

TLE1 as a Diagnostic Immunohistochemical Marker for Synovial Sarcoma Emerging From Gene Expression Profiling Studies

Jefferson Terry, MD,* Tsuyoshi Saito, MD, PhD,† Subbaya Subramanian, PhD,‡
Cindy Ruttan, MSc,* Cristina R. Antonescu, MD,† John R. Goldblum, MD,§
Erinn Downs-Kelly, MD,§ Christopher L. Corless, MD, PhD,|| Brian P. Rubin, MD, PhD,¶
Matt van de Rijn, MD, PhD,‡ Marc Ladanyi, MD,† and Torsten O. Nielsen, MD, PhD*

Abstract: Synovial sarcoma is a soft tissue malignancy defined by the *SYT-SSX* fusion oncogene. Demonstration of the t(X;18) by cytogenetics, fluorescence in situ hybridization or reverse-transcriptase polymerase chain reaction has become the gold standard for diagnosis, but practical considerations limit the availability of these methods. Gene expression profiling studies performed by several independent groups have consistently identified *TLE1* as an excellent discriminator of synovial sarcoma from other sarcomas, including histologically similar tumors such as malignant peripheral nerve sheath tumor. TLE proteins (human homologues of Groucho) are transcriptional corepressors that inhibit Wnt signaling and other cell fate determination signals, and so have an established role in repressing differentiation. We examined the expression of TLE proteins in synovial sarcoma and in a broad range of mesenchymal tumors using tissue microarrays to assess the value of anti-TLE antibodies in the immunohistochemical

confirmation of synovial sarcoma. We demonstrate that TLE expression is a consistent feature of synovial sarcoma using both a well-characterized monoclonal antibody recognizing the TLE family of proteins and a commercially available polyclonal antibody raised against TLE1. Both antibodies gave intense and/or diffuse nuclear staining in 91/94 molecularly confirmed synovial sarcomas. Moderate staining is occasionally seen in schwannoma and solitary fibrous tumor/hemangiopericytoma. In contrast, TLE staining is detected much less frequently and at lower levels, if at all, in 40 other mesenchymal tumors. Our findings establish TLE as a robust immunohistochemical marker for synovial sarcoma, and may have implications for understanding the biology of synovial sarcoma and for developing experimental therapies for this cancer.

Key Words: synovial sarcoma, TLE, immunohistochemistry, microarray, expression profiling

(*Am J Surg Pathol* 2007;31:240–246)

From the *Genetic Pathology Evaluation Centre, British Columbia Cancer Agency, 600 West 10th Avenue, Vancouver, British Columbia, Canada V5Z 4E6; †Department of Pathology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021; ‡Department of Pathology, Stanford University Medical Center, 300 Pasteur Drive, Stanford, CA 94305; §Department of Anatomic Pathology, Cleveland Clinic, 9500 Euclid Avenue, Cleveland, OH 44195; ||Department of Pathology and OHSU Cancer Institute, Oregon Health and Science University, Portland, OR 97239-3098; and ¶Department of Anatomical Pathology, University of Washington Medical Center, Seattle, WA 98195.

Supported by grants from the Terry Fox Foundation (to Torsten O. Nielsen), the National Institute of Health (to Marc Ladanyi), and the US Department of Defense (DoD DAMD17-03-1-0297, to Matt van de Rijn). Torsten O. Nielsen is a scholar of the Michael Smith Foundation for Health Research. Jefferson Terry is a recipient of a Canadian Institutes of Health Research Doctoral Fellowship. T.S. is a recipient of a postdoctoral fellowship from The Uehara Memorial Foundation (Japan). The Genetic Pathology Evaluation Centre is supported by an unrestricted education grant from Sanofi-Aventis.

Reprints: Torsten O. Nielsen, MD, PhD, Anatomical Pathology, Vancouver Coastal Health Research Institute, Room JP1502, 855 West 12th Avenue, Vancouver, British Columbia, Canada, V5Z 1M9 (e-mail: torsten@interchange.ubc.ca).

Reprints also to: Marc Ladanyi, MD, Department of Pathology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021 (e-mail: ladanyim@mskcc.org).

