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TLE1 expression is not specific for synovial sarcoma: a whole section study of 163 soft tissue and bone neoplasms

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TLE1, a transcriptional repressor essential in hematopoiesis, neuronal differentiation and terminal epithelial differentiation, has recently been shown in a single tissue microarray study to be a highly sensitive and relatively specific marker of synovial sarcomas. Expression of TLE1 has not, however, been studied in standard sections of soft tissue and bone tumors. We investigated TLE1 expression in a large series of well-characterized mesenchymal tumors, to more fully characterize the range of TLE1 expression. Standard sections of 163 bone and soft tissue tumors were immunostained for TLE1 (sc-9121, 1:100; Santa Cruz Biochemicals) using the Dako Dual Envision+ detection system. Nuclear positivity was scored as negative (<5% of cell positive), 1+ (5–25% of cells positive), 2+ (25–50% of cells positive), and 3+ (>50% of cells positive). Overall, TLE1 was expressed by 18 of 20 (90%) of synovial sarcoma, with 16 cases (89%) showing 2–3+ positivity. However, TLE1 expression was also seen in 53 of 143 (37%) non-synovial sarcoma, with 36 such cases (25%) showing 2–3+ positivity. TLE1 expression was commonly seen in peripheral nerve sheath tumors, including 33% of neurofibromas, 100% of schwannomas, and 30% of malignant peripheral nerve sheath tumors. Among non-neoplastic tissues, nuclear TLE1 expression was variably present in basal keratinocytes, adipocytes, perineurial cells, endothelial cells and mesothelial cells. Our study confirms the excellent sensitivity of TLE1 for synovial sarcoma. However, TLE1 expression is by no means specific for synovial sarcoma, being present in a number of tumors, which enter its differential diagnosis, in particular tumors of peripheral nerve sheath origin. Heterogeneity of TLE1 expression likely explains the differences between the present standard section study and the earlier TMA study. TLE1 may be of value in the differential diagnosis of synovial sarcoma, but should be used only in the context of a panel of antibodies. Morphology, ancillary immunohistochemistry for traditional markers such as cytokeratins and CD34, and molecular confirmation of synovial sarcoma-associated fusion genes should remain the 'gold standards' for this diagnosis.
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Synovial sarcoma is one of the most common sarcomas in adults, accounting for 6–10% of all soft tissue sarcomas.[1,2] Approximately 70% of synovial sarcoma are of the monophasic fibrous subtype and 30% are biphasic, showing both spindle cell and glandular differentiation.[1,2] Poorly differentiated synovial sarcomas, showing a round cell pattern, account for <5% of synovial sarcoma, and may arise from either monophasic or biphasic synovial sarcomas.[3–5] Although the recognition of biphasic synovial sarcoma is typically straightforward, not requiring ancillary immunostains or molecular genetic tests, the differential diagnosis of monophasic and poorly differentiated synovial sarcoma may be more challenging. Although immunohistochemistry for markers such as cytokeratins, S100 protein, CD34, smooth muscle actin and desmin plays a valuable role in this differential diagnosis, there is overlap in the immunophenotypes of these various tumors, and a definitive diagnosis is not always possible with immunohistochemistry alone. Although molecular diagnostic methods such as reverse transcriptase polymerase chain reaction and fluorescent *in situ* hybridization to detect the synovial sarcoma-specific t(X;18)(*SS18-SSX1-2*) are increasingly used for the definitive diagnosis of synovial sarcoma, these techniques are not yet

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available in all laboratories, and require well-preserved genetic material. Thus there has been continued interest in the development of novel immunohistochemical markers for the diagnosis of synovial sarcoma.

