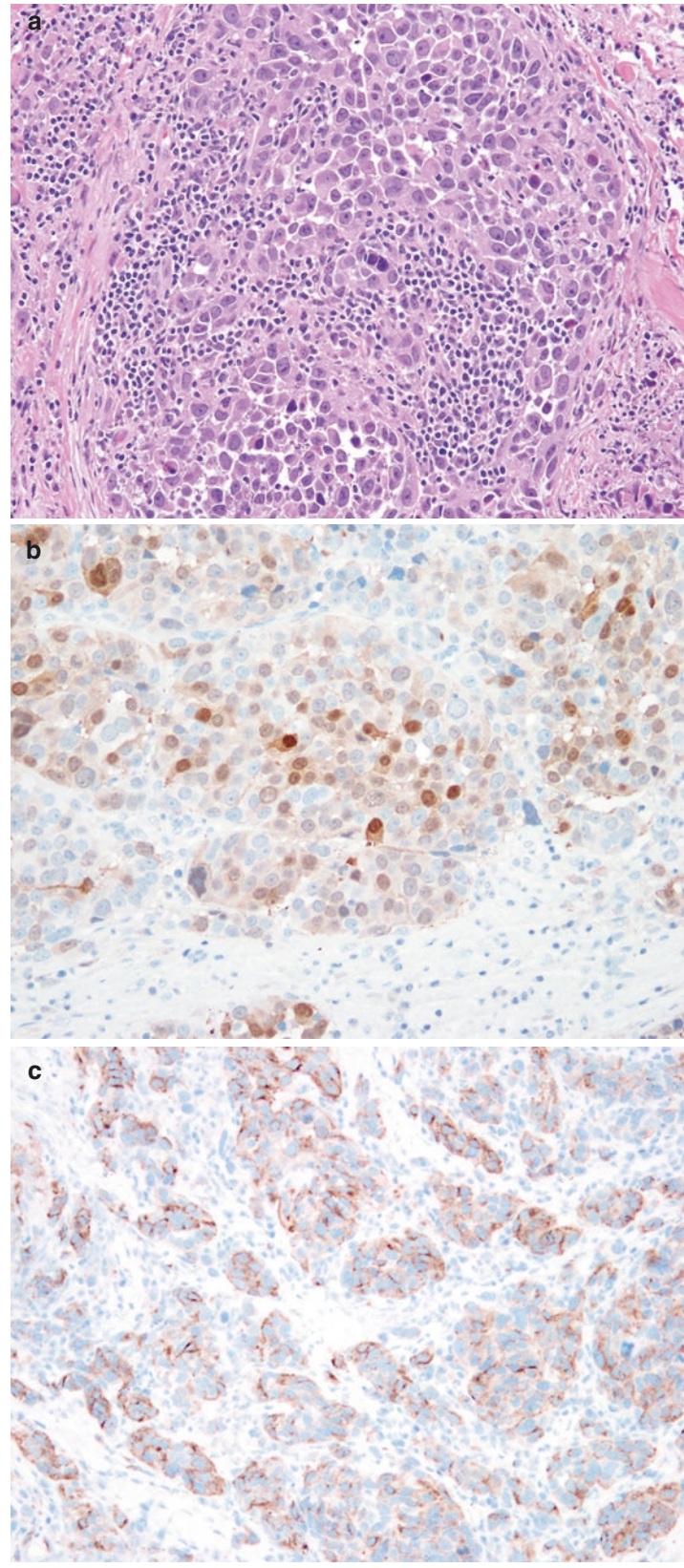


Fig. 2.19 Epithelioid malignant melanoma (**a**), showing expression of S100 protein (**b**). This tumor was also positive for other melanocytic markers, such as HMB45. Robust aberrant expression of keratins was present in this tumor, a potential diagnostic pitfall (**c**)

As noted previously, small-cell melanomas may show a deceptive S100 protein/SOX10-negative, HMB45/Melan-A-positive phenotype, and the absence of S100 protein/SOX10 does not exclude this diagnosis. Aberrant expression of keratins, desmin, and/or neuroendocrine markers may be particularly treacherous in small-cell melanomas.

