Malaysian J Pathol 2016; 38(2) : 117 – 122

ORIGINAL ARTICLE

Transducer-like enhancer of split 1 (TLE1) expression as a diagnostic immunohistochemical marker for synovial sarcoma and its association with morphological features

Ikmal Hisyam BAKRIN *MD, MPath*, Faezahtul Arbaeyah HUSSAIN* *MBBS, MPath* and Sh. Emilia TUAN SHARIF* *MD, MPath*

*Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia and *Department of Pathology, School of Medical Sciences, Universiti Sains Malaysia*

Abstract

Synovial sarcoma (SS) is a malignant soft tissue tumour of uncertain histogenesis which is defined by the translocation t(X;18) that produces the fusion oncogenes SYT-SSX. The emergence of transducer-like enhancer of split 1 (TLE1) as a new immunohistochemical (IHC) marker for SS has offered an alternative to pathologists in differentiating SS from other histological mimics, especially in the setting of limited molecular facilities. We investigated the utility of IHC TLE1 expression against histomorphological features and other IHC markers in SS and non-SS tumours. Twenty-six cases of histologically diagnosed SS and 7 non-SS (for which SS was in the differential diagnosis) were subjected to TLE1 IHC staining, which was graded from 0 to 3+. Of the 26 SS cases, 12 each were biphasic and monophasic types and 2 were poorly-differentiated. TLE1 was expressed in 22/26 (84.6%) SS cases, of which 11/12 (91.7%) were biphasic, 10/12 (83.3%) monophasic and 1/2 (50%) poorly-differentiated tumours. Two of 7 (28.6%) non-SS cases were positive for TLE1. Immunopositivity of SS and non-SS cases for EMA were 20/26 (76.9%) and 2/7 (28.6%) respectively and for CK7 were 7/26 (26.9%) and 0/7 (0%) respectively. All cases were negative for CD34. Consistent histomorphological features for SS included mild nuclear pleomorphism, alternating tumour cellularity, fascicular growth pattern and thick ropy stromal collagen. In conclusion, TLE1 is not a stand-alone diagnostic IHC marker for SS. However, in the absence of molecular studies, it can contribute added diagnostic value in combination with morphological evaluation and other IHC markers such as EMA and CD34.

Key words: synovial sarcoma, TLE1, morphology

INTRODUCTION

Synovial sarcoma is classified as group of soft tissue tumours of uncertain differentiation under the WHO 2013 classification. Despite its name, it is extremely uncommon in joint cavities but arises in areas with no apparent relation to the synovial structure.[1] This tumour commonly leads to diagnostic difficulties due to its morphological features, and mimics many benign and malignant tumours.

Synovial sarcoma is characterized by the translocation t(X;18) that produces the fusion oncogenes SYT-SSX. This translocation involves the fusion of the SYT gene on chromosome 18, and either the SSX1 or SSX2 gene on the X chromosome, or rarely with SSX4.[2] Therefore detection of SYT-SSX fusion gene is considered to be the diagnostic gold standard.[3,4]

Morphologically, synovial sarcoma has two major categories - biphasic and monophasic types. The poorly-differentiated type can be seen in either biphasic or monophasic types. The diagnosis of biphasic synovial sarcoma is fairly straightforward because of the distinctive morphological features of epithelial and spindle cell components in varying proportions. The epithelial components have large, round to oval vesicular nuclei and abundant pale-staining cytoplasm.[1] They have discernible solid, nests or glandular-like structures that may express mucin secretion.[5] The spindle cell component exhibits

Address for correspondence: Ikmal Hisyam Bakrin, Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia. Tel: +603-89472671, Fax: +603-89412787. Email: ikmalhisyam@upm.edu.my

nuclei typically containing fine chromatin without conspicuous nucleoli and minimal cytological pleomorphism with a small amount of indistinct cytoplasm.[6] Generally, these cells are arranged in compact sheets, sweeping fascicles or in a classic herringbone pattern.[1,6,7]