Transducin-like enhancer of split 1 (*TLE1*), one of four members of the *TLE* gene family encoding transcriptional corepressors homologous to the *Drosophila groucho* gene, is involved in control of hematopoiesis, neuronal differentiation and terminal epithelial differentiation.[6–8] *TLE1* also plays an important role in the Wnt/β-catenin signaling pathway, where TLE1 protein competes with and displaces β-catenin, producing TLE1-TCT/LEF complexes that repress transcription.[9–11] The Wnt/β-catenin signaling pathway is known to be associated with synovial sarcoma,[12–14] and *TLE1* has been shown in a variety of DNA microarray studies to be consistently expressed in synovial sarcomas.[12,14,15] Most recently, using tissue microarrays, Terry *et al*[16] have shown TLE1 protein expression to be a sensitive and relatively specific marker of synovial sarcoma in formalin-fixed, paraffin-embedded tissues. However, the sensitivity and specificity of TLE1 have not yet been tested in standard, full-sized tissue sections.

Materials and methods

Standard whole sections of 163 formalin-fixed, paraffin-embedded bone and soft tissue tumors were immunostained for TLE1 (sc-9121, 1:100; Santa Cruz Biochemicals, Santa Cruz, CA, USA) with Dako Background Reducing Diluent, pretreatment in the Dako PTLink module with EDTA pH 8.0 for thirty minutes at 97°C, and Dako Dual Envision+ detection with Dako DAB+ chromogen (Dako Corp, Carpinteria, CA, USA). All available histological and immunohistochemical studies were re-reviewed by two of the authors (KK and ALF) and the diagnoses confirmed. Prior confirmation of the presence of the t(X;18) had been performed in 12–15 monophasic or poorly differentiated synovial sarcomas; the remaining cases were morphologically appropriate, contained scattered cytokeratin-positive cells and were CD34-negative. Only malignant peripheral nerve sheath tumors[1] displaying pleomorphism considerably beyond that seen in synovial sarcomas and lacking cytokeratin expression, or[2] arising in patients with known neurofibromatosis type 1, were included in this study. The tumor subtypes are listed in Table 1. TLE1 expression was scored as 'negative' (<5% of cell positive), '1+' (5–25% of cells positive), '2+' (25–50% of cells positive) and '3+' (>50% of cells positive). Only nuclear staining was considered to represent true TLE1 expression. A genetically confirmed monophasic synovial sarcoma with known 3+ TLE1 expression was used as a positive control; negative controls consisted of substitution of buffer for the primary antibody.

Results

The results are summarized in Table 1. Overall, TLE1 was expressed by 18 of 20 (90%) of synovial

Table 1 Immunohistochemical results

Diagnosis	N	*Negative*	*1+*	*2+*	*3+*	*2–3+ (%)*	*Any positive (%)*
Alveolar soft part sarcoma	2	2	0	0	0	0	0
Acral myxoinflammatory fibroblastic sarcoma	1	0	0	1	0	100	100
Chordoma	10	9	1	0	0	0	10
Clear cell sarcoma	1	1	0	0	0	0	0
Desmoplastic small round cell tumor	1	1	0	0	0	0	0
Endometrial stromal sarcoma	3	0	1	0	2	66	100
Epithelioid sarcoma	6	4	0	1	1	33	33
Ewing sarcoma	4	4	0	0	0	0	0
Fibrosarcoma	3	3	0	0	0	0	0
Fibroma	2	2	0	0	0	0	0
Gastrointestinal stromal tumor	6	6	0	0	0	0	0
Leiomyosarcoma	5	4	0	1	0	20	20
Liposarcoma	24	12	5	4	3	29	50
Low-grade fibromyxoid Sarcoma	1	1	0	0	0	0	0
Lipoma	8	5	2	1	0	13	38
Malignant peripheral nerve sheath tumor	10	4	3	0	3	30	30
Myxofibrosarcoma	3	2	1	0	0	0	33
Neurofibroma	9	6	1	2	0	22	33
Parachordoma/myoepithelioma	3	3	0	0	0	0	0
Rhabdomyosarcoma	13	8	0	2	3	39	39
Schwannoma	11	0	2	6	3	82	100
Solitary fibrous tumor	5	3	1	0	1	20	40
Synovial sarcoma	20	2	1	1	16	85	90
Undifferentiated pleomorphic sarcoma	12	10	0	1	1	17	17
Total	163	92	18	20	33	32	43

