An Update on the Application of Newly Described Immunohistochemical Markers in Soft Tissue Pathology

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• ***Context.***—**During the last 5 to 10 years, significant progress has been made in the molecular characterization of soft tissue tumors, predominantly with the identification of recurrent translocations or amplification of certain genes in different tumor types. Alongside this, translational efforts have identified many novel and diagnostically useful immunohistochemical markers for many of these tumor types.**

Objective.—**This article reviews a select group of recently described immunohistochemical markers of particular use in the evaluation of mesenchymal neoplasms; the underlying biology of the protein product, practical utility, and limitations of each marker are discussed in detail.**

Data Sources.—**Literature review, authors' research data, and personal practice experience serve as sources.**

Conclusions.—**There are many diagnostically useful immunohistochemical markers to help confirm the diagnosis of many different soft tissue tumor types, some of which have reduced the need for additional, and more costly, studies, such as fluorescence in situ hybridization. However, no one marker is 100% specific for a given tumor, and knowledge of potential pitfalls and overlap in patterns of staining among other tumor types is crucial to ensure the appropriate application of these markers in clinical practice.**

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The last 10 years have seen the description of many immunohistochemical markers in the field of soft tissue tumor pathology. As expected, some of these markers prove to be more useful in clinical practice than others, and with time it is generally appreciated that significant overlap in staining patterns can be seen in different tumor types, some of which share similar biology or can be explained by known biologic mechanisms. Perhaps the most interesting aspect pertaining to many of these recently described markers is the method of discovery and the speed at which they have been translated into clinical practice; for example, gene expression profiling studies have identified the protein products TLE1, DOG1, and MUC4 as clinically useful markers for synovial sarcoma, gastrointestinal stromal tumor (GIST), and low-grade fibromyxoid sarcoma, respectively.

In addition, new insights into the biology of several different tumor types are reflected in many recently described immunohistochemical markers, such as tumors with INI-1 loss; tumors with amplification in the region of chromosome 12q13-15, which generally show overexpression of MDM2 and CDK4 (and occasionally STAT6); *MYC* amplification in postradiation angiosarcoma; and metabolic enzyme pathway disturbances in the clinicopathologically distinct group of succinate dehydrogenase–deficient GIST. Fusion protein products resulting from recurrent translocations may also be useful markers, such as STAT6 for solitary fibrous tumor and TFE3 for alveolar soft part sarcoma and a subset of epithelioid hemangioendothelioma. Finally, lineage-specific markers, which tend to show nuclear staining, include ERG as a marker of endothelial differentiation and SOX10 as a marker of neuroectodermal differentiation. The utility of these markers, along with their limitations and potential pitfalls, are discussed in detail in this review and are summarized in the Table.

ERG (Avian v-ets Erythroblastosis Virus E26 Oncogene Homolog)

ERG is a member of the ETS family of transcription factors, which also include ETS-1; Friend leukemia integration site 1 (Fli-1); NERF-2; and TEL; and which are expressed by vascular endothelial cells and defined by a conserved DNA-binding ETS domain that forms a winged helix-turn-helix structural motif.[1] ERG regulates endothelial cell differentiation, angiogenesis, and expression of several endothelial-specific antigens, and it is also required for embryonic stem cells to differentiate into endothelial cells.[2–6] Detection of ERG expression by immunohistochemistry was first described in a subset of prostatic adenocarcinomas, where it was shown to correlate with ERG overexpression via chimeric fusion proteins generated from oncogenic translocations, with *TMPRSS2-ERG* being the most common variant, seen in approximately 50% of all prostate-specific antigen–screened prostate cancers detected in the United States.[7–12]

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Recently Described Immunohistochemical Markers of Soft Tissue Tumors			
Antibody	**Expression Pattern**	**Main Use(s)**	**Comments**
ERG	Nuclear	Confirm endothelial differentiation	Also stains a subset of epithelioid sarcomas, subset of Ewing sarcoma, and 45% of prostatic carcinomas (117 of 261 cases[17]; 30 of 66 cases[13])
MYC	Nuclear	Differentiate postradiation angiosarcoma from APRVP	Expressed in a small subset of primary angiosarcomas (usually head and neck)
MDM2/CDK4	Nuclear	Coexpressed in most well-differentiated and dedifferentiated liposarcomas Coexpressed in intimal sarcoma and parosteal and low-grade central osteosarcoma	MDM2 is positive in up to 64% of MPNSTs (21 of 33 cases[46]), 42% of myxofibrosarcomas (10 of 24 cases[46]), and 29% of embryonal rhabdomyosarcomas (12 of 41 cases[46]); MDM2 positivity is also seen in histiocytes
STAT6	Nuclear (+/− cytoplasmic)	Positive in >97% of SFTs (59 of 60 cases[60]; 54 of 54 cases[61]; 34 of 35 cases[62]; 49 of 49 cases[63])	Expressed in 15% of dedifferentiated liposarcomas (3 of 21 cases[60])
MUC4	Cytoplasmic	Expressed in vast majority of low-grade fibromyxoid sarcoma (49 of 49 cases[72]) and sclerosing epithelioid fibrosarcoma (32 of 41 cases[82]; 14 of 15 cases[83])	Focal positivity seen in synovial sarcoma, ossifying fibromyxoid tumors, and epithelioid GIST Expressed in many carcinomas
DOG1	Cytoplasmic + membranous	Positive in >87% of GISTs (136 of 139 cases[89]; 986 of 1040 cases[90]; 370 of 425 cases[91]) Useful to confirm a diagnosis of GIST in gastric KIT-negative tumors	Various other tumor types reported to show focal staining
SDHB/A	Cytoplasmic, granular	Loss of SDHB expression confirms diagnosis of SDH-deficient GIST Loss of SDHA expression indicative of *SDHA* mutations	Loss of SDHB staining also seen in pheochromocytoma/paraganglioma and rare renal cell carcinomas associated with SDH complex dysfunction
INI1	Nuclear	Loss of expression in 93% of epithelioid sarcomas (127 of 136 cases[131]) and virtually all malignant rhabdoid tumors	Loss of staining also seen in 50% of epithelioid MPNSTs (12 of 24 cases[131]) and 40% of pediatric myoepithelial carcinomas (9 of 22 cases[133])
TLE1	Nuclear	Expression seen in >90% of synovial sarcomas (91 of 94 cases[150]; 249 of 259 cases[155]; 39 of 43 cases[156]; 35 of 35 cases[157]; 18 of 20 cases[158])	Expression often seen in MPNST and SFT (usually, but not always, less diffuse than in synovial sarcoma)
TFE3	Nuclear	Positive in virtually all ASPS	Also positive in Xp11 translocation–type renal cell carcinomas, subset of epithelioid hemangioendotheliomas (with *YAP1-TFE3* fusion gene), and a subset of PEComas
SOX10	Nuclear	Confirm neural crest differentiation—melanocytic tumors, clear cell sarcoma, most benign nerve sheath tumors and 27%–50% of MPNSTs (21 of 78 cases[179]; 38 of 77 cases[182])	Also stains a subset of myoepithelial and salivary gland tumors, and subset of carcinoid tumors and breast carcinomas
NY-ESO-1	Cytoplasmic and nuclear	Expressed in up to 80% of synovial sarcomas (20 of 25 cases[188]; 38 of 50 cases[189]) and 95% of myxoid liposarcomas (36 of 38 cases[192])	Positivity is also seen in melanoma and a variety of carcinomas

Abbreviations: APRVP, atypical postradiation vascular proliferation; ASPS, alveolar soft part sarcoma; GIST, gastrointestinal stromal tumor; MPNST, malignant peripheral nerve sheath tumor; PEComa, perivascular epithelioid cell tumor; SDH, succinate dehydrogenase; SFT, solitary fibrous tumor.

A large study evaluating ERG as an immunohistochemical marker for vascular tumors showed that nuclear positivity for ERG was present in the endothelia of all hemangiomas and lymphangiomas examined.[13] In addition, virtually all subtypes of hemangioendotheliomas were positive for ERG, including kaposiform hemangioendotheliomas, retiform hemangioendotheliomas, and epithelioid hemangioendotheliomas. Expression of ERG was also seen in all Kaposi sarcomas evaluated, and in 96 of 100 angiosarcomas (Figure 1).[13] A wide variety of other nonvascular and nonepithelial mesenchymal, neuroectodermal, and hematopoietic tumors were also evaluated, and the vast majority were negative for ERG, with the exception of 7 of 10 blastic extramedullary myeloid tumors (70%) and 2 of 29 Ewing sarcomas (7%).[13] Another study evaluated ERG in the context of differentiating cutaneous angiosarcoma from other cutaneous neoplasms that arise in sun-damaged skin and may enter the histologic differential diagnosis with angiosarcoma, specifically squamous cell carcinoma, malignant melanoma, and atypical fibroxanthoma. In that study, nuclear ERG expression was 100% sensitive and specific for angiosarcoma, with all 23 cases of angiosarcoma showing distinct nuclear staining, whereas all other tumors evaluated (15 poorly differentiated squamous cell carcinomas, 17 melanomas, 12 atypical fibroxanthomas, and 5 leiomyosarcomas) were negative.[14] From these studies,[13,14] ERG is positive in greater

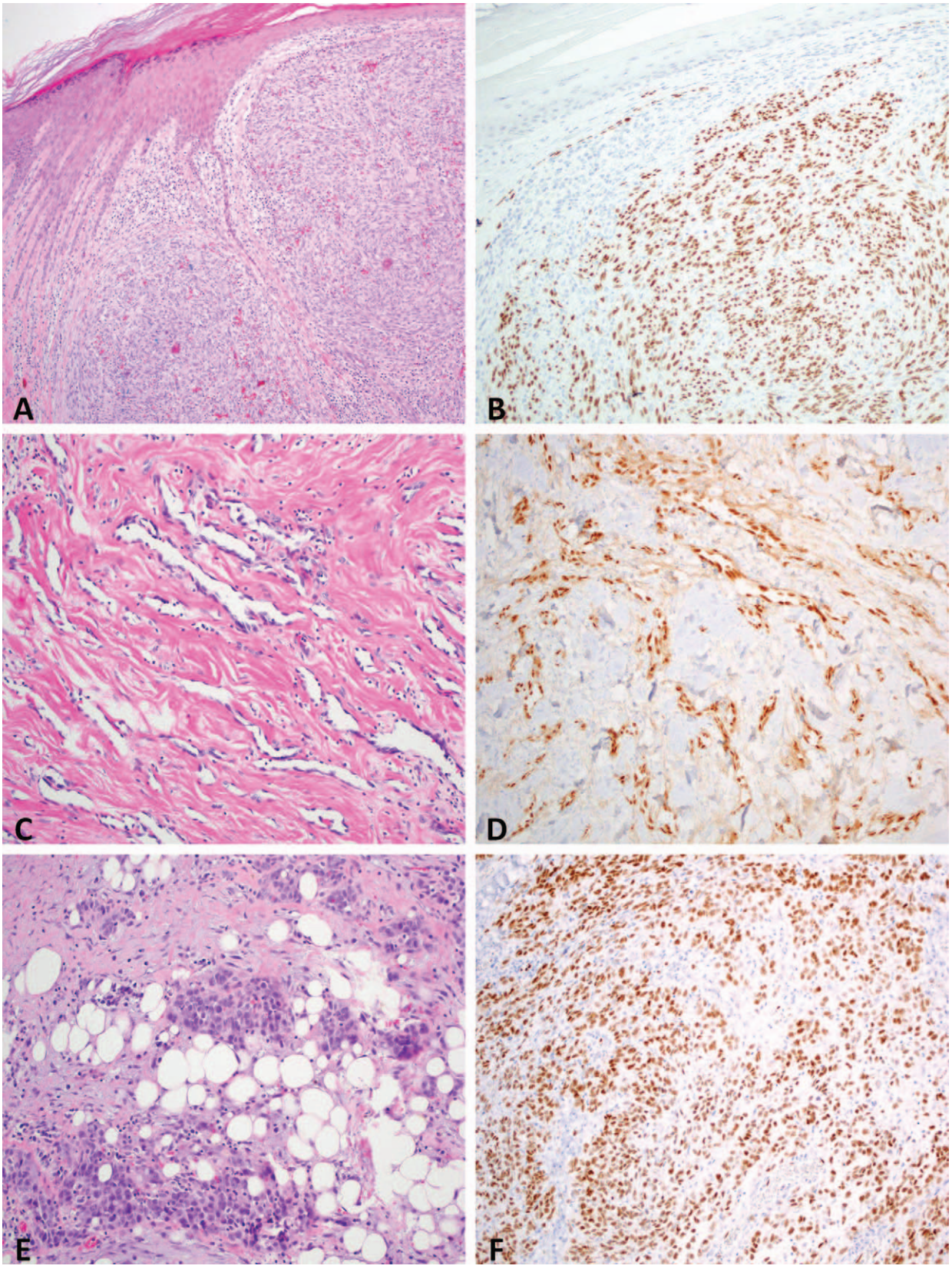

Figure 1. *Nodular Kaposi sarcoma involving dermis composed of fascicles of spindle cells with numerous extravasated red blood cells (A). The tumor cells show diffuse nuclear expression of ERG (B). Vasoformative angiosarcoma composed of complex anastomosing vessels lined by hyperchromatic atypical endothelial cells (C); again, tumor cells show diffuse nuclear expression of ERG (D). Epithelioid angiosarcoma (E), which may mimic poorly differentiated carcinoma or malignant melanoma, is positive for ERG (F), unlike the latter two tumor types (hematoxylin-eosin, original magnifications ×100 [A] and ×200 [C and E]; original magnifications ×100 [B] and ×200 [D and F]).*

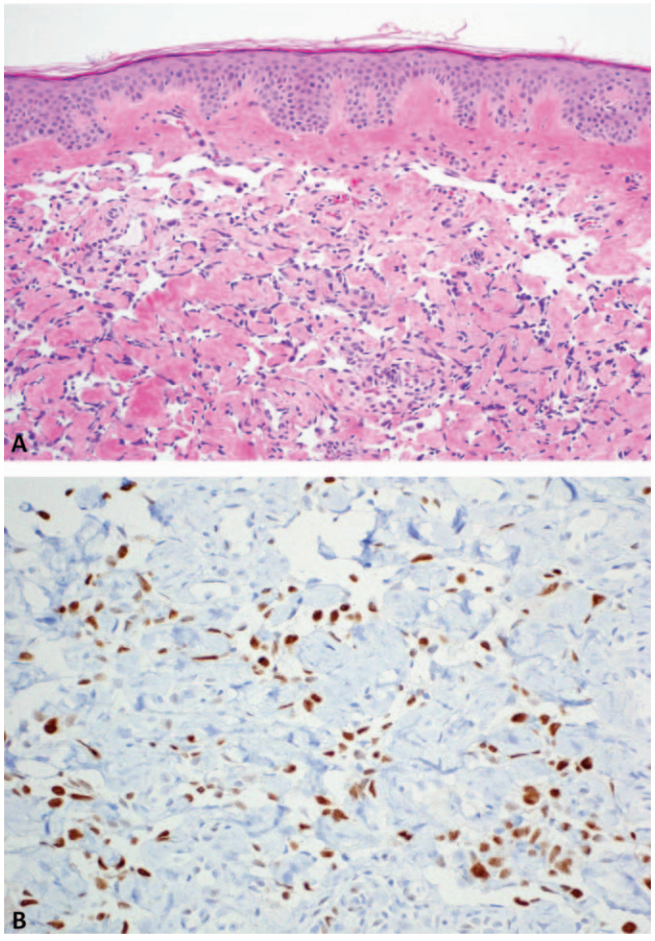

Figure 2. *Postradiation angiosarcoma with a vasoformative growth pattern (A). The tumor cells show diffuse strong nuclear positivity for MYC (B) (hematoxylin-eosin, original magnification ×200 [A]; original magnification ×400 [B]).*

than 95% of angiosarcomas, with a greater sensitivity for angiosarcoma than CD31 and CD34, which are markers routinely used to evaluate for angiosarcoma, and ERG usually shows a diffuse pattern of nuclear staining, which facilitates its interpretation in this context.