Apart from the epithelial and spindle cell features for each variant, there are characteristic stromal features which are useful in diagnosing synovial sarcoma. Thick ropy collagen bundles may be diffusely distributed or form narrow bands or plaque-like masses sometimes separating epithelial and spindle-cell elements.[1,6,7]

The poorly-differentiated synovial sarcoma usually resembles other small round blue cell tumours particularly Ewing sarcoma/PNET, rhabdomyosarcoma and desmoplastic small round cell tumour.[8] Morphological features as proposed by de Silva are cellular tumours with nuclear crowding and irregularity, prominent nucleoli and coarse clumped chromatin, increased mitotic activity (>10/10 high power field) and tumour necrosis.[9]

Currently, the typical immunohistochemical panel used to aid in differentiating synovial sarcoma from other sarcomas consist of CK AE1/AE3, CK7, EMA, BCL-2, CD 99 and CD 34. Previous studies have found that immunopositivity towards EMA, CK AE1/AE3 in combination with CD 34 negativity were the most useful and sensitive markers for diagnosis of monophasic and poorly-differentiated synovial sarcoma.[5,8] However recent studies observed that many of these markers are only sensitive but not specific or vice versa, as seen in one study which reported that EMA was 91% specific but only 64% sensitive.[6]

Recently, transducer-like enhancer of split 1 (TLE1) has been extensively studied as a potentially useful marker of synovial sarcoma.[10,11] TLE1 is a member of the Groucho/TLE gene family and encodes a transcriptional corepressor implicated in many signalling pathways that regulate the survival, haematopoiesis, neuronal differentiation and terminal epithelial differentiation of normal cells.[12,13] A retrospective tissue microarray analysis found that TLE1 was sensitive and specific for synovial sarcoma, supporting its potential use as a diagnostic immunohistochemical marker.[6]

Reaching a correct diagnosis of synovial sarcoma is crucial as its prognosis and management will be different from other soft tissue sarcomas. Since the availability of molecular diagnostic tools such as RT-PCR or FISH is limited in many histopathology laboratories due to practical considerations (e.g. financial constraints and lack of well-trained personal), therefore, there is a necessity to investigate novel markers that are not only cheaper than molecular testing but highly specific and sensitive for synovial sarcoma.

The objective of this study was to investigate the association of transducer-like enhancer of split 1 (TLE1) expression in synovial sarcoma with its morphological features.

MATERIALS AND METHODS

A total of 33 cases were retrieved from the paraffin-embedded tissue archive of the Department of Pathology, Hospital Universiti Sains Malaysia (HUSM) from years 1998 to 2011. Cases retrieved comprised two groups. Group 1 consisted of 26 cases diagnosed as synovial sarcoma (SS) based on histomorphology and immunohistochemistry. Group 2 comprised 7 spindle cell tumours, recorded as non-SS, which were less likely to be SS based on histomorphology and immunohistochemistry. For Group 2, SS was considered in the differential diagnosis. The immunohistochemical profile of all the tumours, based on CK7, EMA and CD34, were retrieved from the original pathology reports. All cases were reviewed by two independent pathologists for histomorphological features of pleomorphism, cellularity, pattern of cell arrangement and stromal features.

TLE1 Immunohistochemistry

Immunohistochemical staining for TLE1 was performed following pressure cooker antigen retrieval (citrate buffer; pH 6.0), using a mouse polyclonal antibody at 1:150 dilution (Abcam, USA) and the EnVision Plus detection system (DAKO, USA). Positivity for TLE1 immunohistochemical staining was based on intensity of nuclear immunoreactivity in tumour cells. It was graded semi-quantitatively.[6,11,12] TLE1 immunoreactivity was graded as 3+ (strong), if over 50% of the tumour cells exhibited strong staining visible at 4x10 objective power, 2+ (moderate) if 26-50% showed strong staining visible at 4x10 objective power or if over 50% of cells clearly stained positive above the background at 10x10 objective power, 1+ (weak) if staining was present below these threshold and 0 (negative) if staining was not visible.