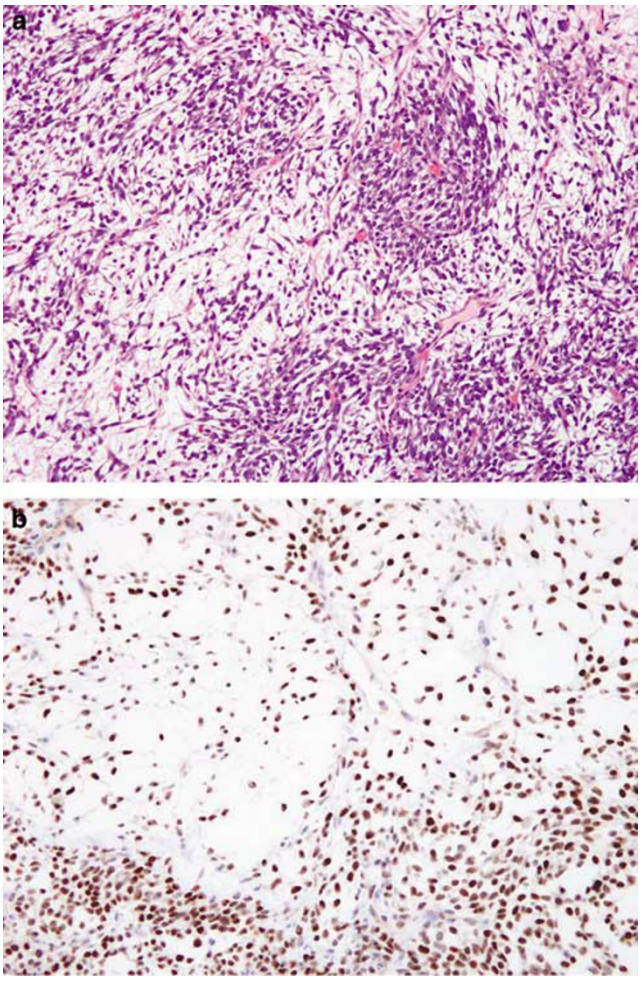

Figure 1 Monophasic synovial sarcoma with myxoid change (**a**), positive for TLE1 expression in both myxoid and non-myxoid areas (**b**).