Expression of ERG occurs in a small subset of Ewing sarcomas (see above), and strong nuclear ERG expression has been found to correlate with *EWSR1-ERG* rearrangement, which is present in a small percentage of Ewing sarcomas compared with the more characteristic *EWSR1-FLI1* rearrangement.[15] *TLS/FUS-ERG* fusion transcripts occur in acute myeloid leukemia and likely account for the detection of ERG positivity in acute myeloid leukemia tissue infiltrates.[13,16,17] Positivity for ERG has also been reported to occur in a significant percentage of epithelioid sarcomas, with 41 of 109 cases (38%) showing ERG positivity in one study.[18] However, ERG reactivity in epithelioid sarcoma is likely dependent on antibody choice, as a subsequent study showed that an antibody to the N-terminus of ERG resulted in positivity in 19 of 28 cases (68%), whereas an antibody to the C-terminus showed focal positivity in only 1 of 29 cases (3%).[19] Whereas CD34 expression is frequent in epithelioid sarcoma, other more specific endothelial markers, such as CD31, are negative, and the characteristic loss of INI1/SMARCB1 expression typically found in epithelioid sarcomas should allow for the distinction of epithelioid sarcoma from vascular tumors in the vast majority of cases.[18]

Other than prostatic adenocarcinoma, very few epithelial malignancies have been reported to show ERG immunoreactivity—1 (of 42) pulmonary large cell undifferentiated carcinoma and 1 (of 27) pleural epithelioid type mesothelioma.[13] *ERG* gene fusions have also been found in myxoid liposarcoma, although ERG immunoreactivity in myxoid liposarcomas has not been detected thus far.[13,16]

ERG is therefore a useful marker for confirming endothelial differentiation in both benign and malignant neoplasms, but expression can also be seen in a subset of epithelioid sarcomas and a small percentage of Ewing sarcomas, as well as approximately 45% to 50% of prostatic carcinomas.

MYC (v-myc Avian Myelocytomatosis Viral Oncogene Homolog)

Cutaneous angiosarcoma arises in four typical clinical settings—chronically sun-damaged skin, particularly the scalp or face; sporadic visceral angiosarcoma; in the setting of chronic lymphedema (eg, after mastectomy in Stewart-Treves syndrome); and in areas of prior therapeutic radiation, such as for the management of breast carcinoma.[20–29] The last two groups are considered "secondary" angiosarcoma. Atypical vascular proliferations, which have been described under various nomenclature designations, occur in areas of prior radiation, are often seen in association with angiosarcoma, and in some cases may be difficult to distinguish from vasoformative angiosarcoma.[22,23,30–40] *MYC* proto-oncogene is a transcription factor located on the long arm of chromosome 8 (8q24.21) and is implicated in cellular proliferation, differentiation, and apoptosis.[41] Nuclear expression of MYC occurs in the vast majority of secondary angiosarcomas (Figure 2), but it is only very rarely seen in primary angiosarcoma, and it is not detected in atypical or benign vascular lesions occurring in irradiated skin.[42–45]

An array-based comparative genomic hybridization study of 22 cases of angiosarcoma (8 primary; 14 secondary to irradiation or chronic lymphedema) found that 10 secondary angiosarcomas (9 associated with radiation therapy and 1 with lymphedema) showed 16 recurrent alterations, with high-level amplifications on 5q35.3 (2), 8q24.21 (8), and 10p12.33 (6), whereas no recurrent alterations were seen in primary angiosarcomas.[42] Given that *MYC* in the region of 8q24.21 was a likely candidate for amplification, in the same study, fluorescence in situ hybridization (FISH) analysis of 28 primary angiosarcomas and 33 secondary angiosarcomas showed high-level *MYC* gene amplification in 18 secondary angiosarcomas (55%; 16 irradiated, 2 lymphedema) but not in primary angiosarcomas ($P < .001$).[42] Another study has shown high-level *MYC* amplification in 100% (20 of 20) of secondary angiosarcomas, as well as coamplification of *FLT4* in 25% (5 of 20) of cases.[43] Atypical postradiation vascular proliferations and other benign vascular lesions of the breast are negative for both *MYC* amplification and MYC overexpression by immunohistochemistry, whereas MYC positivity is consistently detected in postradiation angiosarcomas.[44] A very recent study found that a subset of non–radiation-associated cutaneous angiosarcomas (9 of 38 cases evaluated) showed detectable expression of MYC by immunohistochemistry and that a subset of these cases showed high-level *MYC* gene amplification by FISH,

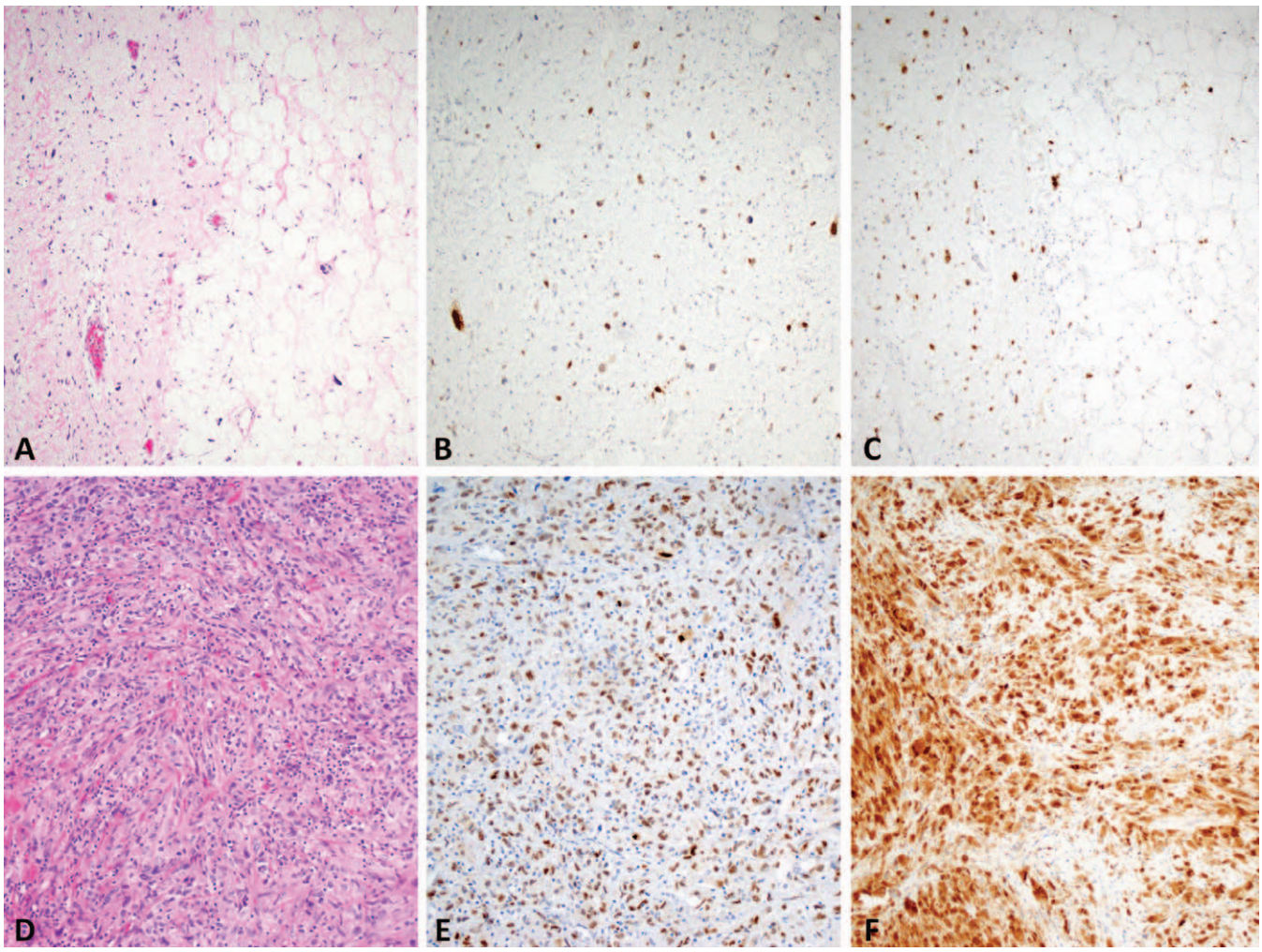

Figure 3. *Well-differentiated liposarcoma with scattered hyperchromatic atypical spindle cells (A). The tumor cells are positive for MDM2 (B) and CDK4 (C). Dedifferentiated liposarcoma with nondistinctive histologic features (D) shows strong diffuse positivity for both MDM2 (E) and CDK4 (F) (hematoxylin-eosin, original magnification ×200 [A and D]; original magnification ×200 [B, C, E, and F]).*

although a small number of MYC immunohistochemistry–negative cases also showed *MYC* gene amplification.[45]

MYC immunohistochemistry is therefore useful in differentiating atypical or benign vascular lesions occurring in irradiated skin from secondary postradiation angiosarcomas.

MDM2 (Murine Double-Minute-Type 2) and CDK4 (Cyclin-Dependent Kinase-4)

Amplification and overexpression of MDM2 is characteristic of well-differentiated and dedifferentiated liposarcoma, and both FISH and immunohistochemistry have become extremely useful tests for confirming the diagnosis of these tumor types, in conjunction with evaluation of CDK4 expression.[46] MDM2 protein is encoded by a gene at chromosome 12q14.3-q15 and acts as an inhibitor of the tumor suppressor effects of p53. Amplification of *MDM2* can be detected by FISH, and overexpression of MDM2 protein can be detected with mouse monoclonal antibodies (clones 2A10 and 1F2; Figure 3). CDK4 is encoded by a gene at chromosome 12q13 and functions in cell cycle progression—CDK4 inhibits the retinoblastoma-1 (*RB1*) gene, and similar to MDM2 is overexpressed in well-differentiated and dedifferentiated liposarcoma (Figure 3). This phenomenon reflects the common presence of ring or giant marker chromosomes that contain amplified material from the q13-15 region of chromosome 12, where both the *CDK4* and *MDM2* genes are located. For both MDM2 and CDK4, only nuclear staining is considered positive. Staining in well-differentiated liposarcoma is often limited in extent and may be present in scattered nuclei only, in contrast to dedifferentiated liposarcoma, where nuclear staining is usually more diffuse.[46,47] Benign lipomatous lesions are negative for both MDM2 and CDK4, as are pleomorphic liposarcoma and myxoid liposarcoma.

Perhaps the most useful role for these two markers is in confirming a diagnosis of dedifferentiated liposarcoma (Figure 3, D through F) when faced with an otherwise nondescript spindle cell or pleomorphic sarcoma in the retroperitoneum, and when a well-differentiated component is not seen. In addition, these markers are often helpful in the distinction between benign lipomas, particularly those with prominent fat necrosis, and atypical lipomatous tumor (well-differentiated liposarcoma), particularly when the latter shows very minimal atypical histologic features.[46–48] However, it must be noted that histiocytes, such as those present in areas of fat necrosis, are frequently MDM2 positive, emphasizing the need for concurrent CDK4

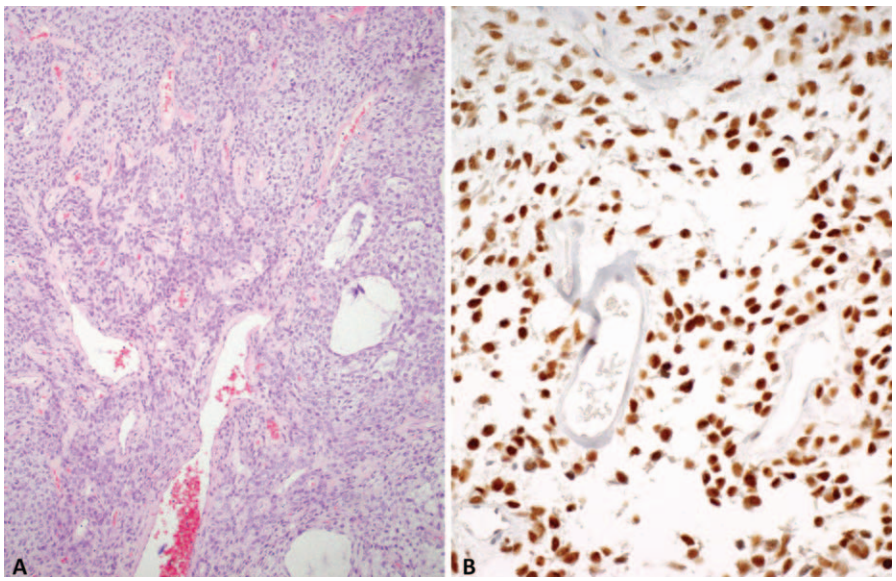

Figure 4. *Solitary fibrous tumor with characteristic hemangiopericytoma-like branching vessels and focally myxoid features (A). STAT6 is diffusely expressed in a nuclear pattern (B) (hematoxylin-eosin, original magnification ×200 [A]; original magnification ×400 [B]).*

evaluation. A small percentage of well-differentiated/dedifferentiated liposarcomas are negative for MDM2 and CDK4 by immunohistochemistry, with one study showing approximately 3% (3 of 105) and 10% (10 of 105) of cases of well-differentiated/dedifferentiated liposarcomas negative for MDM2 and CDK4, respectively, and in such cases where the histologic and clinical/radiologic findings are suggestive of this diagnosis, FISH for *MDM2* amplification should be considered.[46] It is also important to note that overexpression of MDM2 may also be seen in other spindle cell neoplasms—in up to 64% (21 of 33) of malignant peripheral nerve sheath tumors, 42% (10 of 24) of myxofibrosarcomas, and 29% (12 of 41) of embryonal rhabdomyosarcomas—but when combined with coexpression of CDK4, very few of these tumors will express both of these markers.[46] In addition, staining for MDM2 and CDK4 is usually less extensive in these tumor types than in dedifferentiated liposarcoma.