Lymphoblastic lymphoma may be CD45-negative and CD99/FLI-1-positive, which can easily result in a misdiagnosis as Ewing sarcoma. TDT may be critical in arriving at the correct

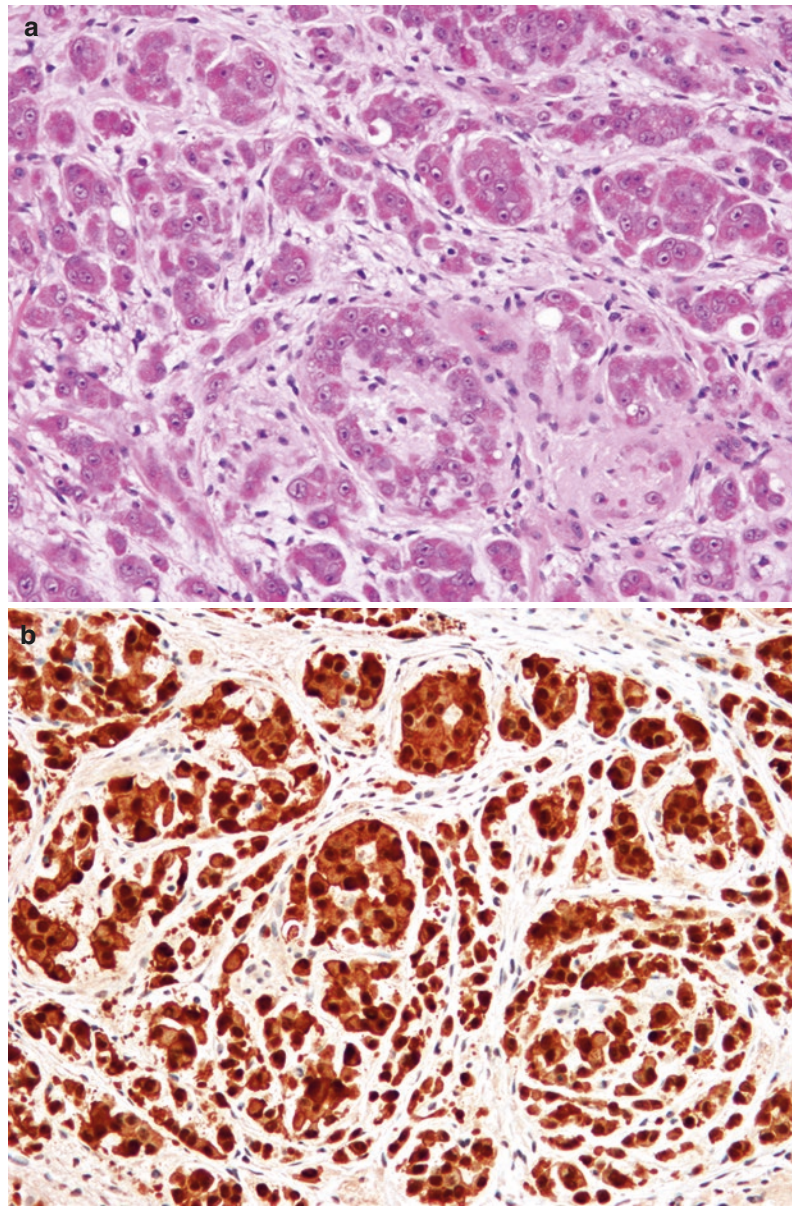
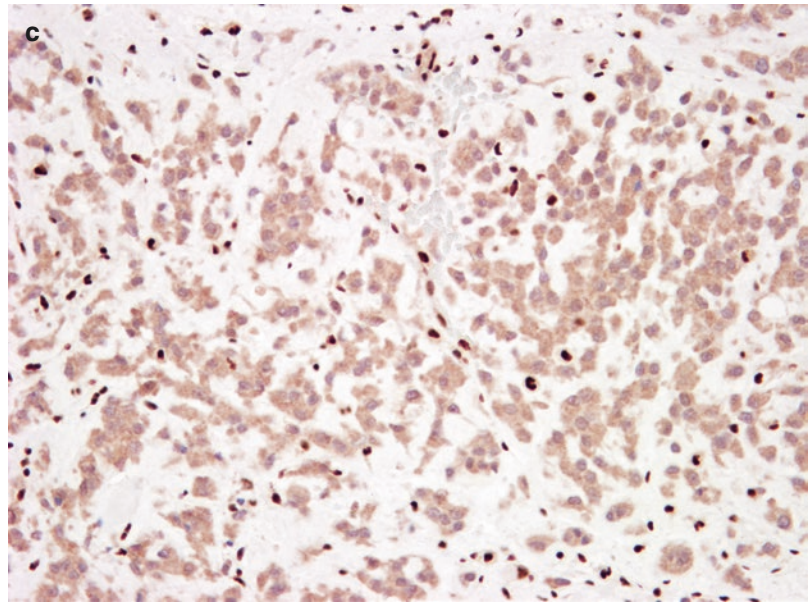