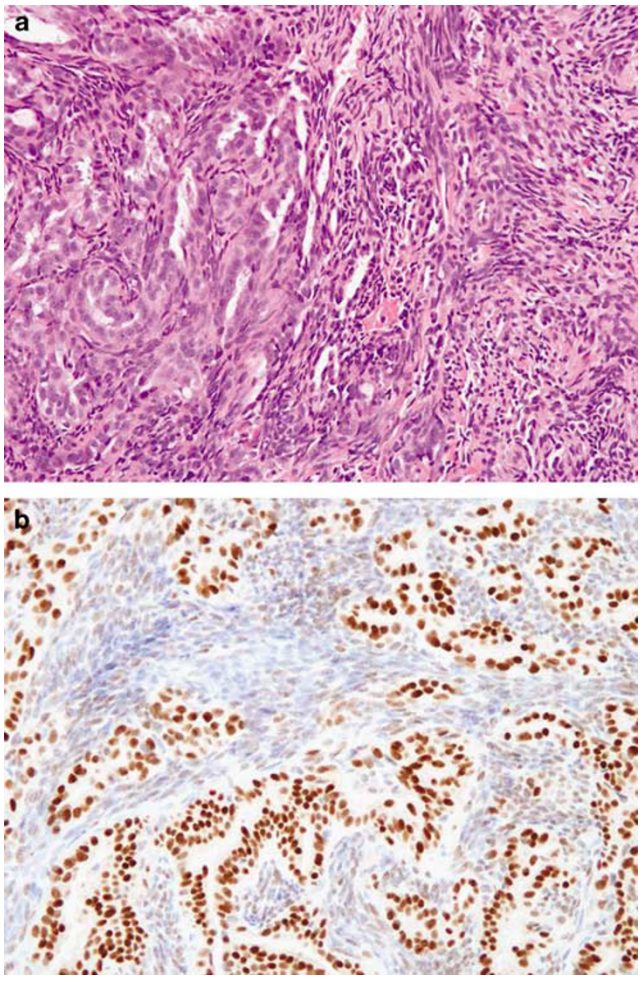

Figure 2 Biphasic synovial sarcoma (**a**) with 3+ TLE1 expression in glandular epithelium (**b**). TLE1 expression is much less frequent in the spindled component of this biphasic tumor.

sarcoma, with 17 cases (85%) showing 2–3+ positivity (Figures 1–3). However, TLE1 expression was also seen in 53 of 143 (37%) non-synovial sarcoma, with 36 such cases (25%) showing 2–3+ positivity. TLE1 expression was commonly seen in peripheral nerve sheath tumors, including 30% of malignant peripheral nerve sheath tumors (Figures 4 and 5), 100% of schwannomas (Figure 6) and 33% of neurofibromas. Figures 7 and 8 illustrate TLE1 expression in other non-synovial sarcomas (alveolar rhabdomyosarcoma and myxoid liposarcoma). In TLE1-positive non-synovial sarcomas expression was often heterogenous, with some fields showing near uniform positivity, and others showing only patchy or even absent positivity. The overall sensitivity and specificity of TLE1 expression for the diagnosis of synovial sarcoma was 85 and 75%, respectively. Among non-neoplastic tissues, nuclear TLE1 expression was occasionally present in basal keratinocytes, adipocytes, perineurial cells, endothelial cells and mesothelial cells.

Discussion

In this study, the first whole section study of TLE1 expression in mesenchymal tumors, we have confirmed and expanded on the previous work of Terry *et al*.[16] In agreement with this earlier tissue microarray study, we have found TLE1 to be a highly sensitive marker of synovial sarcoma, present in close to 90% of cases, typically in >50% of cells. However, we have found TLE1 expression in a somewhat higher percentage of tumors that may enter the differential diagnosis of synovial sarcoma than has previously been reported, with 2–3+ positivity seen in our study in 20% of solitary fibrous tumors/hemangiopericytomas, 82% of schwannomas, 39% of rhabdomyosarcomas, 22% of neurofibromas and 30% of malignant peripheral nerve sheath tumors, as compared with 30, 27, 0, 0 and 5%, respectively, in the study of Terry *et al*. We have also found 2–3+ TLE1 expression in a minority of other mesenchymal tumors (which