Several other tumor types show amplification of *MDM2* and *CDK4*, with corresponding overexpression at the protein level. Intimal sarcoma, a malignant neoplasm arising from large central vessels, most commonly the pulmonary artery, shows *MDM2* amplification and overexpression in 75% (6 of 8) of cases.[49,50] Similarly, cardiac sarcoma, previously considered as representing an otherwise unclassified spindle cell sarcoma, has also been found to show *MDM2* and *CDK4* amplification in most cases, suggesting that it is closely related to intimal sarcoma. This has allowed for improved classification of cardiac sarcomas, and it appears that intimal sarcoma represents the most common primary cardiac sarcoma.[51]

Recent studies have also shown that amplification of *MDM2* and *CDK4* occurs in 67% (10 of 15) of low-grade osteosarcomas (parosteal and central low-grade osteosarcomas) and only 12% (16 of 130) of high-grade osteosarcomas, with corresponding rates of immunohistochemical expression of these proteins.[52] This finding has been shown to be of diagnostic utility; in one study all low-grade osteosarcomas expressed MDM2 and/or CDK4, usually diffusely and with moderate or strong intensity, whereas expression of these markers in benign morphologic mimics of these tumors was extremely limited, with expression seen in only one case of bizarre parosteal osteochondromatous proliferation.[53] Because expression of MDM2 and CDK4 is generally limited to low-grade osteosarcomas and their dedifferentiated counterparts, expression of these two markers in an otherwise nondistinctive high-grade sarcoma of bone may therefore suggest evolution from (dedifferentiation of) a low-grade osteosarcoma.[54]

STAT6 (Signal Transducers and Activators of Transcription 6)

A recurrent *NAB2-STAT6* fusion gene has very recently been identified in the vast majority of solitary fibrous tumors (SFTs), both benign and malignant, using a variety of techniques, including whole-exome and transcriptome sequencing.[55–58] STAT6 is a member of the STAT family of cytoplasmic transcription factors, which regulate gene expression by transmitting signals to the nucleus and binding to specific DNA promoter sequences. STAT6 modulates signaling by interleukin 4 and interleukin 13 in the immune system.[59] STAT signaling is important for normal cellular processes, embryonic development, innate and adaptive immune function, and regulation of cell differentiation, growth, and apoptosis. NAB2 is a transcriptional corepressor, a regulator of the early growth response 1 (EGR1) transcription factor. The *NAB2-STAT6* gene fusion results in variable truncation of the repressor domain of NAB2 with replacement by the transcriptional activation domain of STAT6, and it is thought that the resulting fusion protein translocates to the nucleus, where it acts as a transcriptional activator, inducing expression of EGR target genes and resulting in increased proliferation.[57,60] In the early discovery studies, STAT6 was shown to be overexpressed at the protein level in SFT, whereas NAB2 showed less specific staining compared with other tumor types.[58] A large study of STAT6 expression in 231 tumors showed that nuclear expression of STAT6 is highly sensitive for SFT, with expression seen in more than 95% (59 of 60) of cases examined (Figure 4). Expression of STAT6 was limited in other tumors and was most commonly seen in a subset of dedifferentiated liposarcoma, as well as in 1 (of 10) deep fibrous histiocytoma, in which staining was weak and focal.[60] Other studies have since confirmed the high

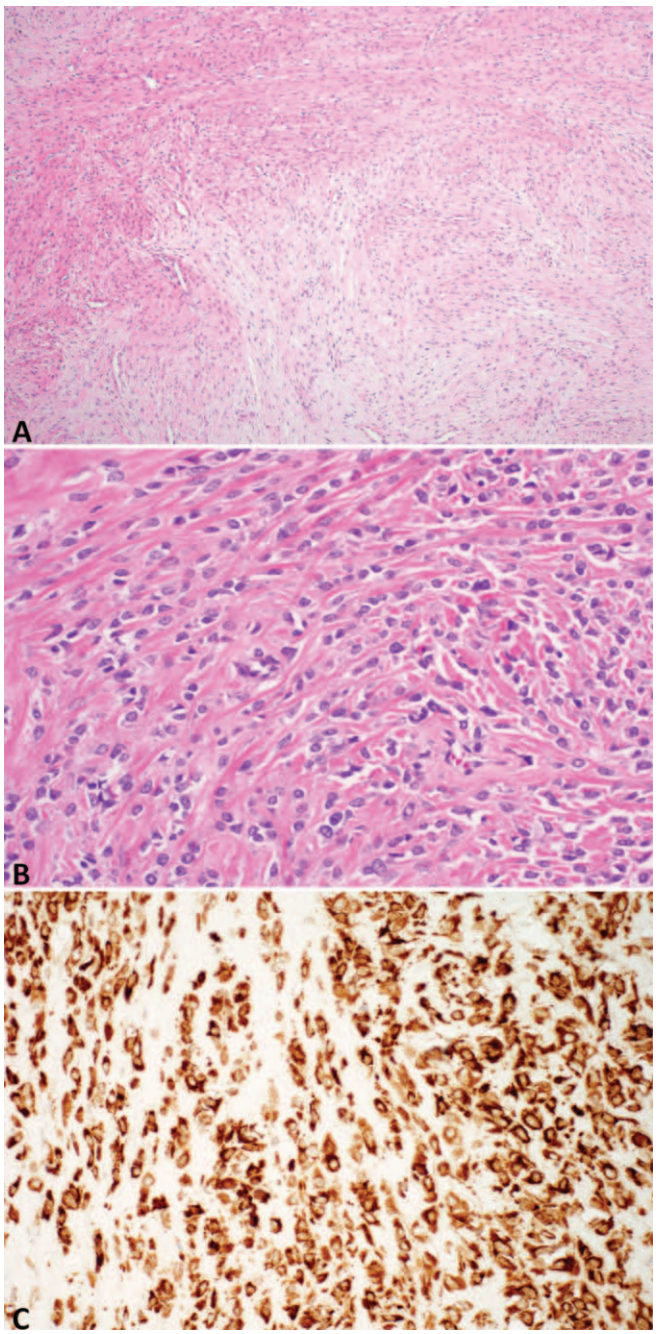

Figure 5. *Low-grade fibromyxoid sarcoma (LGFMS) showing characteristic alternating fibrous and myxoid areas (A), and the related sclerosing epithelioid fibrosarcoma (SEF) composed of cords of epithelioid cells with pale or clear cytoplasm embedded in a densely sclerotic stroma (B). More than 98% of LGFMS and 70% of SEF show diffuse cytoplasmic expression of MUC4 (C) (hematoxylin-eosin, original magnifications ×100 [A] and ×400 [B]; original magnification ×400 [C]).*

sensitivity and specificity of STAT6 for the diagnosis of SFT, with staining seen in a subset of dedifferentiated liposarcomas, and rarely in other tumor types.[60–63,193] The antibodies used differed in some of these studies, accounting for the additional cytoplasmic staining described by some authors; however, nuclear expression is expected in SFT.[61–63] Interestingly, a very recent study has shown a correlation between specific fusion types and morphology, with tumors with *NAB2ex4-STAT6ex2/3* fusion genes corresponding to classic SFTs that arise most often in the pleura of older patients, show diffuse fibrosis, and typically have a benign clinical course. Tumors with the less common NAB2ex6-STAT6ex16/17 fusion occur in younger patients, usually in deep soft tissue, are more cellular, and are associated with aggressive clinical behavior.[64]

Perhaps the greatest potential pitfall in the use of STAT6 for evaluating spindle cell lesions, particularly those in an intra-abdominal/retroperitoneal location, is the presence of STAT6 expression in approximately 7% to 15% of dedifferentiated liposarcomas, which likely represents amplification of the 12q13 region in this tumor type, where *STAT6* is located close to *MDM2* and *CDK4*.[60,62,193] The staining pattern in dedifferentiated liposarcoma is variable and may be focal or diffuse, weak or medium to strong in intensity, unlike the generally strong diffuse pattern seen in solitary fibrous tumor. In addition, unlike the predominantly nuclear pattern of staining seen in solitary fibrous tumor, both cytoplasmic and nuclear expression is common in dedifferentiated liposarcoma.[193] Diffuse expression of MDM2 and CDK4 helps favor a diagnosis of dedifferentiated liposarcoma, and if doubt persists, FISH for *MDM2* amplification may also be useful. Because *STAT6* and *NAB2* are located close together on the long arm of chromosome 12, FISH to demonstrate rearrangement of the genes is technically challenging and not diagnostically useful. The identification of the *NAB2-STAT6* fusion gene in meningeal hemangiopericytoma is further evidence that these lesions are in fact morphologic variants of solitary fibrous tumor.[58] STAT6 nuclear positivity helps distinguish meningeal hemangiopericytomas/solitary fibrous tumor from histologic mimics, because expression was not detected in any (n = 87) meningiomas and the vast majority of potential mimics in a large series of meningeal tumors evaluated for STAT6 expression.[58]

MUC4 (Mucin 4)

MUC4 is a useful marker for low-grade fibromyxoid sarcoma (LGFMS) and sclerosing epithelioid fibrosarcoma (SEF; Figure 5). The mucin 4 (*MUC4*) gene was found to be significantly upregulated in LGFMS compared with other tumor types through gene expression array analysis.[65] MUC4 is a transmembrane glycoprotein that is normally expressed on many epithelial surfaces, where it is thought to serve a protective role, and is also involved in cell proliferation and survival through interacting with the ErbB/HER2 family of growth factor receptors.[66–71] A large study evaluating MUC4 as an immunohistochemical marker for LGFMS evaluated whole-tissue sections of 309 soft tissue tumors, including 49 LGFMSs (all of which showed *FUS* gene rearrangement by FISH), and found expression of MUC4 in all cases of LGFMS, with a diffuse cytoplasmic pattern of staining in tumor cells (Figure 5).[72] However, it should be noted that very rare cases of LGFMS may be negative for MUC4,[73] and if strong suspicion for LGFMS persists, FISH for *FUS* or *EWSR1* rearrangement should be considered.

Sclerosing epithelioid fibrosarcoma, another fibroblastic neoplasm, shows morphologic and molecular overlap with LGFMS, and hybrid tumors showing features of both exist. A subset of SEF has been shown to contain the *FUS-CREB3L2* gene fusion or *FUS* gene rearrangements, characteristic of LGFMS.[74–81] Similar to LGFMS, strong

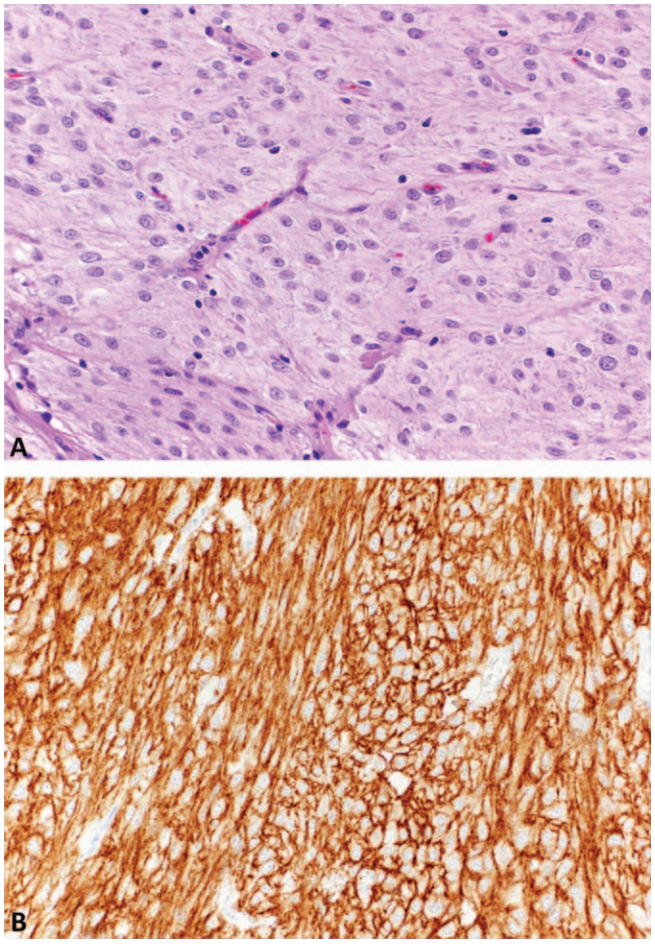

Figure 6. *Gastric gastrointestinal stromal tumor with* PDGFRA *mutation and epithelioid features (A) is frequently negative for KIT, but DOG1 is usually positive, with both cytoplasmic and membranous staining (B) (hematoxylin-eosin, original magnification ×400 [A]; original magnification ×400 [B]).*

diffuse cytoplasmic expression of MUC4 is seen in SEF, occurring in 69% (20 of 29) to up to 90% (9 of 10) of "pure" forms, and virtually all tumors (12 of 12) showing hybrid features of both LGFMS and SEF (Figure 5).[82,83] Recent studies have shown that *EWSR1-CREB3L1* is the predominant gene fusion in SEF; this fusion gene occurs uncommonly in LGFMS.[83,84]

MUC4 positivity is also seen in synovial sarcoma, predominantly in the glandular component of the biphasic subtype, with more limited expression in the spindle cell component of either biphasic or monophasic synovial sarcoma. Focal positivity has also been identified in ossifying fibromyxoid tumors (5 of 17; 29%), epithelioid GISTs (2 of 10; 20%), and some myoepithelial carcinomas (1 of 10; 10%).[82] However, unlike the diffuse strong pattern seen in LGFMS or SEF, staining in these tumor types is generally limited in extent, with either just scattered positive cells or focal staining. Importantly, regarding SEF, in which a poorly differentiated carcinoma may fall into the differential diagnosis, it should be remembered that MUC4 expression is seen in a variety of different carcinomas, such as pancreaticobiliary carcinomas, breast carcinoma, and colonic adenocarcinoma. Helpful features to support a diagnosis of SEF include the presence of characteristic dense sclerotic stroma, within which the tumor cells are embedded and may have pale or clear cytoplasm; the tumor cells of SEF are generally negative for cytokeratins, and in some cases FISH for *EWSR1* and *FUS* gene rearrangement may also help support a diagnosis of SEF.

