Med & Health Dec 2019; 14(2): 41-49 https://doi.org/10.17576/MH.2019.1402.04

ORIGINAL ARTICLE

STAT6 is a Useful Immunohistochemical Adjunct for the Diagnosis of Solitary Fibrous Tumour

AMIZATUL AINI S[1], NUR MAYA SABRINA TL[1], MUHAMAD FAKHRI MS[1], NORAINI MD[2], REENA RAHAYU MZ[1]

[1]Department of Pathology, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia.
[2]Department of Pathology, General Hospital Kuala Lumpur, Jalan Pahang, 50586 Kuala Lumpur, Malaysia

ABSTRAK

Solitary fibrous tumour (SFT) adalah neoplasma fibrotik, juga dikenali sebagai hemangiopericytoma. STAT6 merupakan protein yang berfungsi sebagai transduser isyarat dan penggerak transkripsi keluarga STAT6 dalam fungsi imun dan pertumbuhan sel awal. Perubahan onkogen STAT6 memainkan peranan penting sebagai pemacu tertentu untuk genetik SFT melalui gen gabungan NAB2-STAT6. Tujuan kajian ini adalah untuk membandingkan dan menilai ekspresi STAT6 di antara SFT dan tumor yang menyerupai SFT. Kami menilai sebanyak 30 kes blok tisu parafin yang sebelum ini didiagnosis SFT (n=14) dan tumor yang menyerupai SFT (n=16) selama tempoh 15 tahun. Empat belas kes SFT (100%) menunjukkan ekspresi nuklear STAT6. Semua jenis tumor yang lain adalah negative untuk STAT6 kecuali satu kes liposarcoma dediferensiasi satu tumor stromal gastrointestinal dan satu lipoma yang menunjukkan pewarnaan sitoplasma yang tidak spesifik dan lemah. Ekspresi nuklear STAT6 adalah berguna dan boleh dipercayai sebagai penanda adjunksi SFT apabila diagnosis tidak boleh dibuat melalui kaedah konvensional.

Kata kunci: immunohistokimia, solitary fibrous tumor (SFT), STAT6

ABSTRACT

Solitary fibrous tumour (SFT) is a fibrotic neoplasm, also previously designated as hemangiopericytoma. STAT6 is a protein for signal tranducers and activators of transcription (STAT) family which is involved in immune function and early cell growth. Oncogenic alteration of STAT6 plays an important role for specific driver

Address for correspondence and reprint requests: Dr. Reena Rahayu Md Zin. Department of Pathology, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia. Tel: +603-9145 5359 Email: reenarahayu@ppukm.ukm.edu.my, ayureena@gmail.com

for tumour genesis of solitary fibrous tumour via NAB2-STAT6 fusion gene. The aim of the study was to compare and evaluate STAT6 expression between SFT and other soft tissue histological mimics. We evaluated a total of 30 cases material formalin fixed paraffin embedded tissue block previously diagnosed SFT cases (n=14) and soft tissue tumours mimic cases (n=16) over period of 15 years. Fourteen SFT cases (100%) showed nuclear expression of STAT6, which was diffuse and intense. All other tumour types were negative for STAT6, except for one case of dedifferentiated liposarcoma, one gastrointestinal stromal tumour and one spindle cell lipoma, which showed weak non-specific cytoplasmic staining. Nuclear STAT6 expression is useful and reliable as adjunctive marker for solitary fibrous tumour when the diagnosis is inconclusive by conventional methods.

Keywords: immunohistochemistry, solitary fibrous tumour (SFT), STAT6

INTRODUCTION

Solitary fibrous tumour (SFT) is also known as hemangiopericytoma. The prevalence of soft tissue tumour accounts for about 2% (Goldblum et al. 2013). It was thought originating from the pericytes, a dendritic-like smooth muscle cells encircling the blood vessels when it was first described by Stout and Murray 1942. It was not clear until 1976; when a published paper by Ezinger and Smith described the architectural and cytological criteria for solitary fibrous tumour (Goldblum et al. 2013). The classical histological features composed of staghorn partially hyalinised vessel surrounded by small rounded fusiform cells showing no obvious light microscopic features of differentiation (Fletcher et al. 2013). There are several histological mimics of solitary fibrous tumour such as synovial sarcoma, liposarcoma, desmoids fibromatosis, fibrous histiocytoma, dermatofibrosarcoma protuberans, gastrointestinal stromal tumour, malignant peripheral nerve sheath tumour, schwannoma, and spindle cells lipoma. Solitary fibrous tumour frequently occurs in adult and affects both males and females, equally. It is located at deep soft tissue mainly thigh, pelvic, retroperitoneum and serosal surface (Fletcher et al. 2013).

Recently found, SFTs have recurrent genetic alteration involving chromosome 12q13 and formed NGFI-A binding protein 2 NAB2/ STAT6 fusion gene. When the NAB2-STAT6 fusion gene occurs, it requires STAT6 protein as a transcription factor for tumour proliferation (Doyle et al. 2014).