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Synovial sarcoma is a soft tissue malignancy typically occurring in the limbs of young adults, although it may arise at almost any age and anatomic location.^{10,11} Synovial sarcomas can be segregated into 3 histologic subtypes: monophasic (the most common form, composed entirely of spindle cells), biphasic (displaying both spindle cells and glandular-appearing epithelial components), and poorly differentiated synovial sarcoma (sheets of atypical small blue cells). Morphologic variants can also be identified, such as calcifying and fibrous,¹¹ widening the range of appearances and the differential diagnosis. Demonstration in an appropriate histologic context of t(X;18) by cytogenetics, fluorescence in situ hybridization (FISH) or reverse-transcriptase polymerase chain reaction is considered the gold standard for the diagnosis of synovial sarcoma; however, several practical issues, including cost and the need for specialized equipment and personnel have limited the use of such diagnostic tests.^{5,8} Correct diagnosis of synovial sarcoma on the basis of histology alone can be challenging especially in small biopsies, as monophasic synovial sarcomas can appear similar to other spindle cell tumors

[including malignant peripheral nerve sheath tumor (MPNST), fibrosarcoma, and hemangiopericytoma], and poorly differentiated synovial sarcomas can resemble several tumor types including Ewing sarcoma. Immunoreactivity for epithelial markers such as cytokeratin and epithelial membrane antigen (EMA) is frequently used to aid in differentiating synovial sarcoma from other spindle cell neoplasms; however, these markers not only lack specificity,^{12,27} but also are limited in sensitivity because such epithelial markers are only focally expressed in many synovial sarcomas and are completely negative in a subset of monophasic cases.

The t(X;18) translocation most commonly fuses either the *SSX1* or *SSX2* gene on chromosome X to the *SYT* gene on chromosome 18, resulting in the production of an SYT-SSX fusion protein. The function of SYT-SSX has yet to be fully defined, but combines transcriptional activation (SYT) and repression (SSX) domains and likely drives synovial sarcoma development through dysregulation of gene expression.^{10,19} DNA microarray expression profiling, using different platforms, comparison groups, and informatics approaches, has consistently shown a major association of the Wnt signaling pathway with synovial sarcoma.^{1,3,14,20,21,24,29} One prominent gene related to the Wnt pathway is *TLE1*, which has been found to be a good discriminator of synovial sarcoma in multiple studies (Table 1).^{3,20,23,29} *TLE1* is one of 4 *TLE* (Transducin-Like Enhancer of split) genes that encode human transcriptional repressors homologous to the *Drosophila* corepressor *groucho*³⁰; differential overexpression of *TLE2*, 3 and 4 has also been demonstrated in synovial sarcoma.^{1,3,24} *TLE* proteins are temporally expressed in embryogenesis where they are involved in developmental processes including neurogenesis, body patterning, and hematopoiesis.^{6,30,31,34} The repressive effect of Groucho and *TLE1* is dependent on phosphorylation status and involves histone deacetylase (HDAC) activity.^{4,7,16,26,35} The HDAC inhibitor FK228 has recently been shown to inhibit proliferation of synovial sarcoma, supporting the idea that *TLE1* overexpression

may play an important role in synovial sarcoma pathobiology and identifying *TLE1* as a potential therapeutic target.^{17,18}

The specificity of *TLE1* gene expression in synovial sarcoma, particularly when compared with other sarcomas, suggests that *TLE1* may be clinically exploitable as an immunohistochemical marker. Here, we investigate the protein expression of *TLE* in synovial sarcoma and in a broad range of mesenchymal neoplasms using tissue microarrays, to assess the value of *TLE* as a diagnostic marker for this sarcoma.

MATERIALS AND METHODS

Tumor Samples and Tissue Microarrays

Tissue samples were retrieved from the archives of the Vancouver General Hospital (Vancouver, Canada), Stanford Medical Center (Stanford, CA), University of Washington (Seattle, WA), Cleveland Clinic (Cleveland, OH), Oregon Health and Science University (Portland, OR), and Memorial Sloan-Kettering Cancer Center (MSKCC, New York, NY). Slides corresponding to each archival sample were reviewed by at least 2 staff pathologists with subspecialty expertise in bone and soft tissue tumors, and representative areas in each original tissue block identified. The TA-19 synovial sarcoma tissue microarray has been previously described²⁵ and contains 44 molecularly confirmed synovial sarcomas and 29 other sarcomas with related histologies. The MSKCC synovial sarcoma tissue microarrays contained 52 molecularly confirmed synovial sarcomas each represented by one 3 mm and one 1 mm core (on different arrays). The TA-138 tissue microarray contains 44 cases of NF-1-related MPNST, 24 sporadic MPNST, 15 synovial sarcomas, 8 localized neurofibroma, 24 plexiform neurofibroma, 11 diffuse neurofibroma, 7 cellular schwannoma, 15 typical schwannoma, 4 perineurioma, 10 melanoma, 5 clear cell carcinoma, and 5 cases of dermatofibrosarcoma protuberans with fibrosarcomatous change. The sarcoma tissue microarrays TA-34 and TA-35 contain 421 benign and

TABLE 1. Gene Microarray Expression Profiling Studies Identify *TLE* as a Good Discriminator for Synovial Sarcoma From Other Sarcomas