DOG1 (Discovered on GIST 1)

Gastrointestinal stromal tumors typically harbor gain-of-function mutations in *KIT* or *PDGFRA*, and KIT immunoreactivity is important in the diagnosis of most GISTs; however, approximately 5% to 10% of GISTs are negative for KIT, usually those with *PDGFRA* mutations.[85–88] Gene expression profiling using cDNA microarrays identified overexpression of DOG1 in GIST relative to other tumor types, and immunoreactivity to DOG1 was shown in 98% (136 of 139) of GISTs, regardless of *KIT* or *PDGFRA* mutation status.[89] Expression of DOG1 is typically both cytoplasmic and membranous (Figure 6). Further studies have confirmed that DOG1 is a highly sensitive marker for GIST, being present in more than 87% of cases (986 of 1040 cases[90]; 370 of 425 cases[91]).[89–91] Importantly, DOG1 expression is found in up to 96% of KIT-negative GISTS (24 of 25 cases[92]; 10 of 28 cases[93]; 9 of 10 cases[94]), the most common of which are gastric tumors with *PDGFRA* mutations and epithelioid cytomorphology.[92–94] Approximately 3% (27 of 1040) of all GISTs are negative for both KIT and DOG1, and in cases where the diagnostic suspicion remains high for GIST, mutational analysis for *KIT* and *PDGFRA* mutations is indicated to confirm the diagnosis: 46% (11 of 24) of DOG1-negative GISTs have *KIT* or *PDGFRA* mutations.[90] The specificity of DOG1 for GIST is also relatively high, particularly among other mesenchymal neoplasms that may mimic GIST. Other mesenchymal tumors that show rare and/or focal DOG1 positivity include synovial sarcoma (2.5% to 16%, in 1 of 39 cases[91] and 6 of 37 cases,[90] respectively), uterine type retroperitoneal leiomyomas (5 of 42; 12%),[90] leiomyosarcoma (1 of 326; 0.3%),[91] and some perivascular epithelioid cell tumors (PEComas).[90,91]

DOG1 is also known as Anoctamin-1 (Ano-1), transmembrane protein 16A (TMEM16A), overexpressed in oral (squamous cell) carcinoma 2 (ORAOV2), and tumor-amplified and overexpressed sequence 1 (TAOS1).[95,96] DOG1 positivity is also common in esophageal squamous cell carcinomas and gastric adenocarcinomas, particularly intestinal type, and is less commonly seen in colorectal adenocarcinomas.[90] Infrequent positivity for DOG1 has been reported in a variety of other tumors, including endometrioid adenocarcinoma, acinic cell carcinoma, desmoplastic melanoma, malignant peripheral nerve sheath tumor, Ewing sarcoma, and glomus tumor.[97,98] DOG1 has also recently been found to be positive in the cellular areas of 100% (9 of 9) of chondroblastomas and may have utility in diagnosing this lesion.[99] The combination of both KIT and DOG1 reactivity is most useful in clinical practice in the diagnosis of GIST.[100]

SUCCINATE DEHYDROGENASE A AND B

Approximately 15% of GISTs in adults and more than 90% in children lack *KIT* and *PDGFRA* mutations, so-called wild-type GISTs.[101–103] Wild-type GISTs also include those tumors arising in the Carney triad, the Carney-Stratakis syndrome, and neurofibromatosis 1 (NF1).[104] Carney triad is a nonhereditary syndrome that typically occurs in young females and is characterized by gastric GIST, paraganglio-

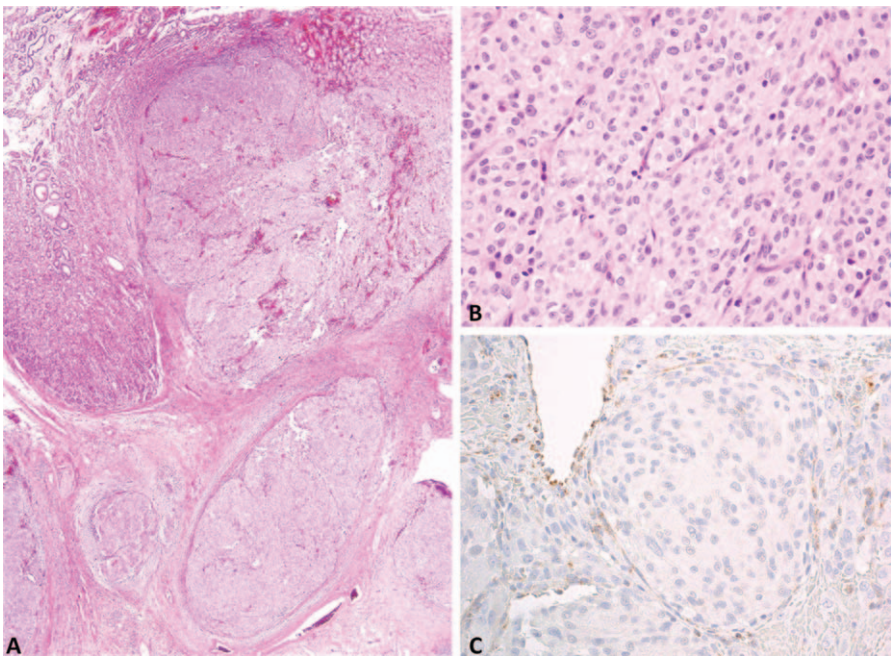

Figure 7. *Succinate dehydrogenase (SDH)–deficient gastrointestinal stromal tumor shows a characteristic multinodular or plexiform growth pattern (A), and tumor cells are usually epithelioid (B). This tumor type shows loss of expression of SDHB in tumor cells, in contrast to surrounding normal cells, such as endothelial cells and inflammatory cells, which show cytoplasmic expression of SDHB (C) (hematoxylin-eosin, original magnifications ×40 [A] and ×400 [B]; original magnification ×400 [C]).*

ma, and pulmonary chondroma; Carney-Stratakis syndrome is inherited in an autosomal dominant fashion and consists of the dyad of gastric GIST and paraganglioma.[105–108] Defects in the succinate dehydrogenase (SDH) metabolic pathway have been found to occur in most pediatric GISTs, a subset of adult "wild-type" GIST, and GISTs occurring in Carney-Stratakis syndrome and Carney triad.[109] These tumors form a clinicopathologically distinct subset of wild-type GISTs known as "SDH-deficient GIST." These tumors arise exclusively in the stomach, particularly the antrum, and show a characteristic multifocal and plexiform growth pattern, and usually a purely or predominantly epithelioid cytomorphology (Figure 7, A and B). Succinate dehydrogenase–deficient GIST is typically positive for KIT and DOG1. Unlike *KIT* mutant GIST, lymph node metastases are common in SDH-deficient GIST, and these tumors tend to be imatinib resistant, yet often show an indolent clinical course.[103,110–112] Succinate dehydrogenase is an enzyme complex composed of four subunit proteins (A, B, C, and D) that is localized in the inner mitochondrial membrane and is involved in the oxidation of succinate to fumarate in the citric acid cycle and the electron transport chain.[113,114] SDHB and SDHA are normally ubiquitously expressed in a granular cytoplasmic pattern. Germline mutations in *SDHA*, *SDHB*, *SDHC*, and *SDHD* were first identified in pheochromocytoma-paraganglioma syndrome, and they result in the destabilization of the SDH complex and loss of SDHB expression by immunohistochemistry (Figure 7, C).[115–117] However, somatic mutations in any of these genes result in loss of SDHB expression, and loss of expression is also seen in tumors where no identifiable mutations are detected, suggesting that dysfunction of the pathway may have arisen by other mechanisms or by other mutations that are not detectable by current methods. Loss of SDHB by immunohistochemistry defines the group of SDH-deficient GIST[118–121] and has been shown to occur with an estimated frequency of 7.5% among all gastric GISTs.[110] Although loss of SDHB expression is detected in 42% (22 of 53) of wild-type GISTs, GISTs with *KIT* or *PDGFRA* mutation consistently show intact SDHB expression.[121]

Germline mutations in *SDHA*, *SDHB*, *SDHC*, and *SDHD* occur in some, but not all, patients with SDH-deficient GIST.[109,120,122,123] Germline mutations in *SDHA* represent the most commonly mutated gene, and loss of expression of SDHA by immunohistochemistry has been shown to reliably predict the presence of *SDHA* mutations.[124] Interestingly, although germline mutations have been identified in association with Carney-Stratakis syndrome, mutations in *SDH* genes have not been found in patients with Carney triad, and the mechanism for deficient SDH function in Carney triad is uncertain at this time.[119,125–127] Wild-type GIST associated with NF1 do not show loss of SDHB expression.[119,121,128]

Given the significant prognostic and predictive implications with regards to clinical course, selection of appropriate therapy (SDH-deficient GIST responds better to second- or third-generation tyrosine kinase inhibitors), and implications for screening of other tumors in both the patient and family members, SDHB immunohistochemistry should be considered for gastric GISTs with an epithelioid cytomorphology and multinodular or plexiform growth pattern. If expression is lost in tumor cells, with normal endothelial cells and inflammatory cells acting as a positive internal control, the patient should be referred for genetic counseling. In addition, if SDHB expression is lost, additional loss of staining for SDHA suggests the presence of *SDHA* mutation.

INI1 (Integrase Interactor 1)

INI1 is the protein product of the gene *hSNF5/INI1/SMARCB1*, located on the long arm of chromosome 22 (22q11.2). INI1 is a core subunit of the SWI/SNF ATP-dependent chromatin remodeling complex, and is ubiquitously expressed in the nuclei of normal cells. INI1 is thought to function as a tumor suppressor. Loss of INI1 function (with corresponding loss of protein expression) can result from mutations or deletions of the *INI1* gene.[129] Loss of INI1 expression is observed in several tumor types. Abnormalities in *INI1* were first described in malignant

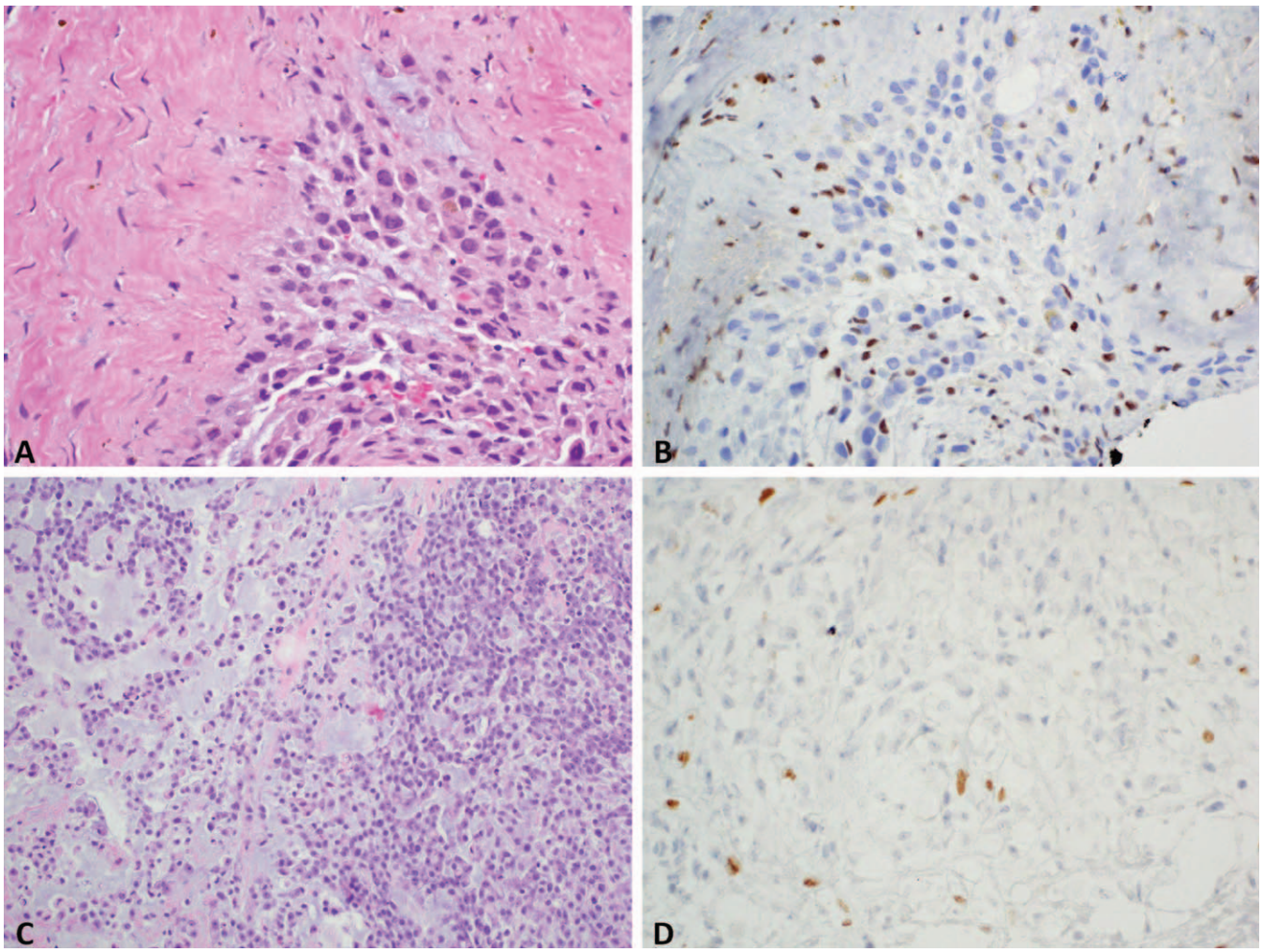

Figure 8. *Proximal-type epithelioid sarcoma, which may mimic carcinoma or melanoma, (A) shows diffuse loss of nuclear INI1, in contrast to surrounding normal cells (B). Approximately 40% of myoepithelial carcinomas (C) in children and 10% in adults show loss of nuclear INI1 expression (D) (hematoxylin-eosin, original magnifications ×400 [A] and ×200 [C]; original magnification ×400 [B and D]).*

rhabdoid tumors (renal, extrarenal, and atypical teratoid/rhabdoid tumor of the central nervous system), and loss of INI1 expression is seen in nearly all of these tumors. Although most are sporadic mutations, a small group of infants with malignant rhabdoid tumor have germline mutations in *INI1/SMARCB1*.[130] Loss of INI1 expression is also seen in approximately 93% (127 of 136) of epithelioid sarcomas, both conventional and proximal-type (Figure 8, A and B).[131] In contrast to malignant rhabdoid tumors, where mutations in *INI1* are the most common cause of loss of function, chromosomal deletions are usually identified in epithelioid sarcomas.[132] In addition to malignant rhabdoid tumors and epithelioid sarcoma, loss of INI1 expression is also seen in 50% (12 of 24) of epithelioid malignant peripheral nerve sheath tumors and in a subset of myoepithelial carcinomas (Figure 8, C and D)—particularly those arising in children (9 of 22; 40%; versus 2 of 22; approximately 10% in adults).[131,133]

Other tumor types reported to show variable loss of INI1 expression include extraskeletal myxoid chondrosarcomas (4 of 24; 17%),[134] poorly differentiated chordoma, undifferentiated hepatoblastoma, and renal medullary carcinoma.[135–138] Approximately 60% of patients with the rare hereditary syndrome of familial schwannomatosis have germline mutations in *INI1/SMARCB1*, which is associated with a "mosaic" pattern of protein loss by immunohistochemistry.[139] Reduced, but not complete loss of, INI1 expression has been reported in synovial sarcoma; the significance of this finding is uncertain.[140,141]

TLE1 (Transducin-Like Enhancer of split 1)