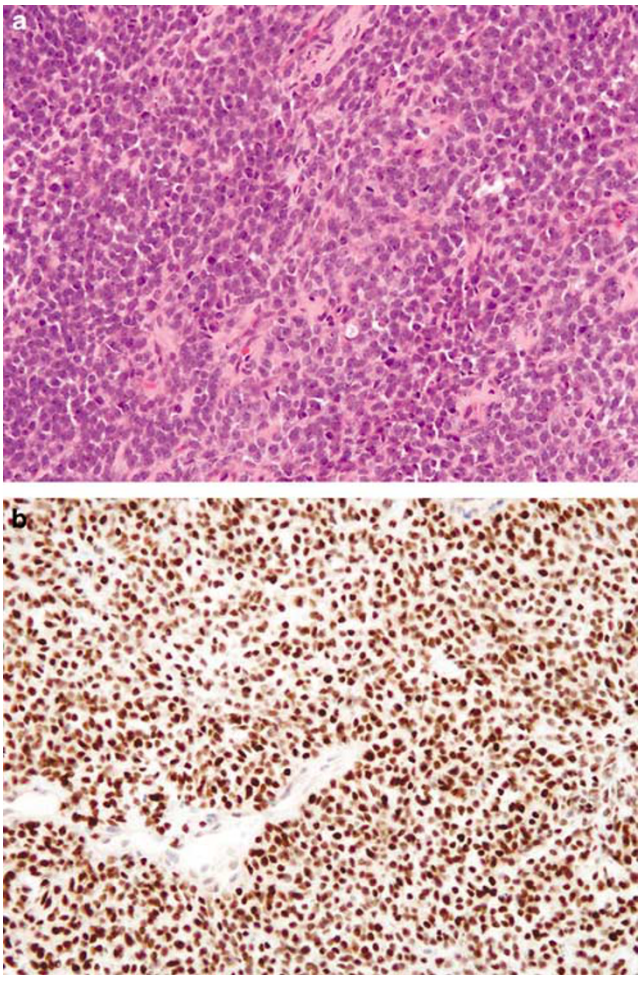

Figure 3 Poorly differentiated synovial sarcoma (**a**) with 3+ TLE1 expression (**b**).

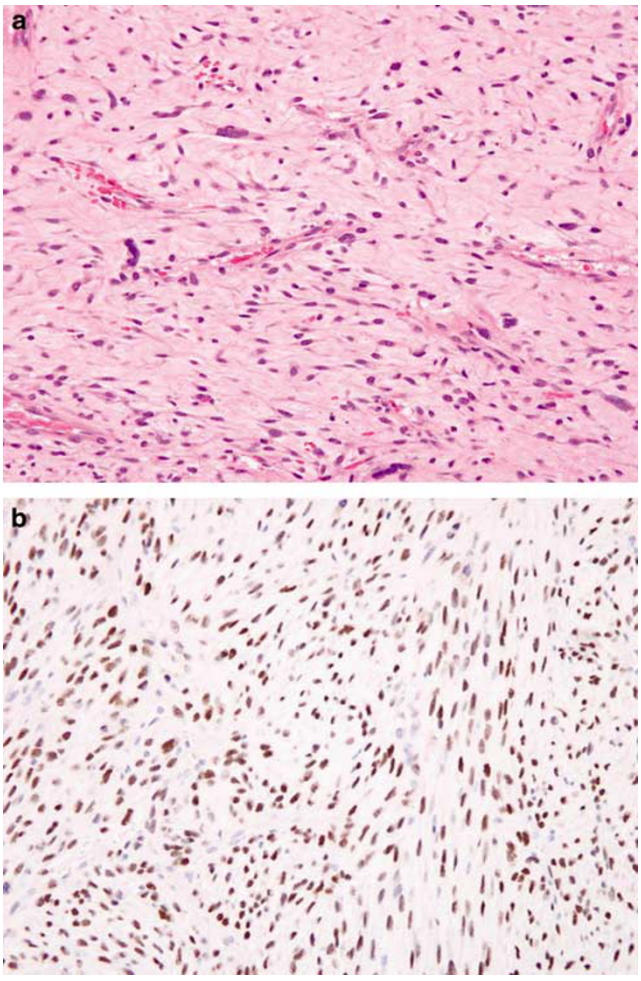

Figure 4 Myxoid and pleomorphic malignant peripheral nerve sheath tumor (**a**) with uniform (3+) expression of TLE 1 (**b**).

would not typically enter the differential diagnosis of synovial sarcoma) including endometrial stromal sarcoma, leiomyosarcoma, lipoma/liposarcoma and undifferentiated pleomorphic sarcoma (so-called 'malignant fibrous histiocytoma'), indicating that TLE1 expression may be more widespread than has been previously recognized.