Study	<i>TLE1</i> Rank*	Array Type	Ranking Parameter	SS Cases	Comparison Group
Baird et al ³	5	12600 cDNA spotted	Weighted <i>P</i> value	16	1 ASPS; 1 CCS; 1 CSa; 5 DFSP; 19 EWS; 7 FSa; 5 GIST; 6 HPCT; 17 LMS; 33 LPS; 38 MFH; 2 MMMT; 5 OSa; 6 BS; 3 MPNST; 6 RMS; 10 NOS
Laé et al ²⁰	1	22215 probe set Affymetrix U133A	<i>P</i> value	46	28 EWS; 28 DSRCT; 23 ARMS; 12 ASPS
Ng et al ²²	4	42000 cDNA spotted	<i>P</i> value	13	24 MPNST
Pretto et al ²⁸	2	12626 probe set Affymetrix U95A	<i>P</i> value	5	6 CCS; 5 DDLS; 5 GIST; 8 FSa; 6 LMS; 11 MFH; 3 PLS; 4 RCLS

*Rank of *TLE1* within total gene list when sorted by ability to positively discriminate synovial sarcoma.

ARMS indicates alveolar rhabdomyosarcoma; ASPS, alveolar soft parts sarcoma; BS, benign schwannoma; CCS, clear cell sarcoma; CSa, chondrosarcoma; DDLS, dedifferentiated liposarcoma; DFSP, dermatofibrosarcoma protuberans; DSRCT, desmoplastic small round cell tumor; EWS, Ewing sarcoma; FSa, fibrosarcoma; GIST, gastrointestinal stromal tumor; HPCT, hemangiopericytoma; LMS, leiomyosarcoma; LPS, liposarcoma; MFH, malignant fibrous histiocytoma; MMMT, malignant mixed Mullerian tumor; MPNST, malignant peripheral nerve sheath tumor; NOS, unclassified sarcoma; OSa, osteosarcoma; PLS, pleomorphic liposarcoma; RCLS, round-cell liposarcoma; RMS, rhabdomyosarcoma; SS, synovial sarcoma.

malignant soft tissue tumor specimens representing over 50 diagnostic entities and have been previously described,³³ as has tissue microarray 03-008, which contains 121 cases of chondroid and osseous tumors.²² For each of these, a tissue microarrayer (Beecher Instruments, MD) was used to extract duplicate 0.6 or 1.0 mm cores from representative areas of each original formalin-fixed, paraffin-embedded tissue block and to transfer to the recipient microarray block, except for one of the MSKCC tissue microarrays for which 3 mm cores were obtained using a manual punch instrument. All tissue samples were collected according to protocols approved by the ethics committees at the contributing institutions.

Molecular Confirmation of Synovial Sarcoma Cases

The presence of t(X;18) in the synovial sarcoma cores and absence of t(X;18) in the nonsynovial sarcoma cores with positive TLE staining in the TA-138, 03-008, TA-34, and TA-39 microarrays were verified using a previously described *SYT* breakapart probe FISH method.³² Briefly, a 6- μ m section was deparaffinized followed by demasking. Differentially labeled DNA BAC probes were hybridized to the samples overnight and the nuclei counterstained. The TA-19 synovial sarcoma microarray has been previously assessed in this manner.³² Synovial sarcoma cases in which the presence of t(X;18) could not be verified were removed. Cases on the MSKCC tissue microarrays were individually validated by *SYT-SSX* reverse-transcriptase polymerase chain reaction on frozen or formalin-fixed paraffin-embedded material, as described previously,² and included 35 *SYT-SSX1* cases and 17 *SYT-SSX2* cases.

Immunohistochemistry

Heat-induced epitope retrieval was performed by heating 4 μ m sections of each tissue microarray for 30 to 40 minutes in 10 mM ethylenediaminetetraacetic acid buffer pH 8. Monoclonal rat anti-human pan-TLE antibody, which recognizes the highly conserved WD-40 domain, has been previously characterized³⁰ and was graciously provided by S. Stifani (Montreal Neurological Institute, Montréal, Canada). Polyclonal rabbit anti-TLE1 (M-101), which also cross-reacts to a lesser extent with TLE2, 3 and 4, was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Each microarray section was hybridized with a 1:2 dilution of monoclonal anti-pan-TLE or a 1:20 dilution of M-101 polyclonal anti-TLE1 using a Ventana automated immunostainer (Ventana, AZ) for 30 minutes followed by washing and hybridization with a 1:1000 dilution of HRP-conjugated anti-rat immunoglobulin G antibody (Abcam, Cambridge, UK). M-101 staining was for comparative purposes also performed manually, using a 1:200 dilution and 4°C overnight incubation. Endogenous peroxidase activity was quenched and antibody visualized by incubation with 3,3'-diaminobenzidine for 10 minutes. TLE immunostaining was graded as "3+" (strong) if greater than 50% of tumor cells per core exhibited intense