TLE1 is a relatively recently described marker of synovial sarcoma. Gene expression profiling studies have shown a major association of the Wnt signaling pathway with synovial sarcoma and overexpression of members of the *TLE* (*T*ransducin-*L*ike *E*nhancer of split) family of genes, particularly *TLE1*, which is one of four *TLE* genes that encode human transcriptional repressors homologous to the *Drosophila* corepressor *groucho*, and is an important component of the Wnt signaling pathway.[142–150] TLE proteins are involved in multiple developmental processes, including lateral inhibition, segmentation, sex determination, eye development, neuronal differentiation, and hematopoiesis.[149,151–154] An early large study using tissue microarrays showed strong positive nuclear staining for TLE1 in 91 of 94 molecularly confirmed synovial sarcomas (97%), including both the epithelial and spindle cell components of biphasic

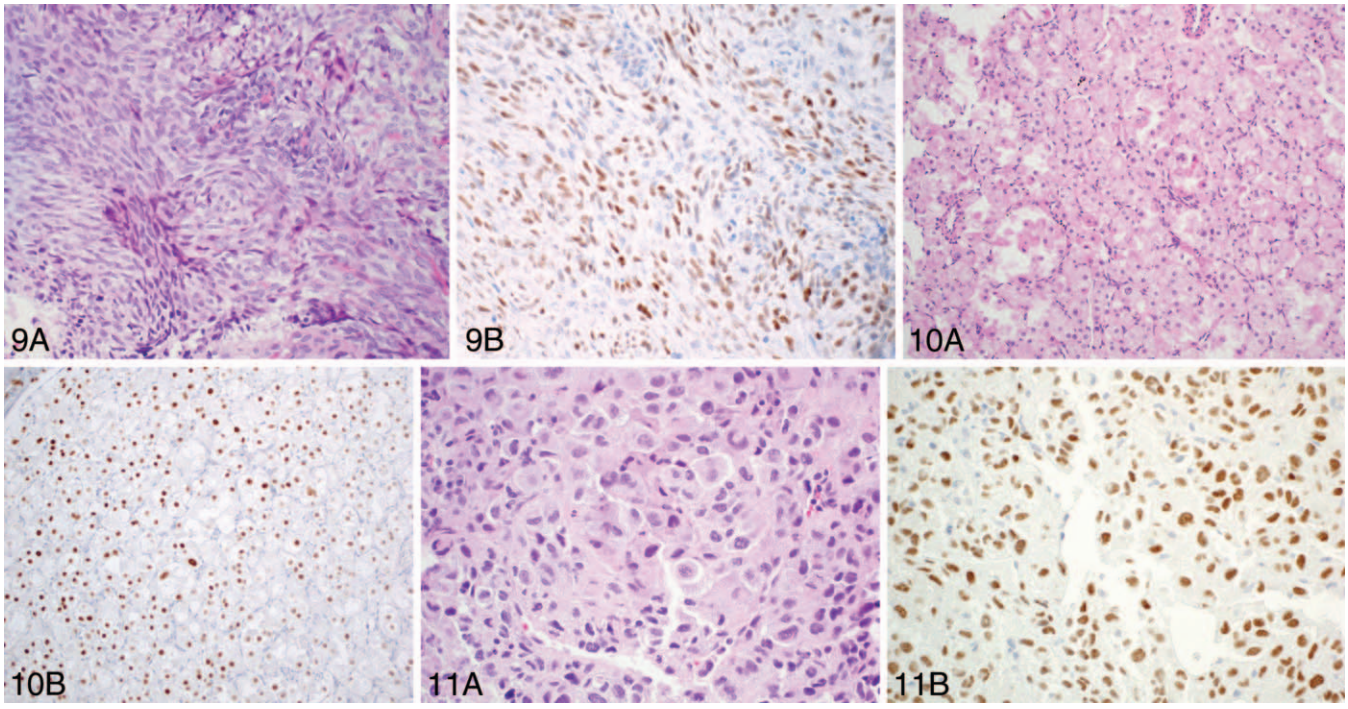

Figure 9. *Monophasic synovial sarcoma (A) shows nuclear expression of TLE1 in approximately 90% of cases (B) (hematoxylin-eosin, original magnification ×400 [A]; original magnification ×400 [B]).*

Figure 10. *Alveolar soft part sarcoma with the classic appearance of large cells with abundant granular palely eosinophilic cytoplasm arranged in a nested growth pattern (A). Nuclear expression of TFE3 is seen in virtually all cases (B) (hematoxylin-eosin, original magnification ×400 [A]; original magnification ×400 [B]).*

Figure 11. *Metastatic malignant melanoma with epithelioid cytomorphology (A) shows diffuse nuclear expression of SOX10 (B) (hematoxylin-eosin, original magnification ×400 [A]; original magnification ×400 [B]).*

synovial sarcoma as well as poorly differentiated variants (Figure 9).[150] Other studies have also shown high sensitivity and specificity of TLE1-positive immunohistochemical staining for molecularly confirmed synovial sarcoma.[155–157] However, one study evaluating 163 soft tissue and bone tumors using whole sections found TLE1 positivity in 18 of 20 synovial sarcomas (90%), but also in 53 of 143 nonsynovial sarcomas (37%).[158]

Regarding other tumors, TLE1 expression is most commonly seen in peripheral nerve sheath tumors and solitary fibrous tumors. Terry et al[150] reported 16 of 88 malignant peripheral nerve sheath tumors (MPNSTs; 18%) with any staining for TLE1, but only 4 tumors (5%) showed more than weak staining. Another study has shown a slightly lower rate of expression of TLE1 in synovial sarcoma (82%; 60 of 73).[159] In that study nuclear reactivity for TLE1 was also observed in 15% (7 of 47) of MPNSTs and 8% (4 of 49) of solitary fibrous tumors, but it was usually only weak in these tumor types. Occasional staining for TLE1 has also been described in clear cell sarcoma, high-grade chondrosarcoma, Ewing sarcoma, rhabdomyosarcoma, GIST, myxofibrosarcoma, and leiomyosarcoma.[150,158] TLE1 expression has also been reported in malignant mesothelioma.[160] However, in most of these tumors, staining is focal and weak to moderate in intensity, unlike the strong diffuse nuclear pattern characteristic of synovial sarcoma. Within nonneoplastic tissue, TLE1 expression has been reported to occur in basal keratinocytes, adipocytes, perineurial cells, endothelial cells, and mesothelial cells.[158]

TFE3 (Transcription Factor Binding to IGHM Enhancer 3)

TFE3 is a member of the microphthalmia (MiT) family of transcription factors, which includes MiTF, TFEB, TFEC, and TFE3, all of which share a common structure consisting of a helix-loop-helix leucine zipper dimerization motif, a transactivation domain, and a basic region involved in DNA binding.[161] The most well-studied MiT transcription factor is MiTF, which is important in the development of melanocytes (survival, growth, and migration), melanogenesis, and osteoclast development; the function of the other MiT family members is less well defined at this time.[161] The utility of MiTF in clinical practice is limited by its low specificity: nuclear expression of MiTF is seen in primary and metastatic melanomas, as well as in some PEComas, clear cell sarcomas, and histiocytic proliferations or neoplasms.[162,163]

Although TFE3 is ubiquitously expressed in humans, native TFE3 protein is usually not detected by routine immunohistochemical methods. Nuclear expression of TFE3 is seen in a variety of different tumors, most of which harbor *TFE3* gene fusions, including alveolar soft part sarcoma, Xp11 translocation renal cell carcinoma, "melanotic" Xp11 translocation renal cell carcinoma, and a subset of PEComas and epithelioid hemangioendotheliomas.[164–171] Although virtually all alveolar soft part sarcomas and Xp11 translocation renal cell carcinomas will show diffuse nuclear TFE3 expression (Figure 10), only a subset of PEComas will show nuclear immunoreactivity for TFE3, with a subset of those cases also harboring a *TFE3* gene rearrangement.[170,172] Moreover, PEComas with a *TFE3* gene fusion have been

found not to harbor the tuberous sclerosis complex (TSC2) alterations characteristic of conventional PEComas, and expression of TFE3 in PEComa is mutually exclusive to expression of MiTF.[173] Although limited by small numbers, cutaneous PEComas do not appear to show reactivity for TFE3 by immunohistochemistry or *TFE3* rearrangement by FISH.[174]

Very recently, a *YAP1-TFE3* fusion gene has been detected in a subset of epithelioid hemangioendothelioma, with corresponding detectable TFE3 nuclear reactivity by immunohistochemistry.[171] Interestingly, epithelioid hemangioendotheliomas with this fusion gene show distinct morphologic appearances, in that they typically have voluminous eosinophilic cytoplasm, show well-formed vascular channels and focally solid growth, and usually arise in young adults.[171]

TFE3 immunohistochemistry is therefore useful in confirming a diagnosis of alveolar soft part sarcoma and Xp11 translocation renal cell carcinoma, and is also expressed in epithelioid hemangioendotheliomas with a distinctive fusion gene and clinicopathologic features, as well as a small subset of PEComas.

SOX10 (Sex-determining Region Y-related HMG-box 10)

SOX10 is a nuclear transcription factor normally expressed in neural crest cells that is crucial for differentiation of Schwann cells and melanocytes.[175–177] Nuclear staining is seen in normal melanocytes, Schwann cells, secretory cells of the eccrine coil, myoepithelial cells, and acinar cells of salivary gland tissue.[178–180] Expression of SOX10 is correspondingly seen in tumors showing neural crest differentiation (ie, melanocytic and nerve sheath tumors), as well as a subset of myoepithelial and salivary gland–type tumors (acinic cell carcinomas, adenoid cystic carcinomas, epithelial-myoepithelial carcinomas, myoepitheliomas/myoepithelial carcinomas, and mixed tumors/pleomorphic adenomas).[179–182] In addition to staining most benign nerve sheath tumors (ie, neurofibroma and schwannoma), recent studies have shown that SOX10 is expressed in 27% to 50% of MPNSTs (21 of 78 cases[179]; 38 of 77 cases[182]), a sensitivity similar to that of S100 protein for this tumor type.[179,182] SOX10 expression is also detected in a large majority of melanocytic neoplasms, including benign nevi (blue, neurotized, dysplastic, Spitz, and nodal capsular) and malignant melanoma (conventional, spindled, desmoplastic, and metastatic), with one study showing SOX10 positivity in 97% of melanomas (76 of 78)[182] and another study showing SOX10 positivity particularly in desmoplastic melanoma (7 of 9; 78%),[179] as well as clear cell sarcoma (4 of 7; 57%[179]; malignant melanoma of soft parts).[179,182,183] Expression of this marker should therefore be interpreted in context, and in general it is not useful in distinguishing melanocytic from neural tumors. However, it can be useful to confirm a diagnosis of melanoma when other melanocytic markers are negative, in the right histologic and clinical context (Figure 11). Karamchandani et al[179] reported expression of SOX10 in 26 of 26 granular cell tumors (100%). The specificity of SOX10 for tumors showing neural crest differentiation (ie, melanocytic and nerve sheath tumors) is greater than that for S100 protein because expression of SOX10 in other mesenchymal and epithelial tumors is limited; expression is found in a subset of myoepithelial and salivary gland tumors, diffuse astrocytomas, and some ductal breast carcinomas.[181–183] Sustentacular cells of paraganglioma/pheochromocytoma and a subset of carcinoid tumors also express SOX10.[182]

NY-ESO-1 (New York Esophageal Squamous Cell Carcinoma 1)

Cancer-testis antigens are a family of antigens normally expressed in adult testicular germ cells, but which have been found to be aberrantly expressed in a variety of tumors. These antigens elicit both humoral and cell-mediated immune responses and are becoming attractive targets for immune-based cancer therapies.[184,185] NY-ESO-1 is encoded by the *CTAG 1B* gene and was initially discovered by serologic analysis of a cDNA expression library of a patient's esophageal carcinoma and autologous serum, and it has since been found to be expressed in melanoma, sarcomas, and various other carcinomas.[186,187]

NY-ESO-1 is expressed in up to 80% (20 of 25 cases[188]; 38 of 50 cases[189]) of synovial sarcomas (both monophasic and biphasic types) by immunohistochemistry, with strong reactivity seen in both the spindle cell and glandular components, and with a predominantly cytoplasmic pattern of staining.[188,189] Expression appears to be limited among other spindle cell tumors, with 10% (2 of 20) of dermatofibrosarcoma protuberans, 3% (1 of 34) of MPNSTs, and 1% (2 of 155) of GISTs showing diffuse positivity. NY-ESO-1 may therefore be useful in the distinction of synovial sarcoma from other spindle cell tumors.

NY-ESO-1 has also been found to be frequently expressed in myxoid liposarcoma,[190,191] with a lower frequency seen in other liposarcoma subtypes.[191] In a study of 138 tumors, including myxoid liposarcoma and other myxoid neoplasms, some of which may fall into the differential diagnosis, such as extraskeletal myxoid chondrosarcoma, 36 of 38 cases of myxoid liposarcoma (95%) were positive for NY-ESO-1, whereas all other tumor types were negative.[192] NY-ESO-1 may therefore also be useful in the distinction of myxoid/round cell liposarcomas from other myxoid neoplasms.

SUMMARY

In summary, many new diagnostic immunohistochemical markers for soft tissue tumors have been described in the last 10 years. This has resulted in improved classification of tumors, identification of clinically and pathologically distinct groups of tumors with prognostic and predictive implications, most notably SDH-deficient GIST, and has also likely reduced the need for additional ancillary molecular studies in routine surgical pathology practice. However, the use of all of these markers requires careful clinical correlation and knowledge of the spectrum of staining in other tumor types, as no one marker is 100% sensitive or specific for a given diagnosis. This article has reviewed the main clinical uses of several newly described immunohistochemical markers that have proved to be of significant clinical utility, along with potential pitfalls and indications where additional molecular studies may be warranted.

References

1. Sato Y. Role of ETS family transcription factors in vascular development and angiogenesis. *Cell Struct Funct*. 2001;26(1):19–24.
2. Baltzinger M, Mager-Heckel AM, Remy P. Xl erg: expression pattern and overexpression during development plead for a role in endothelial cell differentiation. *Dev Dyn*. 1999;216(4–5):420–433.
3. McLaughlin F, Ludbrook VJ, Cox J, von Carlowitz I, Brown S, Randi AM. Combined genomic and antisense analysis reveals that the transcription factor Erg is implicated in endothelial cell differentiation. *Blood*. 2001;98(12):3332–3339.
4. Hewett PW, Nishi K, Daft EL, Clifford Murray J. Selective expression of erg isoforms in human endothelial cells. *Int J Biochem Cell Biol*. 2001;33(4):347–355.

5. Birdsey GM, Dryden NH, Amsellem V, et al. Transcription factor Erg regulates angiogenesis and endothelial apoptosis through VE-cadherin. *Blood*. 2008;111(7):3498–3506.