Ethics review

Approval for the study was obtained from the Human Research Ethics Committee USM (HREC) with the reference number: FWA Reg No: 00007718; IRB Reg. No: 00004494)

RESULTS

Histomorphological findings

Morphologically, the majority of SS cases (84.6%) exhibited mild nuclear pleomorphism. Only 11.5% and 3.8% of cases were observed to have moderate and severe pleomorphism respectively. In contrary, the non-SS group mostly exhibited moderate and severe nuclear pleomorphism (71.4%) which was significantly different from SS with a *p* value of 0.012. More than half (61.5%) of SS cases have predominantly alternated cellularity whereas 85.7% of non-SS cases were predominantly hypercellular (p = 0.026) (Table 1).

Different types of tumour arrangement or patterns were observed in both groups. The fascicular pattern was more common (92.3%) in SS than (57.1%) non-SS tumours (p = 0.021) (Table 2).

Of stroma features, only the presence of thick ropy collagen bundles was significant more common in SS than non-SS cases (p = 0.030). Other tumour patterns and stroma features which were not significant different are summarized in Table 2.

Immunohistochemistry

The immunohistochemical staining results for TLE1 in SS and non-SS cases are summarized in Table 3. Nuclear immunoreactivity was observed in 22 of 26 (84.6%) SS cases, including 11 of 12 (91.7%) biphasic type, 10 of 12 (83.3%) monophasic type and 1 of 2 (50%) poorly differentiated type. Only 2 of 7 (28.6%) of non-SS cases expressed TLE1.

Seventeen of 26 (65.4%) of SS cases showed at least moderate nuclear positivity (score 2+ to 3+), and the percentage had increased to 84.6% (22 of 26 cases) if weak nuclear positivity (score 1+) is also considered as positive. None of the non-SS cases showed strong nuclear positivity (score 3+) as compared to 11 SS cases (p =0.003) (Fig. 1).

From the immunohistochemistry panel available, only EMA was significantly more commonly expressed by SS (76.9%) compared with non-SS (28.6%) cases (p value = 0.016). There were no significant difference in expression of CK AE1/AE3 (p = 0.171) as well as CK 7 (p = 0.122) in both SS and non-SS cases. On the other hand, CD34 expression was completely negative in both SS and non-SS cases (Table 4).

DISCUSSION

In this study, we observed a significant difference in the histomorphology of tumour cells between SS and non-SS cases. However, this was true largely for the biphasic SS which can be easily differentiated from other sarcomas.[5,14] Most monophasic SS displayed only spindle cells with various patterns or arrangement requiring a panel of immunohistochemical stains to rule out other sarcomas.

Though SS is a high grade tumour, most of the cases only showed mild nuclear pleomorphism, a feature in contradiction with the general rule of malignancy. Not many studies have highlighted this feature, but it has been supported by some

TABLE 1: Tumour morphological features (pleomorphism and cellularity) in synovial sarcoma and non-synovial sarcoma cases

	Synovial sarcoma n = 26 n (%)	**Non-synovial sarcoma n = 7 n (%)**	**X^2 (df)**	***p* value**
Pleomorphism			8.78 (2)	**0.012**
Mild	22 (84.6)	2 (28.6)		
Moderate	3 (11.5)	4 (57.1)		
Severe	1 (3.8)	1 (14.3)		
Cellularity			4.93 (1)	**0.026**
Hypercellular	10 (38.5)	6 (85.7)		
Hypocellular	0 (0.0)	0 (0.0)		
Alternate	16 (61.5)	1 (14.3)		

Statistically significant *p* value is set at 0.05

TABLE 2: Tumour morphological features (pattern of tumour and stroma component) in synovial sarcoma and non-synovial sarcoma cases

