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REVIEW AND PERSPECTIVES

Update on selected advances in the immunohistochemical and molecular genetic analysis of soft tissue tumors

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Abstract

Although traditional morphological evaluation remains the cornerstone for the diagnosis of soft tissue tumors, ancillary diagnostic modalities such as immunohistochemistry and molecular genetic analysis are of ever-increasing importance in this field. New insights into the molecular pathogenesis of soft tissue tumors, often obtained from high-throughput sequencing technologies, has enabled significant progress in the characterization and biologic stratification of mesenchymal neoplasms, expanding the spectrum of immunohistochemical tests (often aimed towards recently discovered genetic events) and molecular genetic assays (most often fluorescence in situ hybridization and reverse transcription-polymerase chain reaction). This review discusses selected novel molecular and immunohistochemical assays with diagnostic applicability in mesenchymal neoplasms, with emphasis on diagnosis, refinement of tumor classification, and treatment stratification.

Keywords Immunohistochemistry · Molecular diagnosis · Sarcoma · Soft tissue tumors · BCOR · CIC-DUX4 · EWSR1 · NTRK · SMARCB1 · SMARCA4 · Pathology · Genetics · Targeted therapy

Introduction

Mesenchymal neoplasms are frequently morphologically heterogeneous and often display inconsistent immunoprofiles or immunoprofiles that overlap with other mesenchymal or non-mesenchymal tumors. In recent years, aided by the more widespread application of high-throughput sequencing technologies, there has been marked progress in the molecular characterization of mesenchymal tumors. This has led to an expanded spectrum of commercially available antibodies, often aimed towards identification of immunohistochemical surrogates for various molecular genetic alterations, and new molecular tests (most often fluorescence in situ hybridization (FISH) probes and reverse transcription-polymerase chain reaction (RT-PCR) assays). Immunohistochemistry, the use of antibodies to detect specific epitopes in tissue sections, plays a central role in the diagnosis of most soft tissue neoplasms. While older markers typically delineated the line of differentiation of tumors by detecting cytoplasmic proteins such as intermediate filaments, lineage-associated membrane markers (e.g., CD31 for endothelium) or lineage-associated transcription factors (e.g., myogenin and MyoD1 for skeletal muscle), more recent molecular genetic discoveries have pointed towards newer markers that directly or indirectly detect tumor-specific genetic abnormalities. These include the protein products of gene fusions (e.g., BCOR and CCNB3 in Ewing-like small round cell sarcomas and CAMTA1, FOSB, and TFE3 in vascular tumors); the protein correlates of genetic mutations, deletions, and amplifications (e.g., aberrant beta-catenin expression in desmoid fibromatosis, loss of SMARCB1 and SMARCA4 in epithelioid sarcoma and related tumors, and MDM2 and CDK4 overexpression in atypical lipomatous tumor/well-differentiated liposarcoma); identification of the protein products of genes noted to be overexpressed by expression profiling (e.g., MUC4 in low-grade fibromyxoid sarcoma); and immunohistochemical detection of epigenetic events (e.g., loss of trimethylation of H3K27me3 in malignant

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peripheral nerve sheath tumors (MPNST)). An emerging further application of immunohistochemistry is the detection of mutations in tumors or the germline of patients with neoplasia associated with hereditary cancer predisposition syndromes (e.g., fumarate hydratase deficiency in hereditary renal cell carcinoma-leiomyomatosis syndrome) [1].

The limitations associated with the use of immunohistochemistry should be considered when making any diagnosis; the vast majority of markers show limited specificity, with many antigens expressed by more than one tumor type. Both benign and malignant neoplasms can show anomalous antigen expression, no markers or marker combinations exist that distinguish benign from malignant tumors, and immunohistochemistry should always be interpreted in the context of the complete panel and the morphologic and clinical findings.

Ancillary molecular techniques using formalin-fixed, paraffin-embedded tissues are in widespread routine use in soft tissue tumors diagnosis, with molecular services frequently integrated into or closely affiliated with anatomic pathology laboratories. A subset of soft tissue neoplasms, principally those of uncertain differentiation and which do not have a normal cell counterpart, are better defined by their molecular rather than their immunohistochemical profile. Most diagnostic laboratories utilize FISH to assess for gene rearrangements or fusions and RT-PCR to detect specific fusion transcripts. These techniques are easily applicable on limited material (including core biopsies, cell blocks, and cytologic preparations), and both the turnaround times and cost for these techniques have begun to approximate those for diagnostic immunohistochemistry. It is increasingly evident that the majority of recurrent genetic aberrations are not tumor-specific, even those that have until recently been considered specific for a given entity. For example, *PAX3-FOXO1* fusions, previously thought to be specific for alveolar rhabdomyosarcoma, are seen in a subset of biphenotypic sinonasal sarcomas [2, 3] and *EWSR1-WT1* fusions, initially thought specific for desmoplastic small round cell tumor, have been described in rare pediatric intra-abdominal spindle cell neoplasms resembling leiomyosarcomas [4] and in a very rare, low-grade, small round cell tumor of the cauda equina [5]. Thus, as with immunohistochemistry, molecular genetic findings must be integrated with the clinical, histologic, and immunohistochemical findings.

In this review, we will provide an overview of the recent immunohistochemical and molecular advances made in selected groups of mesenchymal neoplasms, focusing on tests that are of practical significance in their diagnostic work-up. More detailed discussion of many of these immunohistochemical and molecular genetic assays will also be found in the following articles in this special issue.