The differences between our results and those of Terry and co-workers may be explained by our use of whole sections, as opposed to tissue microarrays, inasmuch as TLE1 expression may vary from area to area within a given tumor. It is likely that our use of larger tissue sections resulted in a greater number of '2+' cases, as compared with the study of Terry *et al*, in which such cases might have been scored as '1+' or even negative. Validation of this hypothesis would require whole section study of cases from this earlier study. It is unlikely that the immunohistochemical methods used in this study account for these differences, as our study used the same polyclonal TLE1 antibody as Terry *et al*, at a higher dilution (1:100 *vs* 1:20). It is possible that the Dako Envision+ detection system used in this study is more sensitive than the Ventana system utilized in this earlier study, a hypothesis we are unable to test.

Putting together our results with those of Terry *et al*, it would appear that TLE1 immunohistochemistry should have a somewhat limited role in the diagnosis of monophasic synovial sarcoma, as the differential diagnosis for synovial sarcoma includes a variety of other potentially TLE1-positive spindle cell tumors, most notably malignant peripheral nerve sheath tumor and solitary fibrous tumor. Certainly the finding of strong TLE1 expression in morphologically appropriate, CD34-negative spindle cell tumor with scattered cytokeratin-positive cells is strong evidence in favor of the diagnosis of synovial sarcoma (although in truth one might say the same even without testing such a tumor for TLE1). TLE1 might play a more valuable role in the distinction of poorly differentiated synovial sarcoma from other potentially cytokeratin-positive 'small blue round cell tumors', in particular Ewing sarcoma/primitive neuroectodermal tumor,[17,18] and desmoplastic small round cell tumor,[19] both of which do not appear to express TLE1 protein. Ultimately,

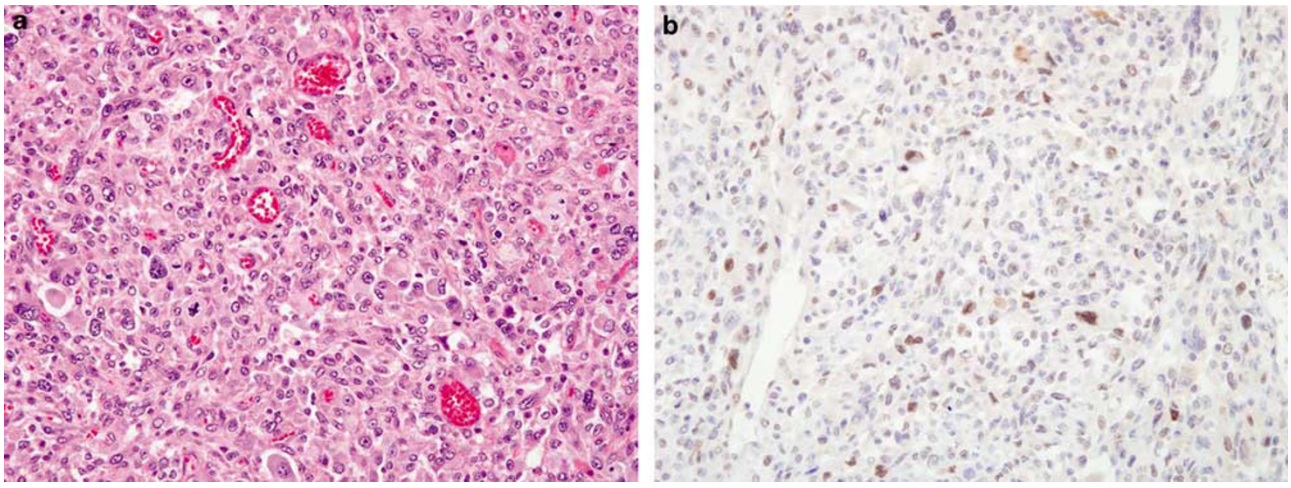

Figure 5 Pleomorphic malignant peripheral nerve sheath tumor (**a**), arising in a patient with neurofibromatosis type 1, with 2+ TLE1 expression (**b**).