nuclear staining visible with a 4 \times low power objective lens, "2+" (moderate) if 10% to 50% exhibited intense nuclear staining obvious at low power or greater than 50% nuclear staining well above background when assessed with 10 \times objective magnification, "1+" (weak) if less than 50% of cells exhibited weak to moderate nuclear staining, and "0" (negative) for no visible nuclear staining. Where scores of duplicate cores were discrepant, the higher score was used. Uninterpretable sets of duplicate cores (ie, no tumor cells, absence of viable cells, or folded/lost tissue core) were excluded from analysis. Tumors with a score of 2+ or 3+ on at least 1 examined tissue microarray core were considered positive for TLE. Sections of each microarray were also stained with hematoxylin and eosin (H&E) using standard methods as an additional histologic reference.

Digital Images

Digital images of immunostained and H&E-stained microarrays were acquired using a BLISS imager (Bacus Laboratories, Lombard, IL). A relational database was constructed that correlates scoring and identification information with images of each core. This information is publicly accessible at <https://www.gpecimage.ubc.ca/tma/web/viewer.phpr#>.

RESULTS

TLE as an Immunohistochemical Marker

The consistent identification of strong *TLE* expression in synovial sarcoma, from several gene expression profiling studies in different laboratories using different DNA microarray platforms (Table 1) led us to investigate its value as a diagnostic immunohistochemical marker. Two antibodies against TLE1, a monoclonal antibody recognizing an epitope in the C-terminal WD-40 domain, previously shown to work in immunohistochemical applications,³⁰ and a commercially available polyclonal antibody raised against TLE1, were tested against 693 cases of adult soft tissue tumors including 94 molecularly validated synovial sarcomas using a tissue microarray format.

Both antibodies gave intense, easy-to-interpret nuclear staining in positive cases. Examples of the 4 grades of staining, as described in Materials and Methods section, are presented in Figure 1. Original images of tissue cores are available for public review at <https://www.gpecimage.ubc.ca/tma/web/viewer.phpr#>, and includes H&E, pan-TLE monoclonal and M101 TLE1 polyclonal immunostains on the same (TA-138) tissue microarray for comparative purposes.

The 2 antibodies (monoclonal anti-pan-TLE and M101 polyclonal anti-TLE1) were tested on sequential sections of the TA-138 tissue microarray and found to be almost equivalent (8 discrepancies among 177 cases, Kappa statistic 0.78, $P < 10^{-25}$). Overall, the intensity of optimized staining with M101 was slightly less than with the monoclonal anti-pan-TLE; all discrepancies in scoring (2 MPNST, 2 solitary fibrous tumor, 1 schwannoma,

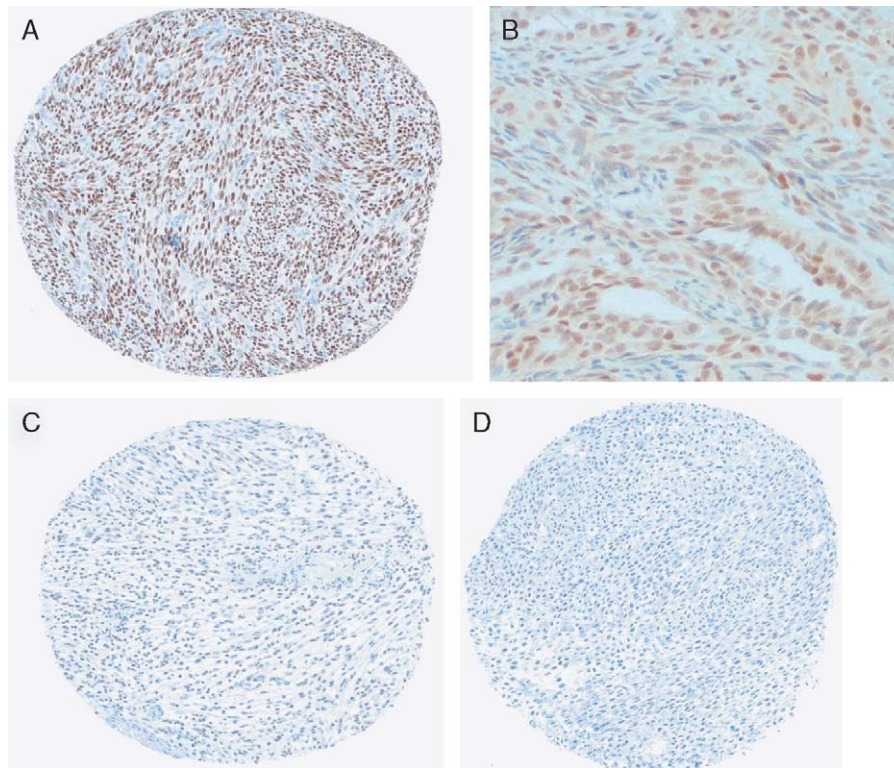


FIGURE 1. TLE immunostaining in representative 0.6 mm cores using pan-TLE antibody. A, 3+ staining in monophasic synovial sarcoma. B, higher power view of 2+ staining in a biphasic synovial sarcoma. C, 1+ staining in MPNST. D, negative (score=0) staining in MPNST.