Epithelioid neoplasms with SMARCB1 and SMARCA4 deficiency

SMARCB1 (INI1/SNF5) and SMARCA4 (BRG1) proteins are subunits of the switch/sucrose non-fermenting (SWI/SNF) chromatin-remodeling complex, which is ubiquitously expressed in all normal cells. This complex plays an essential role in the regulation of transcriptional activity, through ATP-dependent modification of nucleosomes [6, 7]. Other units in this complex include SMARCF1 (ARID1a), SMARCC1 (BAF155), and SMARCC2 (BAF170) [7]. Many SWI/SNF subunits have a tumor-suppressor function, with biallelic inactivation demonstrated as a recurring phenomenon in many cancers, often manifesting with epithelioid or rhabdoid morphology.

SMARCB1 is the product of the *INI1/SMARCB1/BAF47/hSNF5* gene on chromosome 22q11.2 [8–10]. Immunohistochemical loss of SMARCB1 protein is seen in nearly all (~ 98%) renal/extrarenal rhabdoid tumors, in > 90% of (both classical and proximal-type) epithelioid sarcomas [11–20], approximately 70% of epithelioid MPNST [20, 21], in subsets of schwannomas (particularly those associated with schwannomatosis) [21, 22], in approximately 40% of epithelioid schwannomas [23], in 'poorly differentiated' chordomas [24], in about 15% of extraskeletal myxoid chondrosarcoma, and in all renal medullary carcinoma [20]. Demonstration of SMARCB1 loss is of particular value in the distinction of epithelioid sarcoma from poorly differentiated carcinomas, the overwhelming majority of which show retained expression (Fig. 1a, b), and in the distinction of epithelioid malignant peripheral nerve sheath tumors from melanomas, which do not show SMARCB1 loss (Fig. 2a, b).

Although the spectrum of neoplasms harboring *SMARCB1* alterations is incompletely understood, characterization of individual SMARCB1-deficient tumors is important because of emerging therapeutic strategies for these tumors, such as inhibitors of EZH2, a histone-lysine N-methyltransferase enzyme involved in transcriptional repression and DNA methylation, inactivation of which in in vivo models has shown blockade of SMARCB1 loss-driven tumorigenesis [25, 26].

A small proportion of rhabdoid tumors and epithelioid sarcomas that show retained (normal) expression of SMARCB1 instead demonstrate loss of SMARCA4 expression (Fig. 3a, b) [27, 28]. Inactivating mutations of SMARCA4 leading to loss of nuclear SMARCA4 expression are also commonly found in subsets of rare, aggressive neoplasms often showing rhabdoid morphology, including ovarian "small cell carcinoma of hypercalcemic type," [29] undifferentiated carcinomas of various primary sites [30–32],, and in SMARCA4-deficient thoracic sarcomas [33–35].

SMARCA4-deficient thoracic sarcomas are primitive mesenchymal neoplasms arising as compressive mediastinal and/or pulmonary masses in adults, particularly males, often with a

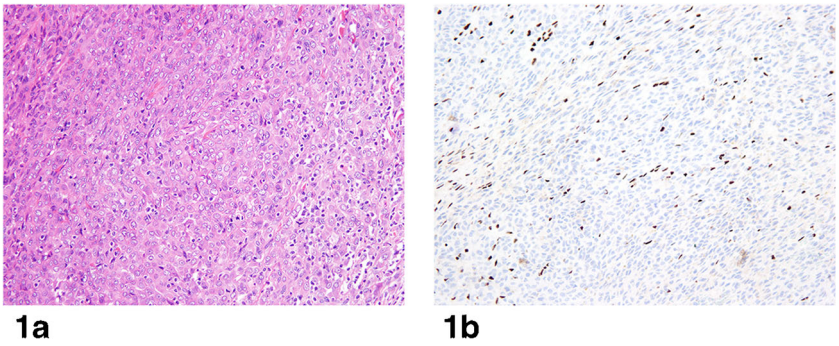

Fig. 1 Epithelioid sarcoma (**a**), showing complete loss of SMARCB1 expression (**b**)

history of smoking. Morphologically, these consist of sheets or patternless arrays of relatively discohesive, epithelioid and rounded, often rhabdoid cells with prominent nucleoli and frequent necrosis, sometimes with stromal myxoid change or desmoplasia. Most show keratin or epithelial membrane antigen expression, as well as SOX2 expression, with CD34 and SALL4 expression in roughly 60% and 30% of cases, respectively. SMARCB1 loss is not seen [36]. Although SMARCA4 loss is seen in roughly 10% of poorly differentiated pulmonary carcinomas, expression profiling studies show SMARCA4-deficient thoracic sarcomas to be distinct from lung carcinomas, but related to malignant rhabdoid tumors and small cell carcinomas of hypercalcemic type [35],. Additionally, SMARCA4-deficient thoracic sarcomas differ from most (but not all) carcinomas by virtue of concurrent inactivation of SMARCA2 and overexpression of SOX2.

Ewing sarcoma-like undifferentiated round cell sarcomas

CIC- and *BCOR*-associated undifferentiated round cell sarcomas represent two emerging classes of primitive round cell sarcomas which show some histologic and immunohistochemical overlap with Ewing sarcoma or atypical Ewing sarcoma, but lack molecular evidence of *EWSR1* gene rearrangements. The distinction between these neoplasms and Ewing sarcoma is prognostically important, as *CIC*-rearranged sarcomas behave more aggressively than Ewing sarcoma (5-year overall survival of 43% compared with 79%) and are associated with poorer responses to Ewing sarcoma-based chemotherapeutic regimens [37–40], whereas *BCOR*-associated sarcomas have a similar 5-year overall survival to Ewing sarcoma (72%) [40].