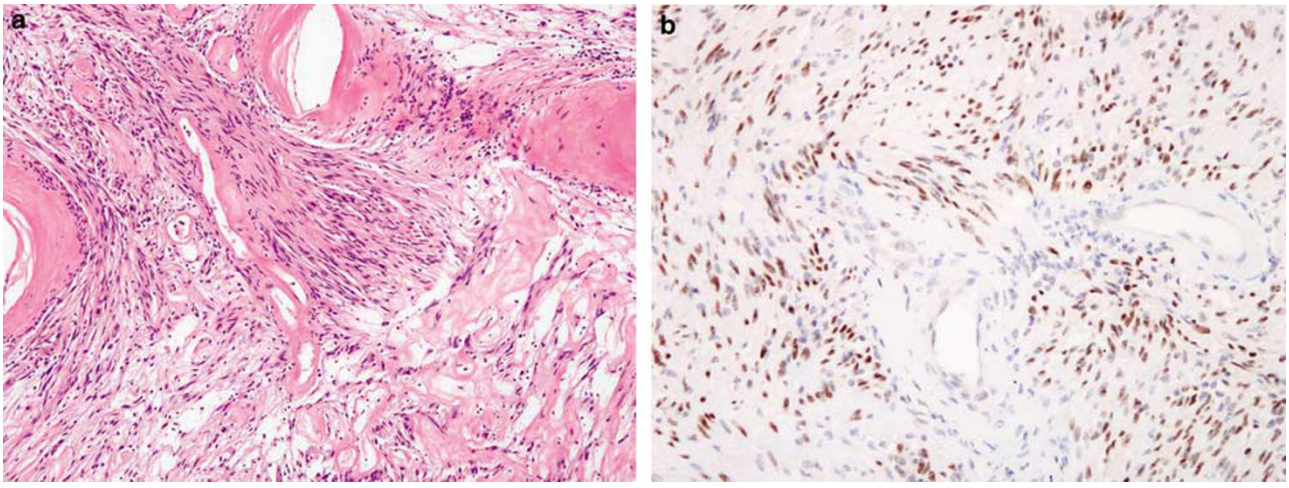

Figure 6 Schwannoma (**a**) with 3+ TLE1 expression (**b**).