6. Yuan L, Le Bras A, Sacharidou A, et al. ETS-related gene (ERG) controls endothelial cell permeability via transcriptional regulation of the claudin 5 (CLDN5) gene. *J Biol Chem*. 2012;287(9):6582–6591.

7. Furusato B, Tan SH, Young D, et al. ERG oncoprotein expression in prostate cancer: clonal progression of ERG-positive tumor cells and potential for ERG-based stratification. *Prostate Cancer Prostatic Dis*. 2010;13(3):228–237.

8. Park K, Tomlins SA, Mudaliar KM, et al. Antibody-based detection of ERG rearrangement-positive prostate cancer. *Neoplasia*. 2010;12(7):590–598.

9. Sun C, Dobi A, Mohamed A, et al. TMPRSS2-ERG fusion, a common genomic alteration in prostate cancer activates C-MYC and abrogates prostate epithelial differentiation. *Oncogene*. 2008;27(40):5348–5353.

10. Mosquera JM, Mehra R, Regan MM, et al. Prevalence of TMPRSS2-ERG fusion prostate cancer among men undergoing prostate biopsy in the United States. *Clin Cancer Res*. 2009;15(14):4706–4711.

11. Tomlins SA, Bjartell A, Chinnaiyan AM, et al. ETS gene fusions in prostate cancer: from discovery to daily clinical practice. *Eur Urol*. 2009;56(2):275–286.

12. Kumar-Sinha C, Tomlins SA, Chinnaiyan AM. Recurrent gene fusions in prostate cancer. *Nat Rev Cancer*. 2008;8(7):497–511.

13. Miettinen M, Wang ZF, Paetau A, et al. ERG transcription factor as an immunohistochemical marker for vascular endothelial tumors and prostatic carcinoma. *Am J Surg Pathol*. 2011;35(3):432–441.

14. McKay KM, Doyle LA, Lazar AJ, Hornick JL. Expression of ERG, an Ets family transcription factor, distinguishes cutaneous angiosarcoma from histological mimics. *Histopathology*. 2012;61(5):989–991.

15. Wang WL, Patel NR, Caragea M, et al. Expression of ERG, an Ets family transcription factor, identifies ERG-rearranged Ewing sarcoma. *Mod Pathol*. 2012; 25(10):1378–1383.

16. Panagopoulos I, Lassen C, Isaksson M, Mitelman F, Mandahl N, Aman P. Characteristic sequence motifs at the breakpoints of the hybrid genes FUS/CHOP, EWS/CHOP and FUS/ERG in myxoid liposarcoma and acute myeloid leukemia. *Oncogene*. 1997;15(11):1357–1362.

17. Kong XT, Ida K, Ichikawa H, et al. Consistent detection of TLS/FUS-ERG chimeric transcripts in acute myeloid leukemia with t(16;21)(p11;q22) and identification of a novel transcript. *Blood*. 1997;90(3):1192–1199.

18. Miettinen M, Wang Z, Sarlomo-Rikala M, Abdullaev Z, Pack SD, Fetsch JF. ERG expression in epithelioid sarcoma: a diagnostic pitfall. *Am J Surg Pathol*. 2013;37(10):1580–1585.

19. Stockman DL, Hornick JL, Deavers MT, Lev DC, Lazar AJ, Wang WL. ERG and FLI1 protein expression in epithelioid sarcoma. *Mod Pathol*. 2014;27(4):496–501.

20. Pincus LB, Fox LP. Images in clinical medicine: the Stewart-Treves syndrome. *N Engl J Med*. 2008;359(9):950.

21. Gonzalez MJ, Koehler MM, Satter EK. Angiosarcoma of the scalp: a case report and review of current and novel therapeutic regimens. *Dermatol Surg*. 2009;35(4):679–684.

22. Fineberg S, Rosen PP. Cutaneous angiosarcoma and atypical vascular lesions of the skin and breast after radiation therapy for breast carcinoma. *Am J Clin Pathol*. 1994;102(6):757–763.

23. West JG, Qureshi A, West JE, et al. Risk of angiosarcoma following breast conservation: a clinical alert. *Breast J*. 2005;11(2):115–123.

24. Stewart FW, Treves N. Lymphangiosarcoma in postmastectomy lymphedema; a report of six cases in elephantiasis chirurgica. *Cancer*. 1948;1(1):64–81.

25. Body G, Sauvanet E, Calais G, Fignon A, Fetissof F, Lansac J. Cutaneous angiosarcoma of the breast following surgery and irradiation of breast adenocarcinoma [in French]. *J Gynecol Obstet Biol Reprod (Paris)*. 1987;16(4): 479–483.

26. Huang J, Mackillop WJ. Increased risk of soft tissue sarcoma after radiotherapy in women with breast carcinoma. *Cancer*. 2001;92(1):172–180.

27. Cozen W, Bernstein L, Wang F, Press MF, Mack TM. The risk of angiosarcoma following primary breast cancer. *Br J Cancer*. 1999;81(3):532–536.

28. Monroe AT, Feigenberg SJ, Mendenhall NP. Angiosarcoma after breast-conserving therapy. *Cancer*. 2003;97(8):1832–1840.

29. Strobbe LJ, Peterse HL, van Tinteren H, Wijnmaalen A, Rutgers EJ. Angiosarcoma of the breast after conservation therapy for invasive cancer, the incidence and outcome: an unforeseen sequela. *Breast Cancer Res Treat*. 1998; 47(2):101–109.

30. Brenn T, Fletcher CD. Radiation-associated cutaneous atypical vascular lesions and angiosarcoma: clinicopathologic analysis of 42 cases. *Am J Surg Pathol*. 2005;29(8):983–996.

31. Brenn T, Fletcher CD. Postradiation vascular proliferations: an increasing problem. *Histopathology*. 2006;48(1):106–114.

32. Rosso R, Gianelli U, Carnevali L. Acquired progressive lymphangioma of the skin following radiotherapy for breast carcinoma. *J Cutan Pathol*. 1995;22(2): 164–167.

33. Diaz-Cascajo C, Borghi S, Weyers W, Retzlaff H, Requena L, Metze D. Benign lymphangiomatous papules of the skin following radiotherapy: a report of five new cases and review of the literature. *Histopathology*. 1999;35(4):319–327.

34. Requena L, Kutzner H, Mentzel T, Duran R, Rodriguez-Peralto JL. Benign vascular proliferations in irradiated skin. *Am J Surg Pathol*. 2002;26(3):328–337.

35. Gengler C, Coindre JM, Leroux A, et al. Vascular proliferations of the skin after radiation therapy for breast cancer: clinicopathologic analysis of a series in favor of a benign process: a study from the French Sarcoma Group. *Cancer*. 2007; 109(8):1584–1598.

36. Mattoch IW, Robbins JB, Kempson RL, Kohler S. Post-radiotherapy vascular proliferations in mammary skin: a clinicopathologic study of 11 cases. *J Am Acad Dermatol*. 2007;57(1):126–133.

37. Patton KT, Deyrup AT, Weiss SW. Atypical vascular lesions after surgery and radiation of the breast: a clinicopathologic study of 32 cases analyzing histologic heterogeneity and association with angiosarcoma. *Am J Surg Pathol*. 2008;32(6):943–950.

38. Leshin B, Whitaker DC, Foucar E. Lymphangioma circumscriptum following mastectomy and radiation therapy. *J Am Acad Dermatol*. 1986;15(5, pt 2):1117–1119.

39. Prioleau PG, Santa Cruz DJ. Lymphangioma circumscriptum following radical mastectomy and radiation therapy. *Cancer*. 1978;42(4):1989–1991.

40. Fisher I, Orkin M. Acquired lymphangioma (lymphangiectasis): report of a case. *Arch Dermatol*. 1970;101(2):230–234.

41. Meyer N, Penn LZ. Reflecting on 25 years with MYC. *Nat Rev Cancer*. 2008;8(12):976–990.

42. Manner J, Radlwimmer B, Hohenberger P, et al. MYC high level gene amplification is a distinctive feature of angiosarcomas after irradiation or chronic lymphedema. *Am J Pathol*. 2010;176(1):34–39.

43. Guo T, Zhang L, Chang NE, Singer S, Maki RG, Antonescu CR. Consistent MYC and FLT4 gene amplification in radiation-induced angiosarcoma but not in other radiation-associated atypical vascular lesions. *Genes Chromosomes Cancer*. 2011;50(1):25–33.

44. Mentzel T, Schildhaus HU, Palmedo G, Buttner R, Kutzner H. Postradiation cutaneous angiosarcoma after treatment of breast carcinoma is characterized by MYC amplification in contrast to atypical vascular lesions after radiotherapy and control cases: clinicopathological, immunohistochemical and molecular analysis of 66 cases. *Mod Pathol*. 2011;25(1):75–85.

45. Shon W, Sukov WR, Jenkins SM, Folpe AL. MYC amplification and overexpression in primary cutaneous angiosarcoma: a fluorescence in-situ hybridization and immunohistochemical study. *Mod Pathol*. 2014;27(4):509–515.

46. Binh MB, Sastre-Garau X, Guillou L, et al. MDM2 and CDK4 immunostainings are useful adjuncts in diagnosing well-differentiated and dedifferentiated liposarcoma subtypes: a comparative analysis of 559 soft tissue neoplasms with genetic data. *Am J Surg Pathol*. 2005;29(10):1340–1347.

47. Dei Tos AP, Doglioni C, Piccinin S, et al. Coordinated expression and amplification of the MDM2, CDK4, and HMGI-C genes in atypical lipomatous tumours. *J Pathol*. 2000;190(5):531–536.

48. Binh MB, Garau XS, Guillou L, Aurias A, Coindre JM. Reproducibility of MDM2 and CDK4 staining in soft tissue tumors. *Am J Clin Pathol*. 2006;125(5): 693–697.

49. Bode-Lesniewska B, Zhao J, Speel EJ, et al. Gains of 12q13-14 and overexpression of mdm2 are frequent findings in intimal sarcomas of the pulmonary artery. *Virchows Arch*. 2001;438(1):57–65.

50. Zhao J, Roth J, Bode-Lesniewska B, Pfaltz M, Heitz PU, Komminoth P. Combined comparative genomic hybridization and genomic microarray for detection of gene amplifications in pulmonary artery intimal sarcomas and adrenocortical tumors. *Genes Chromosomes Cancer*. 2002;34(1):48–57.

51. Neuville A, Collin F, Bruneval P, et al. Intimal sarcoma is the most frequent primary cardiac sarcoma: clinicopathologic and molecular retrospective analysis of 100 primary cardiac sarcomas. *Am J Surg Pathol*. 2014;38(4):461–469.

52. Mejia-Guerrero S, Quejada M, Gokgoz N, et al. Characterization of the 12q15 MDM2 and 12q13-14 CDK4 amplicons and clinical correlations in osteosarcoma. *Genes Chromosomes Cancer*. 2010;49(6):518–525.

53. Yoshida A, Ushiku T, Motoi T, et al. Immunohistochemical analysis of MDM2 and CDK4 distinguishes low-grade osteosarcoma from benign mimics. *Mod Pathol*. 2010;23(9):1279–1288.

54. Yoshida A, Ushiku T, Motoi T, et al. MDM2 and CDK4 immunohistochemical coexpression in high-grade osteosarcoma: correlation with a dedifferentiated subtype. *Am J Surg Pathol*. 2012;36(3):423–431.

55. Chmielecki J, Crago AM, Rosenberg M, et al. Whole-exome sequencing identifies a recurrent NAB2-STAT6 fusion in solitary fibrous tumors. *Nat Genet*. 2013;45(2):131–132.

56. Mohajeri A, Tayebwa J, Collin A, et al. Comprehensive genetic analysis identifies a pathognomonic NAB2/STAT6 fusion gene, nonrandom secondary genomic imbalances, and a characteristic gene expression profile in solitary fibrous tumor. *Genes Chromosomes Cancer*. 2013;52(10):873–886.

57. Robinson DR, Wu YM, Kalyana-Sundaram S, et al. Identification of recurrent NAB2-STAT6 gene fusions in solitary fibrous tumor by integrative sequencing. *Nat Genet*. 2013;45(2):180–185.

58. Schweizer L, Koelsche C, Sahm F, et al. Meningeal hemangiopericytoma and solitary fibrous tumors carry the NAB2-STAT6 fusion and can be diagnosed by nuclear expression of STAT6 protein. *Acta Neuropathol*. 2013;125(5):651–658.

59. Hebenstreit D, Wirnsberger G, Horejs-Hoeck J, Duschl A. Signaling mechanisms, interaction partners, and target genes of STAT6. *Cytokine Growth Factor Rev*. 2006;17(3):173–188.

60. Doyle LA, Vivero M, Fletcher CD, Mertens F, Hornick JL. Nuclear expression of STAT6 distinguishes solitary fibrous tumor from histologic mimics. *Mod Pathol*. 2014;27(3):390–395.

61. Cheah AL, Billings SD, Goldblum JR, Carver P, Tanas MZ, Rubin BP. STAT6 rabbit monoclonal antibody is a robust diagnostic tool for the distinction of solitary fibrous tumour from its mimics. *Pathology*. 2014;46(5):389–395.

62. Koelsche C, Schweizer L, Renner M, et al. Nuclear relocation of STAT6 reliably predicts NAB2/STAT6 fusion for the diagnosis of Solitary Fibrous Tumour [published online ahead of print April 4, 2014]. *Histopathology*. doi: 10.1111/his.12431.

63. Yoshida A, Tsuta K, Ohno M, et al. STAT6 immunohistochemistry is helpful in the diagnosis of solitary fibrous tumors. *Am J Surg Pathol*. 2014;38(4):552–559.

64. Barthelmess S, Geddert H, Boltze C, et al. Solitary fibrous tumors/hemangiopericytomas with different variants of the NAB2-STAT6 gene fusion are characterized by specific histomorphology and distinct clinicopathological features. *Am J Pathol*. 2014;184(4):1209–1218.

65. Moller E, Hornick JL, Magnusson L, Veerla S, Domanski HA, Mertens F. FUS-CREB3L2/L1-positive sarcomas show a specific gene expression profile with upregulation of CD24 and FOXL1. *Clin Cancer Res*. 2011;17(9):2646–2656.

66. Bafna S, Kaur S, Batra SK. Membrane-bound mucins: the mechanistic basis for alterations in the growth and survival of cancer cells. *Oncogene*. 2010;29(20):2893–2904.

67. Chaturvedi P, Singh AP, Batra SK. Structure, evolution, and biology of the MUC4 mucin. *FASEB J*. 2008;22(4):966–981.

68. Carraway KL, Perez A, Idris N, et al. Muc4/sialomucin complex, the intramembrane ErbB2 ligand, in cancer and epithelia: to protect and to survive. *Prog Nucleic Acid Res Mol Biol*. 2002;71:149–185.

69. Carraway KL, Theodoropoulos G, Kozloski GA, Carothers Carraway CA. Muc4/MUC4 functions and regulation in cancer. *Future Oncol*. 2009;5(10):1631–1640.