Fig. 2.20 The morphological features of epithelioid malignant peripheral nerve sheath tumor (**a**) overlap significantly with those of melanoma, and both tumors are typically strongly positive for S100 protein (**b**). However, >50% of epithelioid malignant peripheral nerve sheath tumor also show loss of SMARCB1 expression (**c**), a finding not seen in melanoma

Fig. 2.20 (continued)



diagnosis. Anaplastic large-cell lymphomas, including the small-cell variant, may also be CD45-negative. CD30 is useful here. Myeloid markers should be performed for suspected myeloid malignancies.

Ewing sarcoma usually shows diffuse, membranous expression of CD99 in the absence of expression of other markers. CD99 is, however, a very non-specific marker, expressed by many different "small, round cell tumors." Roughly 20% of Ewing sarcoma show aberrant keratin expression, and rare cases show focal desmin expression. FLI1 and ERG protein expression may be helpful, but are also not specific. Over 95% of Ewing sarcoma show the *EWSR1-FLI1* or *EWSR1-ERG* fusion genes, detectable by RTPCR or FISH. In the appropriate morphological and immunohistochemical context, detection of *EWSR1* rearrangement alone by FISH is supportive. Very rare Ewing sarcoma shows *FUS* rearrangements, however.

Alveolar rhabdomyosarcoma will express desmin in addition to myogenin and MyoD1. Myogenin expression is typically strong and diffuse in alveolar RMS. Many alveolar RMS show aberrant expression of keratins and neuroendocrine markers, such as synaptophysin and CD56. Demonstration of the alveolar RMS-specific *PAX3/PAX7-FOXO1A* fusions by FISH or RT-PCR is confirmatory.

In poorly differentiated synovial sarcoma, keratin expression may be patchy or absent in some cases. EMA and high-molecular-weight keratins may be positive in such cases. CD34 expression is not seen in synovial sarcomas. Uniform, strong, nuclear expression of TLE1 protein is strongly suggestive of poorly differentiated synovial sarcoma and is a useful screening test. Cases showing only TLE1 expression should be confirmed with molecular tests for the *SSX1/2/4-SS18* fusions, either by RTPCR or FISH.

Desmoplastic small round cell tumors characteristically co-express keratins, desmin and vimentin. Carboxy-terminus WT1 antibodies can assist in confirmation of this diagnosis but are not widely available. The WT1 antibodies used in most laboratories are to the amino-terminus end and are not useful for this purpose. Definitive diagnosis may require demonstration of the *EWSR1-WT1* fusion gene by molecular means.

Glomus tumors show an identical phenotype to normal glomus cells, with expression of smooth muscle actin and caldesmon, but not desmin. Abundant pericellular collagen IV