and 3 synovial sarcomas) between the 2 antibodies were cases scored positive by pan-TLE and negative by M101. For rigour, tissue microarrays assessing antibody specificity among various sarcomas were assessed with the more sensitive but less specific monoclonal anti-pan-TLE, and the MSKCC arrays, which only contained synovial sarcoma cases, were assessed with the less sensitive M101. TLE staining results for each tumor type are summarized in Table 2.

Of the 35 bone and soft tissue tumors types with at least 5 cases included in this study, synovial sarcomas were the only type displaying a high proportion of positive TLE staining, with 91/94 (97%) cases exhibiting intense and/or diffuse nuclear staining (ie, 2+ or 3+; Table 2). All cases of biphasic synovial sarcoma exhibited staining in both the epithelial and spindle cell components (Fig. 1B), with the epithelial component showing equivalent or stronger intensity. Four cases had the histology of poorly differentiated synovial sarcoma, and all of these were positive for TLE (3 cases scored as 3+, 1 as 2+). For the synovial sarcoma cases with known *SSX* subtype, all 25 *SYT-SSX1* and all 17 *SYT-SSX2* were positive for TLE. TLE staining was weak (ie, 1+) in only 3/94 synovial sarcomas, whereas none of the synovial sarcomas had absent (score = 0) staining. TLE staining in a core biopsy of a synovial sarcoma is demonstrated in Figure 2.

In contrast to synovial sarcoma, TLE staining was low to absent in other spindle cell tumors, including those in the differential diagnosis of synovial sarcoma. Solitary fibrous tumor/hemangiopericytomas (6/20) and schwannomas (6/22) were occasionally positive, whereas fibroxanthoma (1/4), clear cell sarcoma (1/7), carcinosarcoma (1/7), high-grade chondrosarcoma (1/8), Ewing sarcoma (1/13 cases), MPNST (4/88), gastrointestinal stromal tumor (1/34), and leiomyosarcoma (1/41) were rarely positive for TLE (Table 2). The remaining tumors in this study, including malignant fibrous histiocytoma (synonymously termed pleomorphic undifferentiated sarcoma), fibrosarcoma, and dermatofibrosarcoma protuberans with fibrosarcomatous change, were negative for TLE staining in all examined cases.

DISCUSSION

Distinguishing synovial sarcoma from other spindle cell tumors can present a diagnostic challenge, particularly in those cases that do not exhibit biphasic histology. In these situations, immunohistochemical markers can be valuable in confirming the diagnosis of synovial sarcoma. Many attempts have been made to identify immunomarkers that have high positive predictive value for synovial sarcoma, but to date there have been no markers identified which are both consistently specific and sensitive for this tumor.^{12,15,27} Keratin and/or EMA