CIC-DUX4 fusions appear to represent the most frequent genetic abnormality in primitive small round cell sarcomas lacking *EWSR1* rearrangements, comprising up to two thirds of *EWSR1* rearrangement-negative undifferentiated round cell sarcomas of pediatric and young adult patients [37–39]. In these tumors, *CIC* on 19q13.2 fuses with one of the *DUX4* retrogenes on 4q35 or 10q26.3 [37]. Typically, these show t(4;19)(q35;q13.1) or t(10;19)(q26;q13.1), leading to *CIC-DUX4* fusions [38, 39, 41–43], while smaller numbers harbor *CIC-FOXO4* fusions [42, 44]. CIC-rearranged sarcomas have transcriptional profiles distinct from Ewing sarcoma, suggesting a distinct pathogenesis, [45] and have a preponderance for extremity or truncal soft tissue sites of young adults. Histologically, they are composed of sheets of small to medium-sized, ovoid, rounded to more rarely spindled cells with prominent nucleoli and a moderate amount of pale eosinophilic cytoplasm. Mitotic activity and necrosis may be prominent, and in general, these tumors show greater morphological heterogeneity than does Ewing sarcoma [37]. By immunohistochemistry, roughly 75% of CIC-rearranged sarcomas express CD99, although not generally in the diffuse, membranous pattern seen in Ewing sarcoma [38]. A total of > 95% of *CIC*-rearranged Ewing sarcoma-like sarcomas are positive for WT1 (typically with both nuclear and cytoplasmic

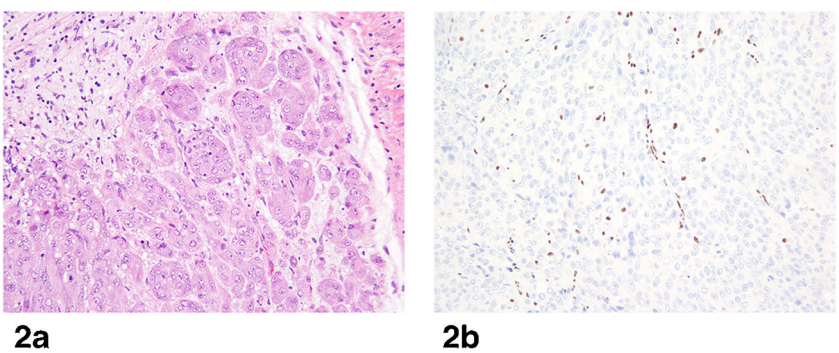

Fig. 2 Although the morphological features of epithelioid malignant peripheral nerve sheath tumor (**a**) overlap significantly with those of malignant melanoma, loss of SMARCB1is seen in many epithelioid malignant peripheral nerve sheath tumors (**b**), but not in melanoma

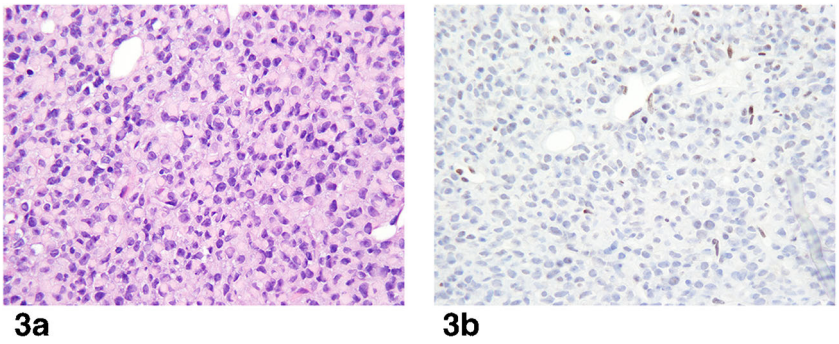

Fig. 3 Malignant rhabdoid tumor, presenting in the back of a middle-aged woman (**a**). Surprisingly, this tumor had retained expression of SMARCB1 (not shown) but exhibited complete loss of SMARCA4 expression (**b**)

expression), using the amino-terminus antibody, [45–49] likely due to transcriptional upregulation of WT1 (Fig. 4a, b) [45]. This contrasts with the absence of WT1 expression in both Ewing sarcoma and *BCOR*-rearranged primitive sarcomas, such that WT1 is helpful in this differential diagnosis [46, 48, 49].

Recently, gene expression profiling studies have identified *ETV4* overexpression in CIC-rearranged tumors, [38, 45] with diffuse nuclear expression of ETV4 seen in > 90% of *CIC*-rearranged sarcomas (Fig. 5a, b). ETV4 expression is not seen in *BCOR*-rearranged sarcomas and is present in only a minority of other round cell sarcomas [46, 49, 50]. Another potentially useful reagent in this differential diagnosis is a monoclonal antibody to the C-terminus of the DUX4 protein, which has been reported to show strong nuclear staining in 5/5 *CIC-DUX4* fusion-positive round cell tumors, with absent expression in all other round cell sarcomas tested (including 20 Ewing sarcomas) [51].

The *BCOR* gene encodes the BCL6 transcriptional repressor [52]. *BCOR*-rearranged sarcomas occur largely in bone or sometimes the deep soft tissues of adolescents or young adults, particularly males [52, 53]. Most cases harbor *BCOR-CCNB3* fusions resulting from inv(X) (p11); other related tumors (which demonstrate similar transcriptional signatures, including high *BCOR* mRNA expression) include those harboring *BCOR-MAML3*, *BCOR* internal tandem duplications, *YWHAE-NUTM2B* and *ZC3H7B-BCOR* [40, 52, 54–57]. These tumors are composed of undifferentiated small round, ovoid, or spindled cells with monomorphic nuclei and fine chromatin, in a highly vascular, myxoid to collagenous stroma [53] In addition to Ewing sarcoma, BCOR-rearranged sarcomas can mimic poorly differentiated or monophasic synovial sarcomas. Immunohistochemically, *BCOR*-rearranged sarcomas typically show some combination of CD99, TLE1 and bcl-2 expression, an immunoprofile that overlaps significantly with Ewing sarcoma, and synovial sarcoma. CyclinD1 and SATB2 are also expressed by most tumors.