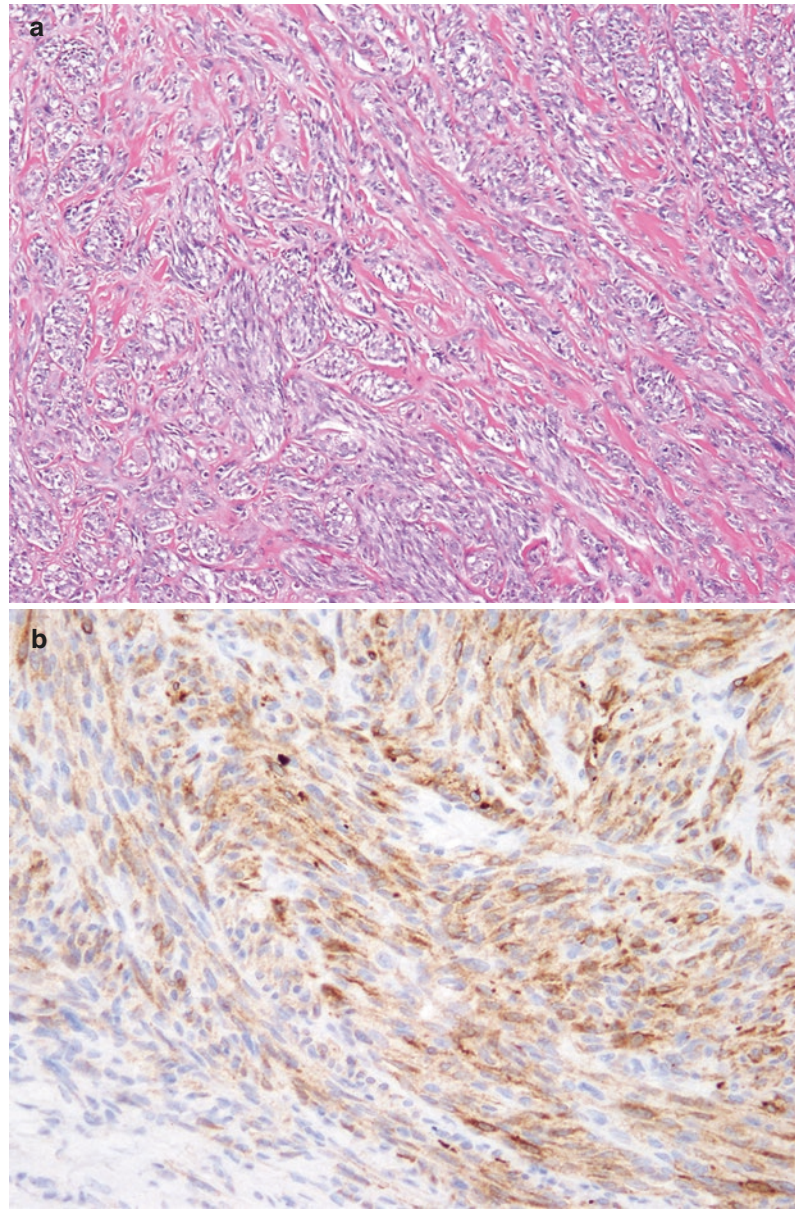


Fig. 2.21 The morphological features of clear cell sarcoma (**a**) are also very similar to those of melanoma. Unfortunately, there are no immunohistochemical markers that distinguish clear cell sarcoma from melanoma, as both are typically positive for S100 protein, SOX10, and more specific markers such as HMB45 (**b**). Molecular testing for the clear cell sarcoma-specific *EWSR1-ATF1* fusion may be required to make this distinction in some cases

expression is a characteristic of glomus tumors. Malignant glomus tumors show the same immunophenotype as do benign glomus tumors but may be extremely difficult to recognize in the absence of a pre-existing benign glomus tumor. Although antibodies to smooth muscle actins are not generally part of the panel of immunostains used to evaluate "small, round cell tumors" in extracutaneous sites, they may be very helpful in the skin.

Other Application of IHC in Cutaneous Mesenchymal Tumors

Adipocytic Tumors

Primary adipocytic tumors of the skin are uncommon, and most are easily diagnosed by morphology alone. The distinction of cutaneous spindle cell/pleomorphic lipoma (SCL/PL) from