Characteristic	Synovial sarcoma (n = 26)		Non-synovial sarcoma (n = 7)		X^2 (df)	*p* value
	Present (%)	Absent (%)	Present (%)	Absent (%)		
Pattern						
Solid	7 (26.9)	19 (73.1)	1 (14.3)	6 (85.7)	0.48 (1)	0.489
Nest	2 (7.7)	24 (92.3)	0 (0.0)	7 (100.0)	0.57 (1)	0.449
Glandular	4 (15.4)	22 (84.6)	0 (0.0)	7 (100.0)	1.23 (1)	0.268
Sheath	8 (30.8)	18 (69.2)	2 (28.6)	5 (71.4)	0.01 (1)	0.911
Fascicle	24 (92.3)	2 (7.7)	4 (57.1)	3 (42.9)	5.31 (1)	**0.021**
Herringbone	9 (34.6)	17 (65.4)	0 (0.0)	7 (100.0)	3.33 (1)	0.068
Stroma						
Myxoid	11 (42.3)	15 (57.7)	1 (14.3)	6 (85.7)	1.87 (1)	0.171
Haemangiopericytic	9 (26.4)	17 (65.4)	1 (14.3)	6 (85.7)	1.08 (1)	0.299
Thick collagen ropy	19 (73.1)	7 (26.9)	2 (28.6)	5 (71.4)	4.72 (1)	**0.030**
Calcification	4 (15.4)	22 (84.6)	0 (0.0)	7 (100.0)	1.23 (1)	0.268
Mast cell	7 (26.9)	19 (73.1)	0 (0.0)	7 (100.0)	2.39 (1)	0.122

Statistically significant *p* value is set at 0.05

authors.[6,7]

We have found that more than half (61.5%) of SS cases have hypercellular alternating with hypocellular areas, a feature similar to malignant peripheral nerve sheath tumour (MPNST). The majority (85.7%) of non-SS cases displayed only hypercellular areas. From these findings, the described pattern can be one of the morphological criteria to be considered, especially if MPNST is in the list of differential diagnosis. This is in agreement with a study which reported that a small percentage of MPNST can also show TLE1 nuclear positivity. However, another study found that up to 30% of MPNST cases can show TLE1 positivity.[12,14] Hence in this type of cases, a panel of immunohistochemistry will be useful to rule out or at least to narrow down the diagnosis.

We also observed that most of the spindle cells

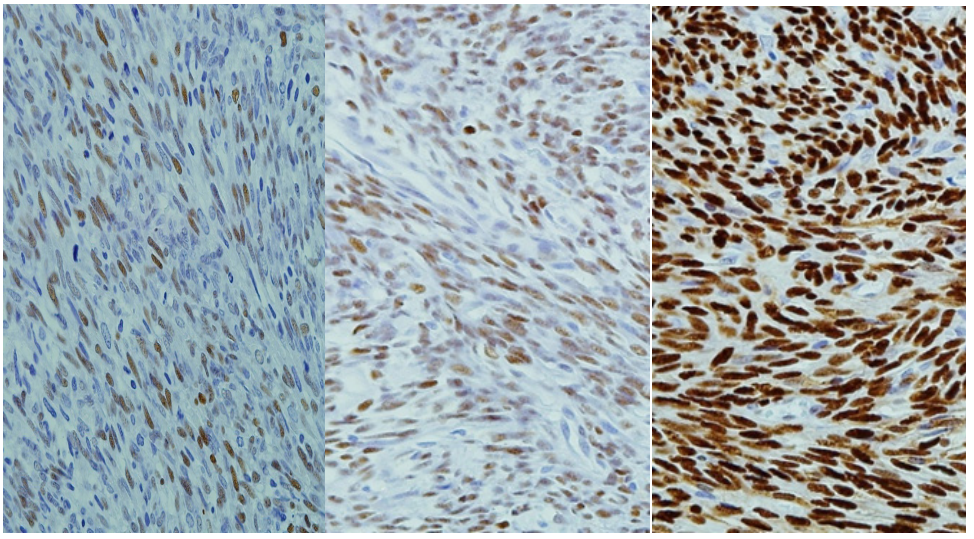

FIG. 1: (Left to right) TLE1 staining: weak (1+), moderate (2+) and strong nuclear positivity (3+) in tumour cells (X 400 magnification)