CCNB3 protein has recently emerged as a potentially useful marker of *BCOR*-rearranged sarcomas, with > 90% of *BCOR-CCNB3* tumors showing nuclear expression [48, 52, 58, 59]. For tumors lacking CCNB3 rearrangements, strong nuclear expression of BCOR protein has been shown in some studies to be a valuable diagnostic adjunct, present in > 95% of neoplasms harboring *BCOR-CCNB3*, *BCOR-MAML3*, or *BCOR* internal tandem duplications, and in related *YWHAE-NUTM2B* tumors (Fig. 6a, b) [48, 58, 60]. The results of BCOR immunohistochemistry have varied somewhat in different studies, with one large study showing BCOR expression in 100% of *BCOR* sarcomas and only 4% of 412 other tumors, including some solitary fibrous tumors, Ewing sarcomas, synovial sarcomas, small cell osteosarcomas, lymphomas and small cell carcinomas [59], and another demonstrating BCOR expression in 49% of synovial sarcomas, including all poorly differentiated forms [40]. Various molecular techniques can be used to assess for *BCOR* gene rearrangements and internal tandem duplications, although these are unlikely to be available outside of tertiary centers.

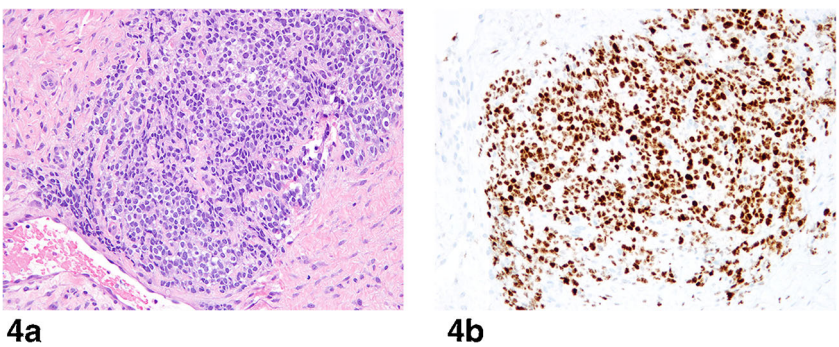

Fig. 4 *CIC*-rearranged sarcoma (**a**), showing diffuse nuclear expression of WT1 protein (**b**). WT1 expression in a round cell tumor should suggest the possibility of CIC-rearranged sarcoma

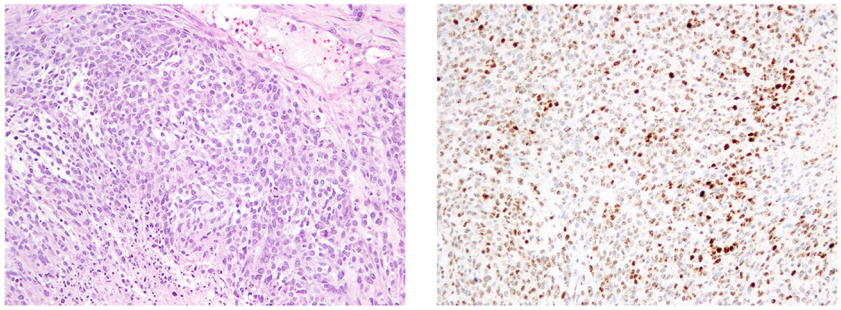

Fig. 5 *CIC*-rearranged sarcoma (**a**), showing strong nuclear expression of ETV4 (**b**)

Epithelioid vascular neoplasms

The morphological and immunohistochemical features of epithelioid hemangioma, epithelioid hemangioendothelioma, pseudomyogenic (epithelioid sarcoma-like) hemangioendothelioma, and epithelioid angiosarcoma overlap to a degree with each other, as well as with those of various non-endothelial, epithelioid neoplasms (e.g., epithelioid sarcoma, carcinoma). Thus, there has been considerable interest in the discovery of novel markers that may assist in this sometimes-difficult differential diagnosis. Recently, the discovery of characteristic, recurrent cytogenetic alterations in these tumors has been translated into useful ancillary molecular genetic and immunohistochemical tests for routine diagnosis.

WWTR1-CAMTA1 and *YAP1-TFE3* gene fusions in epithelioid hemangioendothelioma

Epithelioid hemangioendothelioma (EHE) is a low-grade malignant vascular endothelial neoplasm which can arise at a variety of sites, including the somatic soft tissues, and in lung, liver, and bone, where it is often multifocal. Morphologically, these are composed of cords of epithelioid endothelial cells with pale eosinophilic cytoplasm and often intracytoplasmic vacuoles, within a myxochondroid or hyalinized matrix. EHE express "pan-endothelial" markers, such as CD31, FLI1, and ERG, and are also often keratin-positive, a significant pitfall when the differential diagnosis includes carcinoma. A subset of EHE also shows high nuclear grade and may be difficult to distinguish from conventional angiosarcoma.