TABLE 2. Summary of TLE Immunohistochemistry Results

Tumor Type	n	3+	2+	1+	0	Total Positive	% Positive
Synovial sarcoma	94	70	21	3	0	91	97
Hemangiopericytoma	5	1	1	1	2	2	40
Schwannoma, regular	16	0	5	7	4	5	31
Solitary fibrous tumor	15	0	4	3	8	4	27
Fibroxanthoma	4	0	1	0	3	1	25
Schwannoma, cellular	6	0	1	4	1	1	17
Carcinosarcoma	7	0	1	2	4	1	14
Clear cell sarcoma	7	1	0	1	5	1	14
Chondrosarcoma, high grade	8	0	1	0	7	1	13
Ewing sarcoma	13	1	0	1	11	1	8
GIST	35	0	2	7	26	2	6
MPNST	88	1	3	12	72	4	5
Leiomyosarcoma	41	1	0	4	36	1	2
Angiosarcoma	13	0	0	0	13	0	0
Breast carcinoma	2	0	0	0	2	0	0
Chondroblastoma	4	0	0	1	3	0	0
Chondromyxoid fibroma	2	0	0	0	2	0	0
Chondrosarcoma, myxoid	7	0	0	2	5	0	0
Chondrosarcoma, low grade	23	0	0	0	23	0	0
Chondrosarcoma, mesenchymal	2	0	0	0	2	0	0
DFSP*	17	0	0	1	16	0	0
DSRCT	3	0	0	0	3	0	0
Enchondroma	4	0	0	0	4	0	0
Endometrial stromal sarcoma	11	0	0	1	10	0	0
Epithelioid sarcoma	2	0	0	0	2	0	0
Fibromatosis	17	0	0	0	17	0	0
Fibrosarcoma	3	0	0	0	3	0	0
Giant cell tumor, tenosynovial	9	0	0	0	9	0	0
Glomous tumor	7	0	0	1	6	0	0
Granular cell tumor	8	0	0	0	8	0	0
Vascular tumor†	9	0	0	0	9	0	0
Inflammatory myofibroblastic tumor	3	0	0	0	3	0	0
Kaposi sarcoma	2	0	0	0	2	0	0
Leiomyoma	8	0	0	0	8	0	0
Lipoblastomatosis	2	0	0	0	2	0	0
Liposarcoma, dedifferentiated	14	0	0	1	13	0	0
Liposarcoma, myxoid	4	0	0	1	3	0	0
Liposarcoma, pleomorphic	2	0	0	0	2	0	0
Liposarcoma, well differentiated	11	0	0	1	10	0	0
MFH	56	0	0	5	51	0	0
Melanoma	11	0	0	4	7	0	0
Myxofibrosarcoma, low grade	5	0	0	0	5	0	0
Myxoma	8	0	0	0	8	0	0
Myxosarcoma	4	0	0	1	3	0	0
Neurofibroma, diffuse	12	0	0	4	8	0	0
Neurofibroma, localized	8	0	0	1	7	0	0
Neurofibroma, plexiform	24	0	0	5	19	0	0
Osteosarcoma	16	0	0	1	15	0	0
Perineurioma	4	0	0	0	4	0	0
Rhabdomyosarcoma	13	0	0	2	11	0	0
Sarcoma, NOS	7	0	0	1	6	0	0

*Eight of the DFSP cases contained fibrosarcomatous change.

†Includes 2 cases of epithelioid hemangioendothelioma, 4 capillary hemangiomas, and 3 intramuscular hemangiomas.

DFSP indicates dermatofibrosarcoma protuberans; DSRCT, desmoplastic small round cell tumor; GIST, gastrointestinal stromal tumor; MFH, malignant fibrous histiocytoma; MPNST, malignant peripheral nerve sheath tumor; NOS, not otherwise specified.

immunostains, which are commonly used, are sensitive markers for synovial sarcoma; however, their expression is not specific as many tumors, including MPNST, also express epithelial antigens.^{8,13,27}

Gene expression studies in synovial sarcoma have repeatedly shown overexpression of members of the *TLE* family of genes, particularly *TLE1*, in synovial sarcoma.

Analysis of this gene expression data has identified *TLE1* as one of the best discriminators for synovial sarcoma when compared with histiologically and/or biologically similar sarcomas such as MPNST or Ewing sarcoma. These data, from multiple groups using different expression profiling platforms, suggest that TLE may be a valuable diagnostic marker for synovial sarcoma.



FIGURE 2. Monoclonal antipan-TLE immunostaining of a core needle biopsy, taken from a 6 cm wrist mass in a 49-year-old male. H&E showed a nonpleomorphic spindle cell sarcoma, which was negative for pankeratin, CK7, and EMA by routine diagnostic immunohistochemistry protocols. Subsequent diagnostic FISH assay was positive for a split at the SYT locus, confirming synovial sarcoma 9 days after the TLE immunostaining result. Main image 1.25 ×, inset 40 ×. Full biopsy slide available for viewing at <https://www.gpecimage.ubc.ca/tma/web/viewer.php#r#>.

This study demonstrates that immunohistochemical detection of TLE is not only very highly sensitive for synovial sarcoma, but also specific in the context of other mesenchymal neoplasms. Results are consistent with either a monoclonal antibody recognizing pan-TLE or a commercially available polyclonal antiserum that recognizes TLE1 (and cross-reacts to a lesser extent with TLE2, 3 and 4). We defined positive staining for TLE as moderate to high level staining as described in the Materials and Methods section. Even using stringent criteria, most of the synovial sarcomas (97%) were positive. Other tumors commonly mistaken for synovial sarcoma exhibited lower levels of positive staining for pan-TLE, including schwannomas (27%), Ewing sarcomas (8%), MPNST (5%), and malignant fibrous histiocytoma (0%). Notably, TLE expression distinguishes synovial sarcoma from MPNST, a particularly problematic entity in the differential diagnosis, with a high degree of specificity. The majority of the remaining tumor types exhibit low to absent levels of TLE immunostaining, suggesting that TLE is useful in differentiating synovial sarcoma from these tumors. Since a high proportion of cells within most of the synovial sarcoma tissue microarray cores exhibited TLE staining (ie, 2+ or 3+), high sensitivity on small tissue samples, such as core biopsies, would be seen (Fig. 2). Using synovial sarcoma tissue microarrays, we have now assessed over 35 established and novel immunohistochemical markers of synovial sarcoma,²⁵ none of which perform as well as TLE.