70. Chaturvedi P, Singh AP, Chakraborty S, et al. MUC4 mucin interacts with and stabilizes the HER2 oncoprotein in human pancreatic cancer cells. *Cancer Res*. 2008;68(7):2065–2070.

71. Workman HC, Sweeney C, Carraway KL III. The membrane mucin Muc4 inhibits apoptosis induced by multiple insults via ErbB2-dependent and ErbB2-independent mechanisms. *Cancer Res*. 2009;69(7):2845–2852.

72. Doyle LA, Moller E, Dal Cin P, Fletcher CD, Mertens F, Hornick JL. MUC4 is a highly sensitive and specific marker for low-grade fibromyxoid sarcoma. *Am J Surg Pathol*. 2011;35(5):733–741.

73. Linos K, Bridge JA, Edgar MA. MUC 4-negative FUS-CREB3L2 rearranged low-grade fibromyxoid sarcoma [published online ahead of print March 26, 2014]. *Histopathology*. doi: 10.1111/his.12422.

74. Rekhi B, Deshmukh M, Jambhekar NA. Low-grade fibromyxoid sarcoma: a clinicopathologic study of 18 cases, including histopathologic relationship with sclerosing epithelioid fibrosarcoma in a subset of cases. *Ann Diagn Pathol*. 2011;15(5):303–311.

75. Antonescu CR, Rosenblum MK, Pereira P, Nascimento AG, Woodruff JM. Sclerosing epithelioid fibrosarcoma: a study of 16 cases and confirmation of a clinicopathologically distinct tumor. *Am J Surg Pathol*. 2001;25(6):699–709.

76. Meis-Kindblom JM, Kindblom LG, Enzinger FM. Sclerosing epithelioid fibrosarcoma: a variant of fibrosarcoma simulating carcinoma. *Am J Surg Pathol*. 1995;19(9):979–993.

77. Guillou L, Benhattar J, Gengler C, et al. Translocation-positive low-grade fibromyxoid sarcoma: clinicopathologic and molecular analysis of a series expanding the morphologic spectrum and suggesting potential relationship to sclerosing epithelioid fibrosarcoma: a study from the French Sarcoma Group. *Am J Surg Pathol*. 2007;31(9):1387–1402.

78. Rekhi B, Folpe AL, Deshmukh M, Jambhekar NA. Sclerosing epithelioid fibrosarcoma - a report of two cases with cytogenetic analysis of FUS gene rearrangement by FISH technique. *Pathol Oncol Res*. 2011;17(1):145–148.

79. Folpe AL, Lane KL, Paull G, Weiss SW. Low-grade fibromyxoid sarcoma and hyalinizing spindle cell tumor with giant rosettes: a clinicopathologic study of 73 cases supporting their identity and assessing the impact of high-grade areas. *Am J Surg Pathol*. 2000;24(10):1353–1360.

80. Lane KL, Shannon RJ, Weiss SW. Hyalinizing spindle cell tumor with giant rosettes: a distinctive tumor closely resembling low-grade fibromyxoid sarcoma. *Am J Surg Pathol*. 1997;21(12):1481–1488.

81. Wang WL, Evans HL, Meis JM, et al. FUS rearrangements are rare in 'pure' sclerosing epithelioid fibrosarcoma. *Mod Pathol*. 2012;25(6):846–853.

82. Doyle LA, Wang WL, Dal Cin P, et al. MUC4 is a sensitive and extremely useful marker for sclerosing epithelioid fibrosarcoma: association with FUS gene rearrangement. *Am J Surg Pathol*. 2012;36(10):1444–1451.

83. Arbajian E, Puls F, Magnusson L, et al. Recurrent EWSR1-CREB3L1 gene fusions in sclerosing epithelioid fibrosarcoma. *Am J Surg Pathol*. 2014;38(6):801–808.

84. Lau PP, Lui PC, Lau GT, Yau DT, Cheung ET, Chan JK. EWSR1-CREB3L1 gene fusion: a novel alternative molecular aberration of low-grade fibromyxoid sarcoma. *Am J Surg Pathol*. 2013;37(5):734–738.

85. Rubin BP, Singer S, Tsao C, et al. KIT activation is a ubiquitous feature of gastrointestinal stromal tumors. *Cancer Res*. 2001;61(22):8118–8121.

86. Hirota S, Nishida T, Isozaki K, et al. Gain-of-function mutation at the extracellular domain of KIT in gastrointestinal stromal tumours. *J Pathol*. 2001;193(4):505–510.

87. Heinrich MC, Corless CL, Duensing A, et al. PDGFRA activating mutations in gastrointestinal stromal tumors. *Science*. 2003;299(5607):708–710.

88. Hirota S, Ohashi A, Nishida T, et al. Gain-of-function mutations of platelet-derived growth factor receptor alpha gene in gastrointestinal stromal tumors. *Gastroenterology*. 2003;125(3):660–667.

89. West RB, Corless CL, Chen X, et al. The novel marker, DOG1, is expressed ubiquitously in gastrointestinal stromal tumors irrespective of KIT or PDGFRA mutation status. *Am J Pathol*. 2004;165(1):107–113.

90. Miettinen M, Wang ZF, Lasota J. DOG1 antibody in the differential diagnosis of gastrointestinal stromal tumors: a study of 1840 cases. *Am J Surg Pathol*. 2009;33(9):1401–1408.

91. Espinosa I, Lee CH, Kim MK, et al. A novel monoclonal antibody against DOG1 is a sensitive and specific marker for gastrointestinal stromal tumors. *Am J Surg Pathol*. 2008;32(2):210–218.

92. Kang GH, Srivastava A, Kim YE, et al. DOG1 and PKC-theta are useful in the diagnosis of KIT-negative gastrointestinal stromal tumors. *Mod Pathol*. 2011;24(6):866–875.

93. Liegl B, Hornick JL, Corless CL, Fletcher CD. Monoclonal antibody DOG1.1 shows higher sensitivity than KIT in the diagnosis of gastrointestinal stromal tumors, including unusual subtypes. *Am J Surg Pathol*. 2009;33(3):437–446.

94. Yamamoto H, Kojima A, Nagata S, Tomita Y, Takahashi S, Oda Y. KIT-negative gastrointestinal stromal tumor of the abdominal soft tissue: a clinicopathologic and genetic study of 10 cases. *Am J Surg Pathol*. 2011;35(9):1287–1295.

95. Yang YD, Cho H, Koo JY, et al. TMEM16A confers receptor-activated calcium-dependent chloride conductance. *Nature*. 2008;455(7217):1210–1215.

96. Caputo A, Caci E, Ferrera L, et al. TMEM16A, a membrane protein associated with calcium-dependent chloride channel activity. *Science*. 2008;322(5901):590–594.

97. Hemminger J, Iwenofu OH. Discovered on gastrointestinal stromal tumours 1 (DOG1) expression in non-gastrointestinal stromal tumour (GIST) neoplasms. *Histopathology*. 2012;61(2):170–177.

98. Lopes LF, West RB, Bacchi LM, van de Rijn M, Bacchi CE. DOG1 for the diagnosis of gastrointestinal stromal tumor (GIST): Comparison between 2 different antibodies. *Appl Immunohistochem Mol Morphol*. 2010;18(4):333–337.

99. Akpalo H, Lange C, Zustin J. Discovered on gastrointestinal stromal tumour 1 (DOG1): a useful immunohistochemical marker for diagnosing chondroblastoma. *Histopathology*. 2012;60(7):1099–1106.

100. Novelli M, Rossi S, Rodriguez-Justo M, et al. DOG1 and CD117 are the antibodies of choice in the diagnosis of gastrointestinal stromal tumours. *Histopathology*. 2010;57(2):259–270.

101. Corless CL, Fletcher JA, Heinrich MC. Biology of gastrointestinal stromal tumors. *J Clin Oncol*. 2004;22(18):3813–3825.

102. Janeway KA, Liegl B, Harlow A, et al. Pediatric KIT wild-type and platelet-derived growth factor receptor alpha-wild-type gastrointestinal stromal tumors share KIT activation but not mechanisms of genetic progression with adult gastrointestinal stromal tumors. *Cancer Res*. 2007;67(19):9084–9088.

103. Agaram NP, Laquaglia MP, Ustun B, et al. Molecular characterization of pediatric gastrointestinal stromal tumors. *Clin Cancer Res*. 2008;14(10):3204–3215.

104. Marrari A, Wagner AJ, Hornick JL. Predictors of response to targeted therapies for gastrointestinal stromal tumors. *Arch Pathol Lab Med*. 2012;136(5):483–489.

105. Carney JA, Stratakis CA. Familial paraganglioma and gastric stromal sarcoma: a new syndrome distinct from the Carney triad. *Am J Med Genet*. 2002;108(2):132–139.

106. Carney JA, Sheps SG, Go VL, Gordon H. The triad of gastric leiomyosarcoma, functioning extra-adrenal paraganglioma and pulmonary chondroma. *N Engl J Med*. 1977;296(26):1517–1518.

107. Carney JA. Gastric stromal sarcoma, pulmonary chondroma, and extra-adrenal paraganglioma (Carney Triad): natural history, adrenocortical component, and possible familial occurrence. *Mayo Clin Proc*. 1999;74(6):543–552.

108. Carney JA. Carney triad: a syndrome featuring paraganglionic, adrenocortical, and possibly other endocrine tumors. *J Clin Endocrinol Metab*. 2009;94(10):3656–3662.

109. Janeway KA, Kim SY, Lodish M, et al. Defects in succinate dehydrogenase in gastrointestinal stromal tumors lacking KIT and PDGFRA mutations. *Proc Natl Acad Sci U S A*. 2011;108(1):314–318.

110. Miettinen M, Wang ZF, Sarlomo-Rikala M, Osuch C, Rutkowski P, Lasota J. Succinate dehydrogenase-deficient GISTs: a clinicopathologic, immunohistochemical, and molecular genetic study of 66 gastric GISTs with predilection to young age. *Am J Surg Pathol*. 2011;35(11):1712–1721.

111. Rege TA, Wagner AJ, Corless CL, Heinrich MC, Hornick JL. "Pediatric-type" gastrointestinal stromal tumors in adults: distinctive histology predicts genotype and clinical behavior. *Am J Surg Pathol*. 2011;35(4):495–504.

112. Miettinen M, Lasota J, Sobin LH. Gastrointestinal stromal tumors of the stomach in children and young adults: a clinicopathologic, immunohistochemical, and molecular genetic study of 44 cases with long-term follow-up and review of the literature. *Am J Surg Pathol*. 2005;29(10):1373–1381.

113. Lancaster CR. Succinate:quinone oxidoreductases: an overview. *Biochim Biophys Acta*. 2002;1553(1–2):1–6.

114. Gottlieb E, Tomlinson IP. Mitochondrial tumour suppressors: a genetic and biochemical update. *Nat Rev Cancer*. 2005;5(11):857–866.

115. van Nederveen FH, Gaal J, Favier J, et al. An immunohistochemical procedure to detect patients with paraganglioma and phaeochromocytoma with

germline SDHB, SDHC, or SDHD gene mutations: a retrospective and prospective analysis. *Lancet Oncol*. 2009;10(8):764–771.

116. Nakamura E, Kaelin WG Jr. Recent insights into the molecular pathogenesis of pheochromocytoma and paraganglioma. *Endocr Pathol*. 2006; 17(2):97–106.

117. Gill AJ, Benn DE, Chou A, et al. Immunohistochemistry for SDHB triages genetic testing of SDHB, SDHC, and SDHD in paraganglioma-pheochromocytoma syndromes. *Hum Pathol*. 2010;41(6):805–814.

118. Gaal J, Stratakis CA, Carney JA, et al. SDHB immunohistochemistry: a useful tool in the diagnosis of Carney-Stratakis and Carney triad gastrointestinal stromal tumors. *Mod Pathol*. 2011;24(1):147–151.

119. Gill AJ, Chou A, Vilain R, et al. Immunohistochemistry for SDHB divides gastrointestinal stromal tumors (GISTs) into 2 distinct types. *Am J Surg Pathol*. 2010;34(5):636–644.

120. Pasini B, McWhinney SR, Bei T, et al. Clinical and molecular genetics of patients with the Carney-Stratakis syndrome and germline mutations of the genes coding for the succinate dehydrogenase subunits SDHB, SDHC, and SDHD. *Eur J Hum Genet*. 2008;16(1):79–88.

121. Doyle LA, Nelson D, Heinrich MC, Corless CL, Hornick JL. Loss of succinate dehydrogenase subunit B (SDHB) expression is limited to a distinctive subset of gastric wild-type gastrointestinal stromal tumours: a comprehensive genotype-phenotype correlation study. *Histopathology*. 2012;61(5):801–809.

122. Pantaleo MA, Astolfi A, Indio V, et al. SDHA loss-of-function mutations in KIT-PDGFRA wild-type gastrointestinal stromal tumors identified by massively parallel sequencing. *J Natl Cancer Inst*. 2011;103(12):983–987.

123. Pantaleo MA, Nannini M, Astolfi A, Biasco G. A distinct pediatric-type gastrointestinal stromal tumor in adults: potential role of succinate dehydrogenase subunit A mutations. *Am J Surg Pathol*. 2011;35(11):1750–1752.

124. Wagner AJ, Remillard SP, Zhang YX, Doyle LA, George S, Hornick JL. Loss of expression of SDHA predicts SDHA mutations in gastrointestinal stromal tumors. *Mod Pathol*. 2012;26(2):289–294.

125. Matyakhina L, Bei TA, McWhinney SR, et al. Genetics of carney triad: recurrent losses at chromosome 1 but lack of germline mutations in genes associated with paragangliomas and gastrointestinal stromal tumors. *J Clin Endocrinol Metab*. 2007;92(8):2938–2943.

126. Zhang L, Smyrk TC, Young WF Jr, Stratakis CA, Carney JA. Gastric stromal tumors in Carney triad are different clinically, pathologically, and behaviorally from sporadic gastric gastrointestinal stromal tumors: findings in 104 cases. *Am J Surg Pathol*. 2010;34(1):53–64.

127. Stratakis CA, Carney JA. The triad of paragangliomas, gastric stromal tumours and pulmonary chondromas (Carney triad), and the dyad of paragangliomas and gastric stromal sarcomas (Carney-Stratakis syndrome): molecular genetics and clinical implications. *J Intern Med*. 2009;266(1):43–52.

128. Wang JH, Lasota J, Miettinen M. Succinate dehydrogenase subunit B (SDHB) is expressed in neurofibromatosis 1-associated gastrointestinal stromal tumors (Gists): implications for the SDHB expression based classification of Gists. *J Cancer*. 2011;2:90–93.

129. Hollmann TJ, Hornick JL. INI1-deficient tumors: diagnostic features and molecular genetics. *Am J Surg Pathol*. 2011;35(10):e47–e63.

130. Bourdeaut F, Lequin D, Brugieres L, et al. Frequent hSNF5/INI1 germline mutations in patients with rhabdoid tumor. *Clin Cancer Res*. 2011;17(1):31–38.