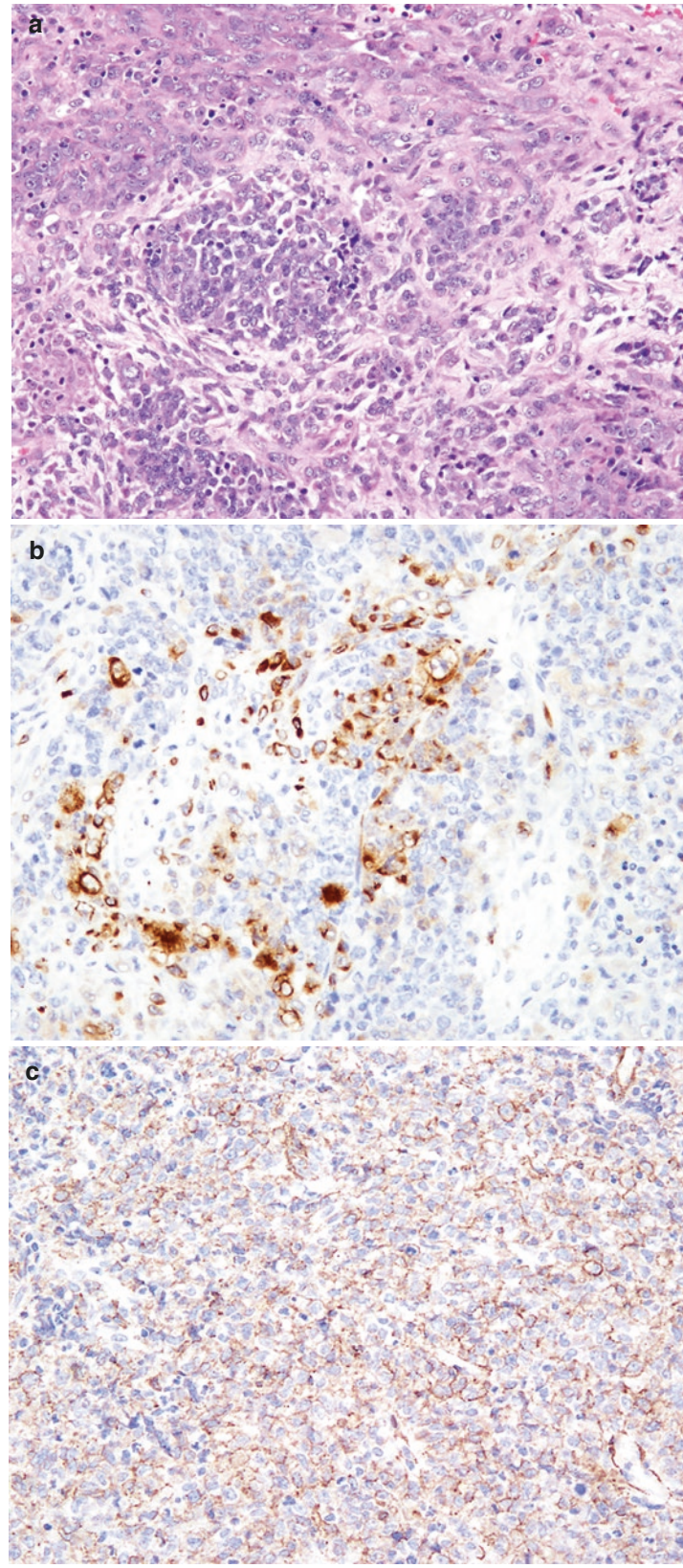


Fig. 2.22 Malignant myoepithelioma (**a**), showing co-expression of keratins (**b**) and smooth muscle actins (**c**)

superficial atypical lipomatous tumor (well-differentiated liposarcoma) (ALT) can at times be challenging, however. At the genetic level, SCL/PL show *RB* gene deletions, with loss of expression of Rb protein. This finding is not seen in ALT, which show instead amplification of the *MDM2* and *CDK4* genes, with overexpression of these proteins. In problematic cases, IHC for RB protein and MDM2 protein may be helpful in distinguishing SCL/PL from ALT. It should be noted that the lipid-laden histiocytes present in fat necrosis are also frequently MDM2 protein-positive, a potential pitfall in the distinction of this nonneoplastic process from ALT.

Atypical Vascular Lesions and Angiosarcomas Arising After Therapeutic Irradiation

In patients who have received therapeutic irradiation (usually, but not always, for breast cancer), the distinction of atypical vascular lesions (AVL) from well-differentiated angiosarcoma may be extremely difficult. Recent data indicates that postirradiation angiosarcoma frequently shows amplification of the *MYC* gene, with overexpression of MYC protein, whereas AVL lack these findings (Fig. 2.23). Demonstration of strong, uniform MYC protein expression in the nuclei of

Table 2.7 Screening panel for small, blue, round cell tumors

Antibody to	Merkel cell carcinoma	Melanoma	Lymphoma	ES	RMS	PDSS
Keratin	Positive	Variable	Negative	Variable	Rare	Positive
Keratin 20	Positive	Negative	Negative	Negative	Negative	Negative
Merkel cell polyoma virus	Positive	Negative	Negative	Negative	Negative	Negative
S-100 protein/SOX10	Negative	Positive	Negative	Variable	Rare	Variable
CD45	Negative	Negative	Positive	Negative	Negative	Negative
TdT	Variable	Negative	Positive	Negative	Negative	Negative
Desmin	Negative	Variable	Negative	Rare	Positive	Negative
CD99	Negative	Negative	Variable	Positive	Variable	Positive