The prominence of *TLE1* and other components of the Wnt/ β -catenin signaling pathway in synovial sarcoma gene expression studies suggest that they may well play an important role in the development of this tumor. The observation that high levels of nuclear-localized β -catenin are a feature of synovial sarcoma,²² and recent experimental data showing that *SYT-SSX* expression leads to

accumulation of β -catenin in a nuclear complex that includes SYT-SSX, provides evidence that downstream components of this pathway that mediate its effects on gene transcription are probably important in the pathogenesis of synovial sarcoma.²⁸ The present study demonstrates protein-level correlation of *TLE1* mRNA overexpression in synovial sarcoma. TLE1 competes with activated β -catenin for TCF/LEF transcription factors in the nucleus; TLE1 binding displaces β -catenin to form transcriptionally repressive TLE1-TCF/LEF complexes.^{4,9} β -catenin and SYT-SSX2 interact to potentiate β -catenin-mediated transcription,²⁸ suggesting that TLE1 overexpression may represent a compensatory response to excessive β -catenin signaling or serve to limit transcriptional activation to certain genes. TLE is also known to associate with other transcription factors, such as HES, where it fulfills a similar role as an adaptor molecule within multiprotein complexes involved in transcriptional repression.⁶ In this context, TLE may serve to repress genes involved in differentiation and maintain the relatively undifferentiated histopathologic state seen in synovial sarcoma.

TLE functions to repress transcription via recruitment of HDAC activity.⁶ Of interest, recent data have shown strong growth inhibition of synovial sarcoma models that were treated with clinically applicable HDAC inhibitors.^{17,18} Further functional studies are required to delineate the role of TLE proteins in synovial sarcoma pathogenesis.

In conclusion, TLE1 expression is a consistent and prominent feature of synovial sarcoma, whereas it is low to absent in other tumors in the differential diagnosis. Reproducible immunohistochemical staining of TLE1 with negligible background can be obtained with both monoclonal and commercially available polyclonal anti-TLE1 antibodies. TLE1 is a sensitive and specific immunohistochemical marker for synovial sarcoma,

performing better than other known immunohistochemical markers, and can significantly aid in the pathologic diagnosis of this tumor.