131. Hornick JL, Dal Cin P, Fletcher CD. Loss of INI1 expression is characteristic of both conventional and proximal-type epithelioid sarcoma. *Am J Surg Pathol*. 2009;33(4):542–550.

132. Sullivan LM, Folpe AL, Pawel BR, Judkins AR, Biegel JA. Epithelioid sarcoma is associated with a high percentage of SMARCB1 deletions. *Mod Pathol*. 2012;26(3):385–392.

133. Gleason BC, Fletcher CD. Myoepithelial carcinoma of soft tissue in children: an aggressive neoplasm analyzed in a series of 29 cases. *Am J Surg Pathol*. 2007;31(12):1813–1824.

134. Kohashi K, Oda Y, Yamamoto H, et al. SMARCB1/INI1 protein expression in round cell soft tissue sarcomas associated with chromosomal translocations involving EWS: a special reference to SMARCB1/INI1 negative variant extraskeletal myxoid chondrosarcoma. *Am J Surg Pathol*. 2008;32(8):1168–1174.

135. Mobley BC, McKenney JK, Bangs CD, et al. Loss of SMARCB1/INI1 expression in poorly differentiated chordomas. *Acta Neuropathol*. 2010;120(6): 745–753.

136. Calderaro J, Moroch J, Pierron G, et al. SMARCB1/INI1 inactivation in renal medullary carcinoma. *Histopathology*. 2012;61(3):428–435.

137. Cheng JX, Tretiakova M, Gong C, Mandal S, Krausz T, Taxy JB. Renal medullary carcinoma: rhabdoid features and the absence of INI1 expression as markers of aggressive behavior. *Mod Pathol*. 2008;21(6):647–652.

138. Trobaugh-Lotrario AD, Tomlinson GE, Finegold MJ, Gore L, Feusner JH. Small cell undifferentiated variant of hepatoblastoma: adverse clinical and molecular features similar to rhabdoid tumors. *Pediatr Blood Cancer*. 2009;52(3): 328–334.

139. Boyd C, Smith MJ, Kluwe L, Balogh A, Maccollin M, Plotkin SR. Alterations in the SMARCB1 (INI1) tumor suppressor gene in familial schwannomatosis. *Clin Genet*. 2008;74(4):358–366.

140. Arnold MA, Arnold CA, Li G, et al. A unique pattern of INI1 immunohistochemistry distinguishes synovial sarcoma from its histologic mimics. *Hum Pathol*. 2013;44(5):881–887.

141. Kohashi K, Oda Y, Yamamoto H, et al. Reduced expression of SMARCB1/INI1 protein in synovial sarcoma. *Mod Pathol*. 2010;23(7):981–990.

142. Allander SV, Illei PB, Chen Y, et al. Expression profiling of synovial sarcoma by cDNA microarrays: association of ERBB2, IGFBP2, and ELF3 with epithelial differentiation. *Am J Pathol*. 2002;161(5):1587–1595.

143. Baird K, Davis S, Antonescu CR, et al. Gene expression profiling of human sarcomas: insights into sarcoma biology. *Cancer Res*. 2005;65(20):9226–9235.

144. Henderson SR, Guiliano D, Presneau N, et al. A molecular map of mesenchymal tumors. *Genome Biol*. 2005;6(9):R76.

145. Nagayama S, Katagiri T, Tsunoda T, et al. Genome-wide analysis of gene expression in synovial sarcomas using a cDNA microarray. *Cancer Res*. 2002; 62(20):5859–5866.

146. Nielsen TO, West RB, Linn SC, et al. Molecular characterisation of soft tissue tumours: a gene expression study. *Lancet*. 2002;359(9314):1301–1307.

147. Ng TL, Gown AM, Barry TS, et al. Nuclear beta-catenin in mesenchymal tumors. *Mod Pathol*. 2005;18(1):68–74.

148. Pretto D, Barco R, Rivera J, Neel N, Gustavson MD, Eid JE. The synovial sarcoma translocation protein SYT-SSX2 recruits beta-catenin to the nucleus and associates with it in an active complex. *Oncogene*. 2006;25(26):3661–3669.

149. Stifani S, Blaumueller CM, Redhead NJ, Hill RE, Artavanis-Tsakonas S. Human homologs of a *Drosophila* Enhancer of split gene product define a novel family of nuclear proteins. *Nat Genet*. 1992;2(2):119–127.

150. Terry J, Saito T, Subramanian S, et al. TLE1 as a diagnostic immunohistochemical marker for synovial sarcoma emerging from gene expression profiling studies. *Am J Surg Pathol*. 2007;31(2):240–246.

151. Chen G, Courey AJ. Groucho/TLE family proteins and transcriptional repression. *Gene*. 2000;249(1–2):1–16.

152. Swingler TE, Bess KL, Yao J, Stifani S, Jayaraman PS. The proline-rich homeodomain protein recruits members of the Groucho/Transducin-like enhancer of split protein family to co-repress transcription in hematopoietic cells. *J Biol Chem*. 2004;279(33):34938–34947.

153. Yao J, Liu Y, Lo R, Tretjakoff I, Peterson A, Stifani S. Disrupted development of the cerebral hemispheres in transgenic mice expressing the mammalian Groucho homologue transducin-like-enhancer of split 1 in postmitotic neurons. *Mech Dev*. 2000;93(1–2):105–115.

154. Liu Y, Dehni G, Purcell KJ, et al. Epithelial expression and chromosomal location of human TLE genes: implications for notch signaling and neoplasia. *Genomics*. 1996;31(1):58–64.

155. Knosel T, Heretsch S, Altendorf-Hofmann A, et al. TLE1 is a robust diagnostic biomarker for synovial sarcomas and correlates with t(X;18): analysis of 319 cases. *Eur J Cancer*. 2010;46(6):1170–1176.

156. Chuang HC, Hsu SC, Huang CG, Hsueh S, Ng KF, Chen TC. Reappraisal of TLE-1 immunohistochemical staining and molecular detection of SS18-SSX fusion transcripts for synovial sarcoma. *Pathol Int*. 2013;63(12):573–580.

157. Jagdis A, Rubin BP, Tubbs RR, Pacheco M, Nielsen TO. Prospective evaluation of TLE1 as a diagnostic immunohistochemical marker in synovial sarcoma. *Am J Surg Pathol*. 2009;33(12):1743–1751.

158. Kosemehmetoglu K, Vrana JA, Folpe AL. TLE1 expression is not specific for synovial sarcoma: a whole section study of 163 soft tissue and bone neoplasms. *Mod Pathol*. 2009;22(7):872–878.

159. Foo WC, Cruise MW, Wick MR, Hornick JL. Immunohistochemical staining for TLE1 distinguishes synovial sarcoma from histologic mimics. *Am J Clin Pathol*. 2011;135(6):839–844.

160. Matsuyama A, Hisaoka M, Iwasaki M, Iwashita M, Hisanaga S, Hashimoto H. TLE1 expression in malignant mesothelioma. *Virchows Arch*. 2010;457(5):577–583.

161. Haq R, Fisher DE. Biology and clinical relevance of the micropthalmia family of transcription factors in human cancer. *J Clin Oncol*. 2011;29(25):3474–3482.

162. Miettinen M, Fernandez M, Franssila K, Gatalica Z, Lasota J, Sarlomo-Rikala M. Microphthalmia transcription factor in the immunohistochemical diagnosis of metastatic melanoma: comparison with four other melanoma markers. *Am J Surg Pathol*. 2001;25(2):205–211.

163. Busam KJ, Iversen K, Coplan KC, Jungbluth AA. Analysis of microphthalmia transcription factor expression in normal tissues and tumors, and comparison of its expression with S-100 protein, gp100, and tyrosinase in desmoplastic malignant melanoma. *Am J Surg Pathol*. 2001;25(2):197–204.

164. Argani P, Antonescu CR, Illei PB, et al. Primary renal neoplasms with the ASPL-TFE3 gene fusion of alveolar soft part sarcoma: a distinctive tumor entity previously included among renal cell carcinomas of children and adolescents. *Am J Pathol*. 2001;159(1):179–192.

165. Argani P, Antonescu CR, Couturier J, et al. PRCC-TFE3 renal carcinomas: morphologic, immunohistochemical, ultrastructural, and molecular analysis of an entity associated with the t(X;1)(p11.2;q21). *Am J Surg Pathol*. 2002;26(12): 1553–1566.

166. Argani P, Ladanyi M. Translocation carcinomas of the kidney. *Clin Lab Med*. 2005;25(2):363–378.

167. Argani P, Lui MY, Couturier J, Bouvier R, Fournet JC, Ladanyi M. A novel CLTC-TFE3 gene fusion in pediatric renal adenocarcinoma with t(X;17)(p11.2; q23). *Oncogene*. 2003;22(34):5374–5378.

168. Ladanyi M, Lui MY, Antonescu CR, et al. The der(17)t(X;17)(p11;q25) of human alveolar soft part sarcoma fuses the TFE3 transcription factor gene to ASPL, a novel gene at 17q25. *Oncogene*. 2001;20(1):48–57.

169. Argani P, Aulmann S, Karanjawala Z, Fraser RB, Ladanyi M, Rodriguez MM. Melanotic Xp11 translocation renal cancers: a distinctive neoplasm with overlapping features of PEComa, carcinoma, and melanoma. *Am J Surg Pathol*. 2009;33(4):609–619.

170. Argani P, Aulmann S, Illei PB, et al. A distinctive subset of PEComas harbors TFE3 gene fusions. *Am J Surg Pathol*. 2010;34(10):1395–1406.
171. Antonescu CR, Le Loarer F, Mosquera JM, et al. Novel YAP1-TFE3 fusion defines a distinct subset of epithelioid hemangioendothelioma. *Genes Chromosomes Cancer*. 2013;52(8):775–784.
172. Argani P, Lal P, Hutchinson B, Lui MY, Reuter VE, Ladanyi M. Aberrant nuclear immunoreactivity for TFE3 in neoplasms with TFE3 gene fusions: a sensitive and specific immunohistochemical assay. *Am J Surg Pathol*. 2003;27(6):750–761.
173. Malinowska I, Kwiatkowski DJ, Weiss S, Martignoni G, Netto G, Argani P. Perivascular epithelioid cell tumors (PEComas) harboring TFE3 gene rearrangements lack the TSC2 alterations characteristic of conventional PEComas: further evidence for a biological distinction. *Am J Surg Pathol*. 2012;36(5):783–784.
174. Llamas-Velasco M, Mentzel T, Requena L, Palmedo G, Kasten R, Kutzner H. Cutaneous PEComa does not harbour TFE3 gene fusions: immunohistochemical and molecular study of 17 cases. *Histopathology*. 2013;63(1):122–129.
175. Kelsh RN. Sorting out Sox10 functions in neural crest development. *Bioessays*. 2006;28(8):788–798.
176. Paratore C, Goerich DE, Suter U, Wegner M, Sommer L. Survival and glial fate acquisition of neural crest cells are regulated by an interplay between the transcription factor Sox10 and extrinsic combinatorial signaling. *Development*. 2001;128(20):3949–3961.
177. Kuhlbrodt K, Herbarth B, Sock E, Hermans-Borgmeyer I, Wegner M. Sox10, a novel transcriptional modulator in glial cells. *J Neurosci*. 1998;18(1):237–250.
178. Shin J, Vincent JG, Cuda JD, et al. Sox10 is expressed in primary melanocytic neoplasms of various histologies but not in fibrohistiocytic proliferations and histiocytoses. *J Am Acad Dermatol*. 2012;67(4):717–726.
179. Karamchandani JR, Nielsen TO, van de Rijn M, West RB. Sox10 and S100 in the diagnosis of soft-tissue neoplasms. *Appl Immunohistochem Mol Morphol*. 2012;20(5):445–450.
180. Ohtomo R, Mori T, Shibata S, et al. SOX10 is a novel marker of acinus and intercalated duct differentiation in salivary gland tumors: a clue to the histogenesis for tumor diagnosis. *Mod Pathol*. 2013;26(8):1041–1050.
181. Naujokas A, Charli-Joseph Y, Ruben BS, et al. SOX-10 expression in cutaneous myoepitheliomas and mixed tumors. *J Cutan Pathol*. 2014;41(4):353–363.
182. Nonaka D, Chiriboga L, Rubin BP. Sox10: a pan-schwannian and melanocytic marker. *Am J Surg Pathol*. 2008;32(9):1291–1298.
183. Mohamed A, Gonzalez RS, Lawson D, Wang J, Cohen C. SOX10 expression in malignant melanoma, carcinoma, and normal tissues. *Appl Immunohistochem Mol Morphol*. 2013;21(6):506–510.
184. Scanlan MJ, Gure AO, Jungbluth AA, Old LJ, Chen YT. Cancer/testis antigens: an expanding family of targets for cancer immunotherapy. *Immunol Rev*. 2002;188:22–32.
185. Ayyoub M, Taub RN, Keohan ML, et al. The frequent expression of cancer/testis antigens provides opportunities for immunotherapeutic targeting of sarcoma. *Cancer Immun*. 2004;4:7.
186. Chen YT, Scanlan MJ, Sahin U, et al. A testicular antigen aberrantly expressed in human cancers detected by autologous antibody screening. *Proc Natl Acad Sci U S A*. 1997;94(5):1914–1918.
187. Nicholaou T, Ebert L, Davis ID, et al. Directions in the immune targeting of cancer: lessons learned from the cancer-testis Ag NY-ESO-1. *Immunol Cell Biol*. 2006;84(3):303–317.
188. Jungbluth AA, Chen YT, Stockert E, et al. Immunohistochemical analysis of NY-ESO-1 antigen expression in normal and malignant human tissues. *Int J Cancer*. 2001;92(6):856–860.
189. Lai JP, Robbins PF, Raffeld M, et al. NY-ESO-1 expression in synovial sarcoma and other mesenchymal tumors: significance for NY-ESO-1-based targeted therapy and differential diagnosis. *Mod Pathol*. 2012;25(6):854–858.
190. Pollack SM, Jungbluth AA, Hoch BL, et al. NY-ESO-1 is a ubiquitous immunotherapeutic target antigen for patients with myxoid/round cell liposarcoma. *Cancer*. 2012;118(18):4564–4570.
191. Hemminger JA, Ewart Toland A, Scharschmidt TJ, et al. The cancer-testis antigen NY-ESO-1 is highly expressed in myxoid and round cell subset of liposarcomas. *Mod Pathol*. 2013;26(2):282–288.
192. Hemminger JA, Iwenofu OH. NY-ESO-1 is a sensitive and specific immunohistochemical marker for myxoid and round cell liposarcomas among related mesenchymal myxoid neoplasms. *Mod Pathol*. 2013;26(9):1204–1210.
193. Doyle LA, Tao D, Mariño-Enríquez A. STAT6 is amplified in a subset of dedifferentiated liposarcoma. *Mod Pathol*. 2014;27(9):1231–1237.