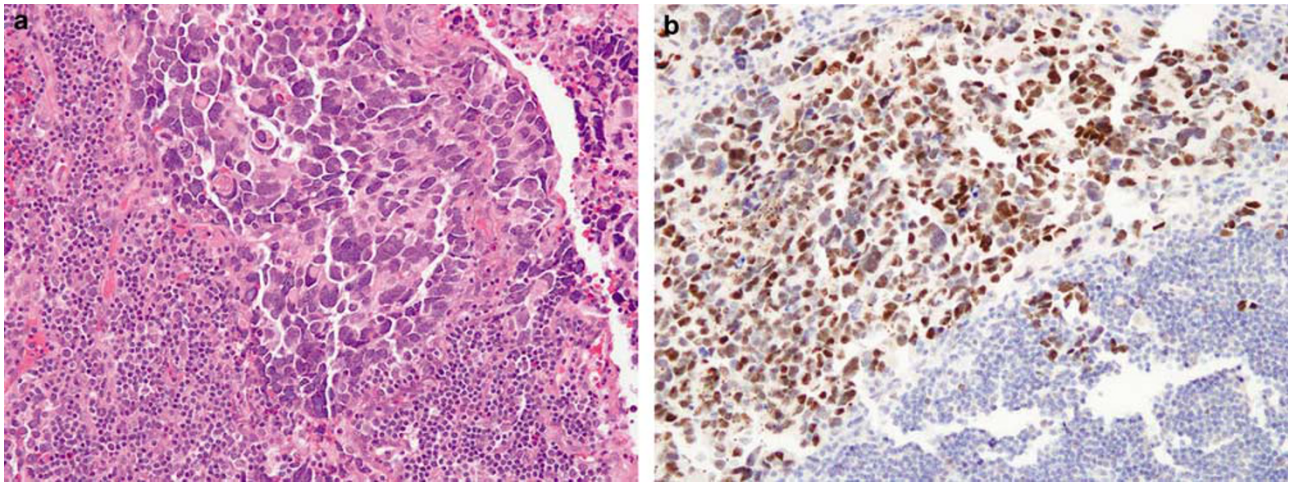

Figure 7 Alveolar rhabdomyosarcoma metastatic to a lymph node (**a**), with 3+ TLE1 expression (**b**).

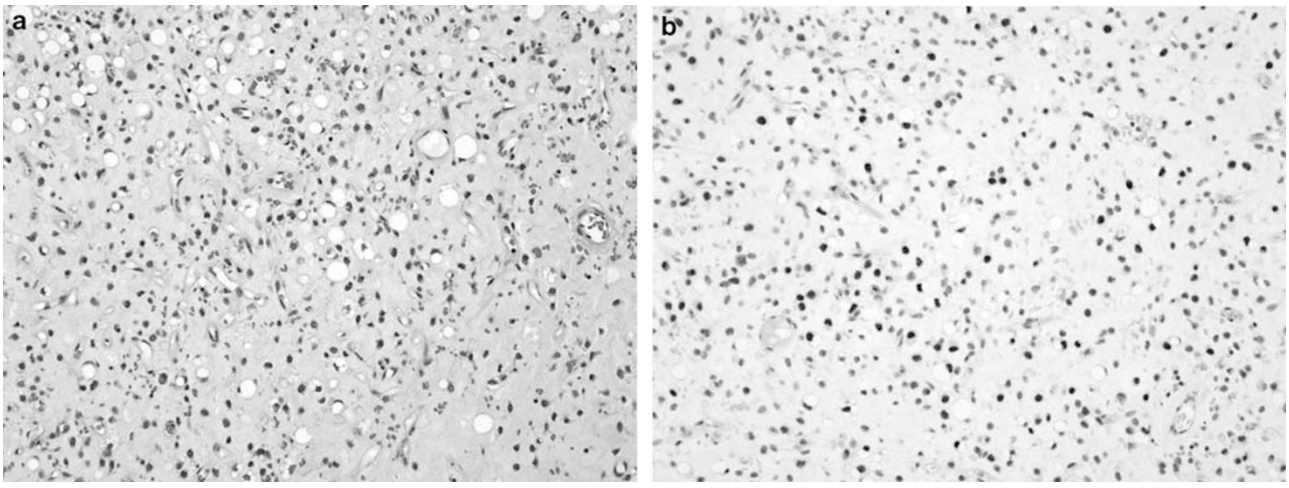

Figure 8 Low-grade myxoid liposarcoma (**a**) with 2+ TLE1 expression (**b**).

however, it is difficult to see any advantage of TLE1 immunohistochemistry over RT–PCR or FISH detection of synovial sarcoma-specific genetic events (eg, t(X;18)(*SS18-SSX1-2*), especially as such tests are available in an increasing number of laboratories, readily performed in formalin-fixed, paraffin-embedded tissues, highly sensitive, and to date absolutely specific for the diagnosis of synovial sarcoma.[20–26]

In summary, using conventional whole tissue sections, we have identified strong TLE1 expression in the overwhelming majority of synovial sarcomas, as well as in a considerable number of other spindle cell tumors that may enter the differential diagnosis of synovial sarcoma. Although TLE1 immunohistochemistry may play a limited role in the diagnosis of synovial sarcoma when used in the context of a panel of morphology and traditional immunohistochemical markers, molecular confirmation of synovial sarcoma-associated fusion genes should remain the 'gold standard' for this diagnosis in problematic cases.

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