EHE is characterized genetically by *WWTR1-CAMTA1* gene fusions, resulting from the reciprocal t (1; 3) (p36; q23-25) [61, 62]. *CAMTA1* encodes a transcription factor expressed normally only in brain tissue [63]. Diffuse nuclear immunohistochemical expression of CAMTA1 using a rabbit polyclonal antibody (Novus Biologicals, Littleton, CO) is highly sensitive and specific for EHE and has been found in > 85% (including both conventional types and those with morphologically high-grade features), while absent in morphologic mimics such as epithelioid angiosarcoma (Fig. 7a, b) [64, 65]. It is important to use this particular polyclonal antibody, as other CAMTA1 antibodies lack specificity [66]. A smaller subset of EHE are associated with *YAP1-TFE3* gene fusions; these typically present in young adults and are morphologically distinct, with a greater propensity for solid growth, cells containing voluminous eosinophilic cytoplasm and in some cases well-formed vascular channels, a feature absent in classical EHE [67]. These neoplasms are immunohistochemically negative for CAMTA1, and instead show diffuse nuclear expression of TFE3 [65]. It should be cautioned, however, that TFE3 immunohistochemistry is not at all specific for *TFE3* rearrangement, and FISH is the preferred test for the demonstration of this molecular genetic event [68].

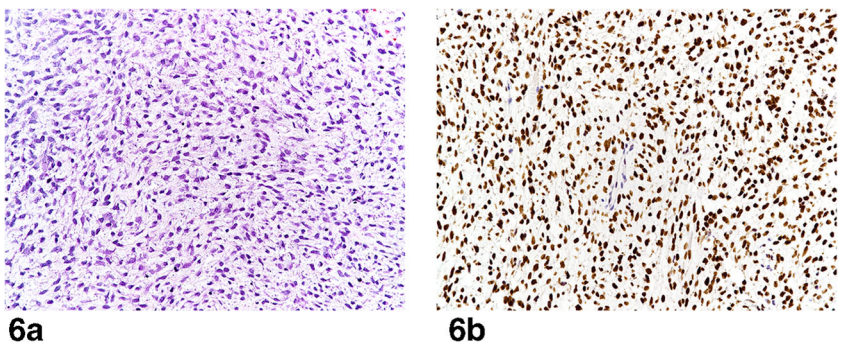

Fig. 6 *BCOR*-rearranged primitive myxoid sarcoma (**a**), strongly positive for BCOR protein by immunohistochemistry (**b**)

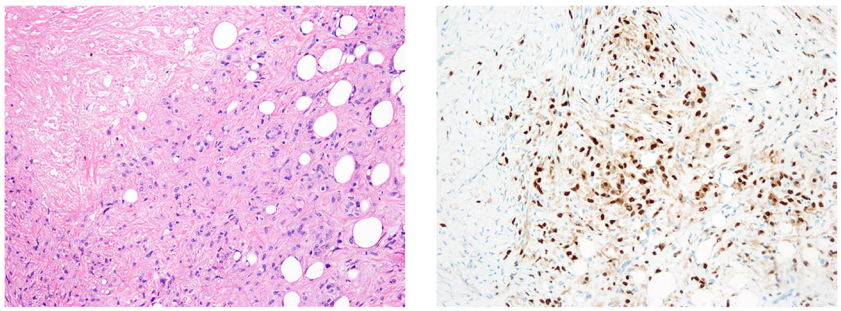

Fig. 7 Epithelioid hemangioendothelioma (**a**), positive for CAMTA1 by immunohistochemistry (**b**). CAMTA1 expression is highly specific for epithelioid hemangioendothelioma, among endothelial tumors

FOSB gene rearrangements in pseudomyogenic (epithelioid sarcoma-like) hemangioendothelioma and epithelioid hemangioma

Pseudomyogenic hemangioendothelioma is a neoplasm of intermediate biologic potential that typically presents in the limbs of young adult males, characteristically occurring in a multicentric distribution in different tissue planes. Histologically, it is composed of loose fascicles of plump spindled and epithelioid cells with eosinophilic cytoplasm and expresses both keratins and endothelial markers, but not CD34 [69, 70]. It can be confused with a variety of neoplasms, including epithelioid sarcoma and various myoid tumors, although it demonstrates retained SMARCB1 expression and generally lacks expression of myogenous markers.

The *FOSB* gene shows recurrent rearrangements in pseudomyogenic hemangioendothelioma and in epithelioid hemangioma, and this gene and its paralogue *FOS* are also recurrently rearranged in osteoblastomas [71]. FOSB is a member of the Fos transcription factor family (which includes FOS, FOSL1, and FOSL2) and takes part in a range wide of biologic processes, including adaption to stress and oncogenesis [72]. *SERPINE1-FOSB* fusions associated with t (7; 19) (q22; q13) are characteristic of pseudomyogenic hemangioendothelioma, [73, 74]; a yet to be fully defined percentage of cases show *FOSB* rearrangements with *ACTB* or *WWTR1* instead [75, 76]. *SERPINE1* is highly expressed in vascular cells; fusion with *FOSB* leads to its overexpression by placing it under transcriptional control of the *SERPINE1* promoter [77]. *FOSB* rearrangements are also present in epithelioid hemangiomas, especially those with atypical histologic features such as solid growth pattern, increased cellularity, nuclear atypia and necrosis, with most cases showing fusion to *ZFP36*, or more rarely to *WWTR1* or an unknown partner [78],. *FOS* rearrangements occur in approximately up to one third of epithelioid hemangiomas, most frequently those in bone or showing solid/cellular histology. These rearrangements do not seem to be a feature of cutaneous "angiolymphoid hyperplasia with eosinophilia" [79].

FOSB immunohistochemistry has been shown to be a useful surrogate marker for the presence of *FOSB* rearrangements in pseudomyogenic hemangioendothelioma and epithelioid hemangiomas [80–82]. Strong and diffuse nuclear expression is present in > 95% of pseudomyogenic hemangioendotheliomas, with weaker expression noted in small numbers of angiosarcomas, EHE, and nodular/proliferative fasciitis (Fig. 8a, b) [82]. FOSB expression has also been shown in 54–100% of epithelioid hemangiomas [83], including cutaneous neoplasms, with absent expression in various benign and malignant mimics (Fig. 9a, b).