REFERENCES

- 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;161:1587–1595.
- Antonescu CR, Kawai A, Leung DH, et al. Strong association of SYT-SSX fusion type and morphologic epithelial differentiation in synovial sarcoma. *Diagn Mol Pathol.* 2000;9:1–8.
- Baird K, Davis S, Antonescu CR, et al. Gene expression profiling of human sarcomas: insights into sarcoma biology. *Cancer Res.* 2005;65:9226–9235.
- Brantjes H, Roose J, van De Wetering M, et al. All Tcf HMG box transcription factors interact with Groucho-related co-repressors. *Nucleic Acids Res.* 2001;29:1410–1419.
- Chang CC, Shidham VB. Molecular genetics of pediatric soft tissue tumors: clinical application. *J Mol Diagn.* 2003;5:143–154.
- Chen G, Courey AJ. Groucho/TLE family proteins and transcriptional repression. *Gene.* 2000;249:1–16.
- Chen G, Fernandez J, Mische S, et al. A functional interaction between the histone deacetylase Rpd3 and the corepressor groucho in *Drosophila* development. *Genes Dev.* 1999;13:2218–2230.
- Coindre JM, Pelmus M, Hostein I, et al. Should molecular testing be required for diagnosing synovial sarcoma? A prospective study of 204 cases. *Cancer.* 2003;98:2700–2707.
- Daniels DL, Weis WI. Beta-catenin directly displaces Groucho/TLE repressors from Tcf/Lef in Wnt-mediated transcription activation. *Nat Struct Mol Biol.* 2005;12:364–371.
- dos Santos NR, de Bruijn DR, van Kessel AG. Molecular mechanisms underlying human synovial sarcoma development. *Genes Chromosomes Cancer.* 2001;30:1–14.
- Fisher C. Synovial sarcoma. *Ann Diagn Pathol.* 1998;2:401–421.
- Folpe AL, Schmidt RA, Chapman D, et al. Poorly differentiated synovial sarcoma: immunohistochemical distinction from primitive neuroectodermal tumors and high-grade malignant peripheral nerve sheath tumors. *Am J Surg Pathol.* 1998;22:673–682.
- Golouh R, Vuzevski V, Bracko M, et al. Synovial sarcoma: a clinicopathological study of 36 cases. *J Surg Oncol.* 1990;45:20–28.
- Henderson SR, Guiliano D, Presneau N, et al. A molecular map of mesenchymal tumors. *Genome Biol.* 2005;6:R76.
- Hui P, Li N, Johnson C, et al. HMGA proteins in malignant peripheral nerve sheath tumor and synovial sarcoma: preferential expression of HMGA2 in malignant peripheral nerve sheath tumor. *Mod Pathol.* 2005;18:1519–1526.
- Husain J, Lo R, Grbavec D, et al. Affinity for the nuclear compartment and expression during cell differentiation implicate phosphorylated Groucho/TLE1 forms of higher molecular mass in nuclear functions. *Biochem J.* 1996;317:523–531.
- Ito T, Ouchida M, Morimoto Y, et al. Significant growth suppression of synovial sarcomas by the histone deacetylase inhibitor FK228 in vitro and in vivo. *Cancer Lett.* 2005;224:311–319.
- Kwan W, Lubieniecka J, Terry J, et al. Effect of depsipeptide (NSC 630176), a histone deacetylase inhibitor, on human synovial sarcoma in vitro. *ASCO 2005 Annual Meeting.* 2005.
- Ladanyi M. Fusions of the SYT and SSX genes in synovial sarcoma. *Oncogene.* 2001;20:5755–5762.
- Laé M, Saito T, Barr F, et al. Expression profiling of pediatric sarcomas with chimeric transcription factors: a study of 153 samples [USCAP Web site]. March 8, 2004. Available at: <http://www.abstracts2view.com/uscap06>. Accessed November 1, 2006.
- Nagayama S, Katagiri T, Tsunoda T, et al. Genome-wide analysis of gene expression in synovial sarcomas using a cDNA microarray. *Cancer Res.* 2002;62:5859–5866.
- Ng TL, Gown AM, Barry TS, et al. Nuclear beta-catenin in mesenchymal tumors. *Mod Pathol.* 2005;18:68–74.
- Nielsen T, Rubin B, Ruttan C, et al. Expression of Groucho/Transducin-like enhancer of split protein distinguishes synovial sarcoma from malignant peripheral nerve sheath tumor [Connective Tissue Oncology Society Web site]. 2005. Available at: <http://www.ctos.org/meeting/2005/program.html>. Accessed February 14, 2006.
- Nielsen TO, West RB, Linn SC, et al. Molecular characterisation of soft tissue tumours: a gene expression study. *Lancet.* 2002;359:1301–1307.
- Nielsen TO, Hsu FD, O'Connell JX, et al. Tissue microarray validation of epidermal growth factor receptor and SALL2 in synovial sarcoma with comparison to tumors of similar histology. *Am J Pathol.* 2003;163:1449–1456.
- Nuthall HN, Husain J, McLaren KW, et al. Role for Hes1-induced phosphorylation in Groucho-mediated transcriptional repression. *Mol Cell Biol.* 2002;22:389–399.
- Pelms M, Guillou L, Hostein I, et al. Monophasic fibrous and poorly differentiated synovial sarcoma: immunohistochemical reassessment of 60 t(X;18)(SYT-SSX)-positive cases. *Am J Surg Pathol.* 2002;26:1434–1440.
- Pretto D, Barco R, Rivera J, et al. The synovial sarcoma translocation protein SYT-SSX2 recruits beta-catenin to the nucleus and associates with it in an active complex. *Oncogene.* 2006. [Epub ahead of print].
- Segal NH, Pavlidis P, Antonescu CR, et al. Classification and subtype prediction of adult soft tissue sarcoma by functional genomics. *Am J Pathol.* 2003;163:691–700.
- Stifani S, Blaumueller CM, Redhead NJ, et al. Human homologs of a *Drosophila* Enhancer of split gene product define a novel family of nuclear proteins. *Nat Genet.* 1992;2:119–127.
- Swingler TE, Bess KL, Yao J, et al. 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;279:34938–34947.
- Terry J, Barry TS, Horsman DE, et al. Fluorescence in situ hybridization for the detection of t(X;18)(p11.2;q11.2) in a synovial sarcoma tissue microarray using a breakapart-style probe. *Diagn Mol Pathol.* 2005;14:77–82.
- West RB, Harvell J, Linn SC, et al. Apo D in soft tissue tumors: a novel marker for dermatofibrosarcoma protuberans. *Am J Surg Pathol.* 2004;28:1063–1069.
- Yao J, Liu Y, Lo R, et al. 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;93:105–115.
- Yochum GS, Ayer DE. Pfl, a novel PHD zinc finger protein that links the TLE corepressor to the mSin3A-histone deacetylase complex. *Mol Cell Biol.* 2001;21:4110–4118.