TABLE 3: Scoring of TLE1 staining in types of synovial sarcoma and non-synovial sarcoma

Types of sarcoma	Scoring				Total
	0 n (%)	1+ n (%)	2+ n (%)	3+ n (%)	
Synovial sarcoma					26
Monophasic	2 (16.7)	3 (25.0)	2 (16.7)	5 (41.7)	12
Biphasic	1 (8.3)	1 (8.3)	5 (41.7)	5 (41.7)	12
Poorly differentiated	1 (50.0)	0 (0.0)	0 (0.0)	1 (50.0)	2
Non-synovial sarcoma	5 (71.4)	0 (0.0)	2 (28.6)	0 (0.0)	7

in SS cases were mainly arranged in fascicular pattern in both monophasic and biphasic types.[1,6,7] Even though this was significantly different from non-SS cases, many differential diagnoses still need to be considered under this type of cellular pattern including MPNST, leiomyosarcoma, fibrosarcoma and fibrosarcomatous variant of DFSP.[1,8,14]

Apart from the type of tumour cells and pattern of tumour cell arrangement, characteristic stroma features which are sometimes was neglected by the pathologist are actually very helpful to differentiate SS from other histological mimics. In this study, thick ropy collagen bundles were significantly more often encountered in SS than non-SS cases. This hyaline band, even though it can be seen in any type of SS, is significantly associated with the biphasic SS.[9]

In terms of reactivity for TLE1, we found that 84.6% of SS cases were positive for TLE1 (1+ to 3+). However if we only considered 2+ and 3+ as positive, 65.4% of SS cases were positive in this study. This finding was similar to that of Foo *et al*, who found that only 60% of synovial sarcoma were positive for TLE1, if 2+ and 3+ nuclear staining were considered to be the positive criteria.[14]

Two (28.6%) of seven non-SS cases showed 2+ nuclear staining for TLE1. The morphology of these two cases were spindle tumour cells in fascicles and small round cells mixed with occasional spindle cells. Both of these cases displayed moderate nuclear pleomorphism. One of the cases had thick ropey collagen bundle (small round cells mixed with occasional spindle cells), while the other case showed high mitotic figures (>20 per 10 high power fields) (spindle tumour cells in fascicles). Their immunohistochemical profiles were not suggestive of SS except for positivity for TLE1 and vimentin. We believe both of these cases were indeed high grade SS based on tumour cell pleomorphism and high mitotic rate.[1,7] However, the diagnosis was not supported by immunohistochemical panel markers. TLE1 on the other hand was able to pick up these cases in view of its high sensitivity compared to others markers.[6]

By comparing the EMA and TLE1 immunohistochemistry, TLE1 is more readable. TLE1 produced intense nuclear immunoreactivity in a high proportion of cells, in contrast to a focal staining pattern of EMA in SS. This provides more readily interpretable results on

TABLE 4: Immunohistochemical profile of synovial sarcoma and non-synovial sarcoma

IHC Test	Synovial sarcoma n = 26		Non-synovial sarcoma n =7		X^2 (df)	*p* value
	Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)		
CK AE1/AE3	15 (57.7)	11 (42.3)	2 (28.6)	5 (71.4)	1.87 (1)	0.171
EMA	20 (76.9)	6 (23.1)	2 (28.6)	5 (71.4)	5.80 (1)	**0.016**
CK7	7 (26.9)	19 (73.1)	0 (0.0)	7 (100.0)	2.39 (1)	0.122
TLE1	22 (84.6)	4 (15.4)	2 (28.6)	5 (71.4)	8.73 (1)	**0.003**

Statistically significant *p* value is set at 0.05

small tissue samples such as tru-cut biopsies in which diagnosis is crucial for further patient management.