Non-Ewing sarcoma tumors with *EWSR1* gene rearrangements

First shown to be rearranged in Ewing sarcoma and thought specific for this neoplasm, it is now established that the *EWSR1* gene can fuse with a broad variety of partners. The *FUS* gene is highly homologous with *EWSR1*, and these can

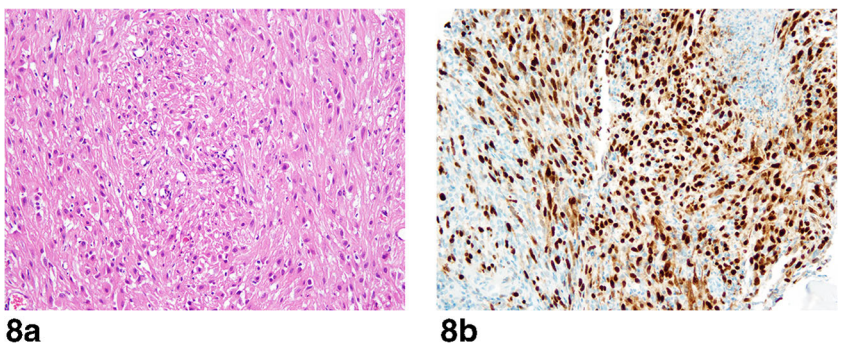

Fig. 8 Pseudomyogenic hemangioendothelioma (**a**), displaying diffuse FOSB expression (**b**), reflecting the underlying *SERPINE1-FOSB* fusions usually seen in these rare lesions

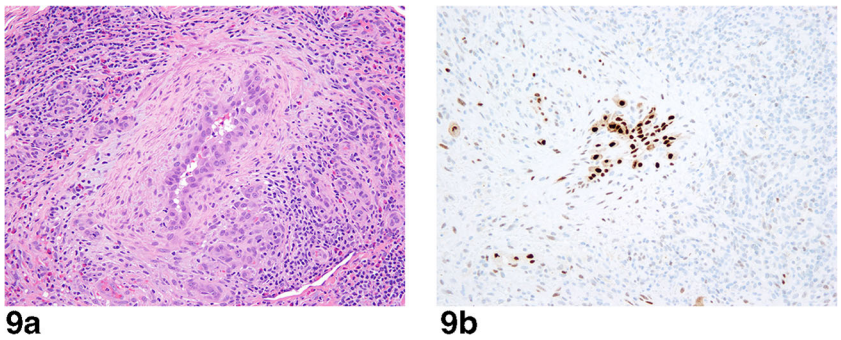

Fig. 9 Epithelioid hemangioma (**a**), demonstrating FOSB expression (**b**)

serve as alternative binding partners in a number of translocation-associated tumors, [84] including angiomatoid fibrous histiocytoma (AFH), myxoid liposarcoma, low-grade fibromyxoid sarcoma, and sclerosing epithelioid fibrosarcoma. It is increasingly evident that most gene fusions are rarely specific for a given tumor type, and that the same fusion can generate neoplasms that are clinically and pathologically diverse; identical gene fusions might lead to the formation of phenotypically different neoplasms due to specific influences of different anatomic locations on similar progenitor cells [85], or due to occurrence within different progenitor cells that activate specific sets of transcription factors. This is illustrated by the finding of *EWSR1-ATF1* and/or *EWSR1-CREB1* gene fusions in AFH [86–89], clear cell sarcoma (of tendons and aponeuroses) (CCS), malignant gastrointestinal neuroectodermal tumor (MGNET) [51, 90–92], primary pulmonary myxoid sarcoma [93], and hyalinizing clear cell carcinoma (HCCC) of salivary gland [94]. *EWSR1-ATF1* fusion has also been reported in a soft tissue myoepithelial tumor [95], and an angiosarcoma of the parotid gland [96]. *EWSR1* has a propensity for fusing with genes encoding members of the CREB family of transcription factors. The *ATF1* gene encodes the cyclic AMP-dependent transcription factor ATF1, a member of the cyclic-AMP response element binding protein (CREB)-ATF transcription factor family, which bind to cAMP-inducible promoters. CREB proteins are related functionally to ATF, and *CREB1* is an alternative gene to *ATF1* in AFH and CCS [89].

More recently, a novel group of myxoid mesenchymal neoplasms has been described [97] that predominantly arises at intracranial sites in children or young adults and are associated with either *EWSR1-CREM* or *EWSR1-CREB1* fusions [97, 98]. These tumors are lobulated and typically composed of cords or reticular arrangements of uniform ovoid cells within prominent myxoid/microcystic stroma, with rare mitotic figures [98], and often "sunburst" amianthoid fibers. These tumors show variable expression of desmin, epithelial membrane antigen, GLUT1 and CD99. *EWSR1-CREM* fusions