ES Ewing sarcoma, *PDSS* poorly differentiated synovial sarcoma, *RMS* rhabdomyosarcoma, *SCCA* small-cell carcinoma

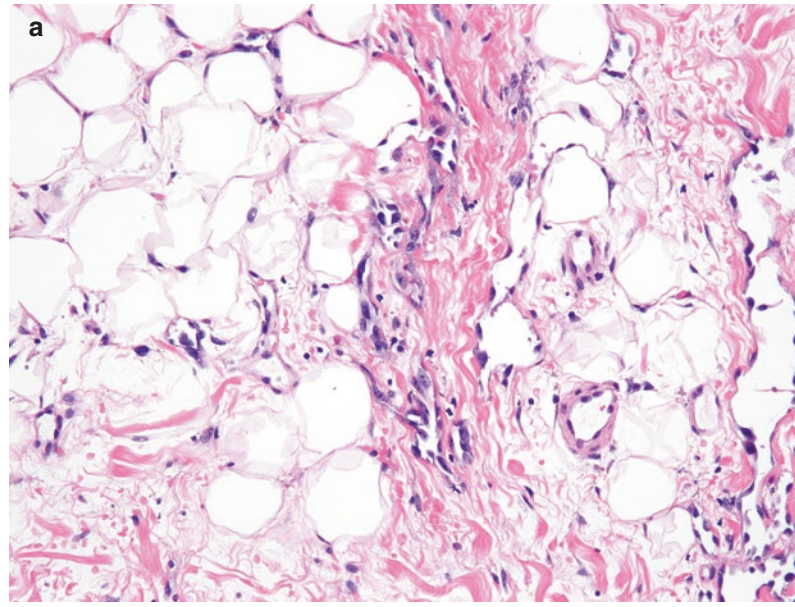
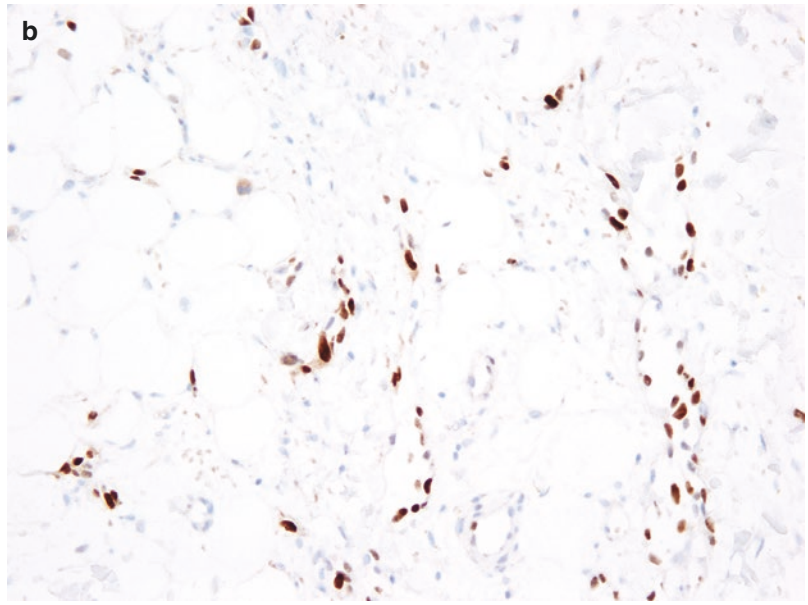


Fig. 2.23 Postirradiation angiosarcoma of the skin of the breast (**a**). Strong nuclear immunoreactivity for MYC protein was present (**b**), further supporting classification of this tumor as angiosarcoma, as opposed to an atypical vascular lesion following irradiation

Fig. 2.23 (continued)



endothelial cells lining the vascular channels in question may be helpful in confirming a diagnosis of angiosarcoma in selected cases, although caution is urged in interpreting this finding. This is because some cases may show MYC staining as the result of polysomy of chromosome 8, rather than MYC gene amplification, and because this immunostain can be difficult to interpret or may label only scattered endothelial cells. There may be some benefit in performing both MYC IHC and FISH for *MYC* amplification. It is important to keep in mind that *MYC* amplification is not a feature of primary cutaneous angiosarcoma, and MYC IHC is not helpful in this setting.

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