TLE1 must be cautiously interpreted especially when it shows mild nuclear staining and a heterogenous staining pattern. It is worth mentioning that in non-SS cases, TLE1 expression was rather heterogenous with some fields showing near uniform positivity and some areas showing only patchy or even absent positivity.[12]

The interpretation of TLE1 positivity should be correlated with its morphological features along with other immunohistochemistry panel markers. The findings of strong TLE1 expression in appropriate histological context, positive scattered cytokeratin or EMA and negative CD34 are strongly suggestive and favour the diagnosis of SS.[5]

In conclusion, TLE1 immunohistochemistry for SS can be very useful to distinguish SS from histological mimics. Good nuclear staining without background or cytoplasmic staining makes interpretation easier than other immunohistochemistry panel markers. While molecular confirmation should remain the gold standard in diagnosing SS, TLE1 offers the pathologist a valuable tool in combination with other immunohistochemistry markers and histomorphology particularly when access to molecular testing is not available.

ACKNOWLEDGEMENT

This work was part of a requirement for a Master of Pathology programme and was funded by a Universiti Sains Malaysia short term grant.

REFERENCES

1. Weiss SW, Goldblum JR. Enzinger and Weiss's Soft Tissue Tumors. 5th ed. USA: Mosby Elsevier; 2008.
2. Jain S, Xu R, Prieto VG, Lee P. Molecular classification of soft tissue sarcomas and its clinical applications. Int J Clin Exp Pathol. 2010; 3: 416-28.
3. Mikami Y, Nakajima M, Hashimoto H, Kuwabara K, Sasao Y, Manabe T. Primary poorly differentiated monophasic synovial sarcoma of the lung. A case report with immunohistochemical and genetic studies. Pathol Res Pract. 2003; 199: 827-33.
4. Amary MF, Berisha F, Bernardi Fdel C, *et al*. Detection of SS18-SSX fusion transcripts in formalin-fixed paraffin-embedded neoplasms: analysis of conventional RT-PCR, qRT-PCR and dual color FISH as diagnostic tools for synovial sarcoma. Mod Pathol. 2007; 20: 482-96.
5. Fisher C. Synovial sarcoma. Ann Diagn Pathol. 1998; 2: 401-21.
6. 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: 1743-51.
7. Kempson RL, Rouse RV. Surgical Pathology Criteria: Synovial Sarcoma [Internet]. 2007 [updated 2012 August 3; cited 2013]. Available from: http://surgpathcriteria.stanford.edu/softmisc/synovial_sarcoma/.
8. Pelmus M, Guillou L, Hostein I, Sierankowski G, Lussan C, Coindre JM. Monophasic fibrous and poorly differentiated synovial sarcoma: immunohistochemical reassessment of 60 t(X;18) (SYT-SSX)-positive cases. Am J Surg Pathol. 2002; 26: 1434-40.
9. de Silva MV, McMahon AD, Paterson L, Reid R. Identification of poorly differentiated synovial sarcoma: a comparison of clinicopathological and cytogenetic features with those of typical synovial sarcoma. Histopathology. 2003; 43: 220-30.
10. Baird K, Davis S, Antonescu CR, *et al*. Gene expression profiling of human sarcomas: insights into sarcoma biology. Cancer Res. 2005; 65: 9226-35.
11. 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: 240-6.
12. 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: 872-8.
13. Seo SW, Lee H, Lee HI, Kim HS. The role of TLE1 in synovial sarcoma. J Orthop Res. 2011; 29: 1131-6.
14. Foo WC, Cruise MW, Wick MR, Hornick JL. Immunohistochemical staining for TLE1 distinguishes synovial sarcoma from histologic mimics. Am J Clin Pathol. 2011; 135: 839-44.