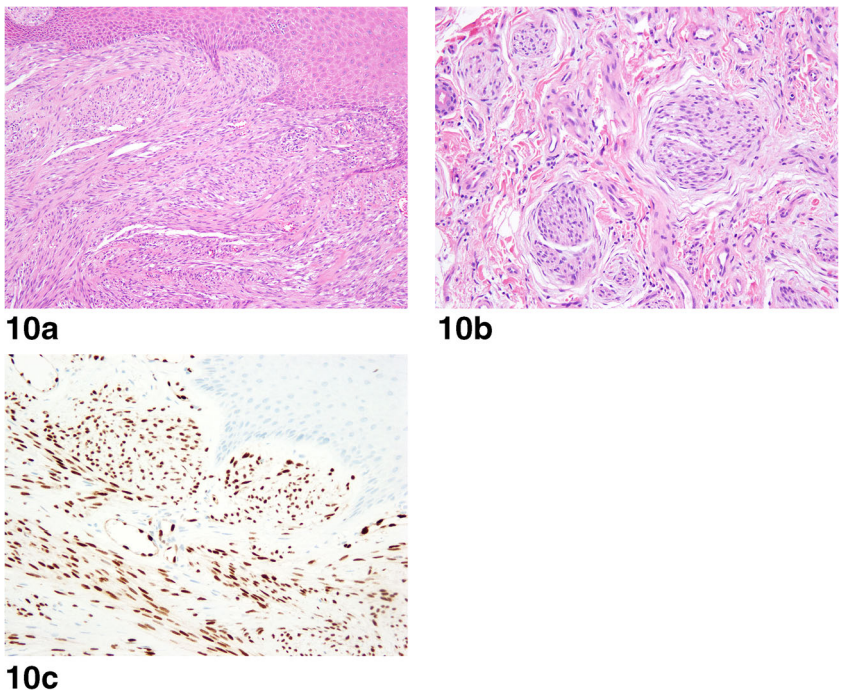

Fig. 10 *EWSR1-SMAD3* fibroblastic tumor, presenting in the foot of a young woman (**a**). Higher power view of this same tumor, showing an infiltrative proliferation of compact fascicles of cytologically uniform spindled cells (**b**). Diffuse expression of ERG protein is characteristic of *EWSR1-SMAD3* tumors, for unknown reasons, and may be a valuable diagnostic clue (**c**)

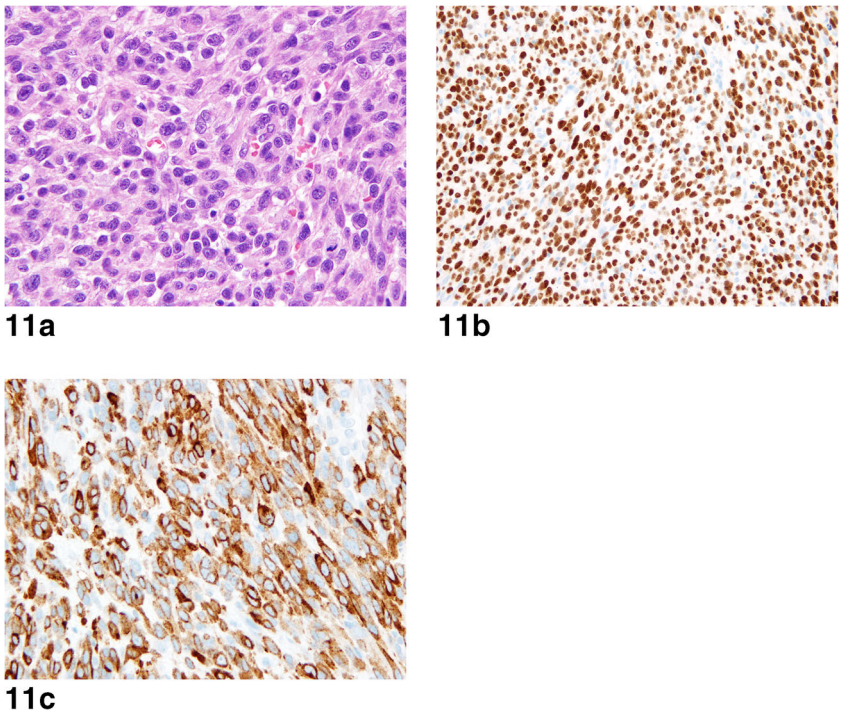

Fig. 11 *FUS-TFCP2* fusion-positive epithelioid rhabdomyosarcoma of the mandible (**a**), strongly positive for MyoD1 (**b**), and ALK protein (**c**). ALK expression is commonly seen in these very rare, recently described rhabdomyosarcomas of bone

are also found in some clear cell carcinomas in the tongue, lung, and nasopharynx with the morphology of HCCC, and *CREM* likely serves as an alternative gene to *CREB1* and *ATF* in partnering with *EWSR1* [99].

EWSR1-NFATC2 sarcomas present as primary bone or soft tissue tumors, with a predilection for the long bones of adult males, and have potential for local and distant recurrence, with *FUS-NFATC2* tumors reported exclusively within the long bones [100]. Their histology is variable, from round cells in a trabecular or pseudoacinar pattern in a myxoid or collagenous matrix, to tumors of short spindle cells with nuclear pleomorphism. There is variable expression of CD99, with frequent keratin positivity, and consistent high-level amplification of the *EWSR1-NFATC2* fusion gene. These tumors have different gene expression profile patterns from Ewing sarcoma, share a distinct DNA methylation signature and harbor characteristic copy number alterations, and are resistant to Ewing sarcoma-specific chemotherapy, such that these should be considered separately from Ewing sarcomas [101–103].

EWSR1-SMAD3 fusions have recently been reported in a small number of clinicopathologically distinctive fibroblastic/myofibroblastic neoplasms [104, 105]. These are typically small tumors which occur in superficial soft tissue, predominantly in acral sites or the lower limb, with a wide age range (but predominantly in adult women). These neoplasms can recur locally, although metastases have not been as yet reported. Histologically, tumors often display a nodular growth pattern with zonation, with peripheral hypercellular areas of bland, fibroblastic-like spindle cells in short fascicles, and hypocellular central areas of hyalinization, small calcifications, and sometimes infarction. For unknown reasons, these unusual tumors are characterized by strong, diffuse nuclear expression of ERG protein, but are negative for smooth muscle actin, S100 protein, CD31 and CD34 (Fig. 10a–c) [104, 105].

Recently, *EWSR1/FUS–TFCP2* fusions have been identified in an aggressive group of primary intraosseous rhabdomyosarcomas predominantly occurring in young adults at

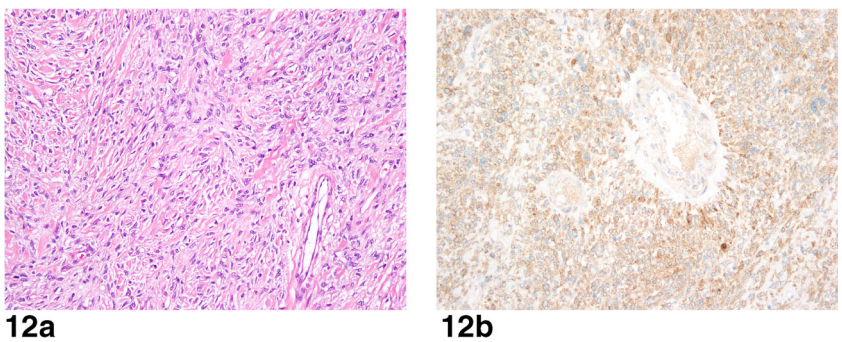

Fig. 12 *NTRK*-rearranged spindle cell tumor of the small intestine in a child (**a**), positive for TRK protein by immunohistochemistry (**b**)

multiple osseous sites including the pelvis, femur, chest wall, maxilla, skull, and sphenoid bone [100, 106, 107]. These distinctive rhabdomyosarcomas are composed of sheets and short fascicles of epithelioid cells with monotonous rounded nuclei and prominent nucleoli, with variably fibrous stroma and focal sclerosis, or may display hybrid spindled and epithelioid features, with moderate amounts of eosinophilic cytoplasm and mild atypia [100, 106]. Rarer cases with *MEIS1-NCOA2* fusions have more primitive, fascicular spindle cell features [106]. As would be expected, these novel rhabdomyosarcoma subtypes express desmin, MyoD1, and myogenin. Cases with *TFCP2* fusions can also express epithelial markers, ALK and TERT, the latter representing potential therapeutic targets (Fig. 11a–c) [100, 106]. By expression profiling, these rare tumors do not cluster with other rhabdomyosarcomas, or with other neoplasms having *EWSR1/FUS* fusions [100].

Mesenchymal tumors with *NTRK* fusions

NTRK1, NTRK2, and NTRK3 are neurotrophic receptor tyrosine kinase genes that, respectively, encode the tropomyosin receptor kinases, TRKA, TRKB, and TRKC. Identification of *NTRK* gene rearrangements is of clinical importance, as the development of selective NTRK inhibitors has enabled the potential for targeted therapy of neoplasms with *NTRK* rearrangements [108]. This group encompasses infantile fibrosarcoma with *ETV6-NTRK3* fusion (also found in mesoblastic nephroma, secretory carcinoma of breast and salivary glands, and leukemias, and, much more rarely, small numbers of inflammatory myofibroblastic tumors, gastrointestinal stromal tumors, and Ewing sarcoma), and various other neoplasms typically comprising fibroblastic-appearing spindle cells with a spectrum of histologic appearances and clinical behaviors. Members of this family of tumors include the recently described "lipofibromatosis-like neural tumor", which arises in the subcutis in various locations, shows *NTRK1* gene rearrangements variably fused to *ETV6*, *TFG*, or *TPM4*, and retains the NTRK3 kinase domain [108]. These lesions show variable histologic features ranging from low- to intermediate-grade morphology with a patternless proliferation of monomorphic spindle cells with stromal bands and perivascular hyalinized collagen, to those with high-grade fascicular spindle cell sarcoma morphology, somewhat resembling fibrosarcoma or MPNST [108]. Tumors with *LMNA-NTRK1* or *TPM3-NTRK1* fusions and various other *NTRK*-rearranged sarcomas have been reported at various locations (including in soft tissue, bone, and viscera) and show a variety of histologic patterns. Despite their variable morphology, *NTRK*-rearranged sarcomas typically show patchy positivity for CD34 and S100 protein, without SOX10 expression. Immunohistochemistry using a pan-TRK antibody has shown diffuse expression of pan-TRK to be highly sensitive, although not entirely specific, for neoplasms with *NTRK* rearrangements (Fig. 12a, b) [109–111]. Variant staining patterns are seen in tumors with *NTRK1* and *NTRK2* fusions and those with fusions of *NTRK3* [110]. As small molecule inhibitors of TRK activity such as larotrectinib have shown promising efficacy in the treatment of patients with *NTRK*-rearranged neoplasms [112], diffuse pan-TRK expression by immunohistochemistry may represent a potential means of rapid selection of patients amenable to TRK-targeted therapy, although data is very limited.

Conclusions

The spectrum of diagnostic immunohistochemical markers directed at protein surrogates of recurrent molecular genetic aberrations in soft tissue tumors continues to expand and has already had significant diagnostic impact. Immunohistochemistry for these "molecular surrogates" may also serve to potentially guide targeted therapy. In selected cases, immunohistochemistry may even be able to replace molecular diagnostic confirmation of certain genetic events, although as with any technique, perfect sensitivity and specificity are lacking, and these tests must be interpreted in the context of all other available clinical, morphologic, and ancillary information.

Authors' contributions Dr Folpe contributed to the writing of manuscript and the production of the figures. Dr. Thway contributed to the writing of the manuscript.

Compliance with ethical standards

Drs. Folpe and Thway attest that this manuscript was produced in accordance with the ethical standards of the institutional research committees of Mayo Clinic and The Royal Marsden NHS Trust and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Conflict of interest The authors declare that they have no conflict of interest.

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