Robinson et al. (2013) identified the NAB2-STAT6 gene fusion by integrative sequencing in 51 cases (100%) of solitary fibrous tumour. Chmielecki et al. (2013) investigated solitary fibrous tumour via whole genome exon sequencing data in 53 cases and revealed NAB2-STAT6 fusion gene in 29 out of 53 cases (55%). Vogels et

al. (2014) showed that NAB2-STAT6 fusion gene was found in 19/28 cases (68%). Cheah et al. (2014) studied expression of STAT6 immunoreactivity in 54 cases of solitary fibrous tumour. It showed 100% sensitivity and was not expressed by other histological mimic. Voges et al. (2014) studied nuclear STAT6 expression in 28 cases of solitary fibrous tumour. All 28 cases (100%) showed nuclear expression of STAT6.

Doyle et al. (2014) demonstrates in large cases of solitary fibrous tumour showed 98% nuclear expression to STAT6. In this study 231 soft tissue tumours were analysed, including 60 cases of solitary fibrous tumour and the rest were benign, malignant mesenchymal neoplasms and sarcomatoid mesotheliomas. A total of 59 out of 60 solitary fibrous tumour (98%) showed nuclear expression of STAT6. The other tumours were negative for STAT6 except 3 dedifferentiated liposarcoma and one deep fibrous histiocytoma. Demicco et al. (2015) did an extensive survey on STAT6 expression in 2021 mesenchymal tumours. Strong nuclear STAT6 was expressed in 285 out of 2021 tumours. It revealed 206 out of 241 cases (86%) of solitary fibrous tumour showing strong nuclear expression of STAT6. Other tumours showed some nuclear and cytoplasmic staining such as 49/408 (12%) well differentiated/ dedifferentiated liposarcoma, 8/65 (12%) unclassified sarcoma, and 14/184 desmoid tumour. In this study, expression of STAT6 in solitary fibrous tumour was limited to the nucleus.

The aim of the study was to observe and validate reliability of STAT6 expression as diagnostic adjunct in solitary fibrous tumour.

MATERIALS AND METHODS

Patient Selections and Clinical Data

We retrospectively selected all cases diagnosed as solitary fibrous tumour between 2000-2015 in the Department of Pathology, Universiti Kebangsaan Malaysia Medical Centre and Hospital Kuala Lumpur. We selected some of the soft tissue tumours mimicking cases, based on literature such as synovial sarcoma, liposarcoma, desmoids fibromatosis, fibrous histiocytoma, dermatofibrosarcoma protuberans, gastrointestinal stromal tumor, malignant peripheral nerve sheath tumour, schwannoma, and spindle cells lipoma. The representative hematoxylin and eosin stained slides were reviewed. One representative slide of tumour was selected.

Methods

Paraffin embedded tissue sections were cut (3um-thick), dried, deparaffinized and rehydrated using standard procedures. STAT6 antibody was applied to a representative section of the cases as per manufacturer's protocol with heat-induced epitope retrieval followed by incubation with the primary antibody rabbit monoclonal to STAT6 antibody (Clone YE361, Product Code No: Ab32520, Abcam England) was used at a dilution of 1:1000. Normal kidney tissue and

tumour tissue was used as the positive control tissue. EnVisionTM FLEX, high pH and DAB chromogen solution was used for visualization. The slides were then stained lightly with hematoxylin. All slides were then evaluated by a trainee and a pathologist who were blinded for the pathologic data.

Interpretation of Results

STAT6 expression was evaluated according to manufacturer's recommendation and referring to the previous study by using STAT6 rabbit monoclonal antibody. Only nuclear staining of STAT6 was considered positive. Scoring was based on percentage of nuclear positive for the presence of STAT6. Staining will be scored as 0 for absent staining; 1+ (1-25%); 2+ (26-50%); 3+ (>50%) (Doyle et al. 2014) (Table 1). Intensity was scored as weak, moderate or strong. Immunohistochemical evaluation was performed by two observers (one trainee and one pathologist) independently. In cases with discrepancy, a final score was determined by consensus after re-examination.

Data Processing and Analysis

All data and results were processed and analyzed statistically using Statistical Package for Society Study (SPSS) version 23.0 statistics software. The STAT6 expression were analysed by using Chi square test. A p value of <0.05 was considered as statistically significant with confidence interval of 95%.

RESULTS

Clinical Data

Fourteen solitary fibrous tumours were studied. Among the cases, there were six males and eight females with a median age of 54 years (range 30-88 years). These arose from a variety of anatomical sites including brain, neck, orbit, submandibular, preauricular, pelvis, spine, abdomen, kidney and small bowel (Table 2). Ten cases were diagnosed benign, while four cases were malignant. Histologically, all cases showed typical features of SFT with a patternless architecture of alternating hypo and hypercellular areas of spindle shape cells (Figure 1A). Hemangiopericytoma-like vessels were visible in all cases (Figure 1B). Some

Table 1: The following scoring approach in the assessment of STAT6 immunostaining was used. Adapted from Doyle et al. (2014).

Score	Staining pattern	Percentage of nuclear positivity
0	No staining, cytoplasmic or background non-specific staining	0
1+	Weak nuclear staining	1-25%
2+	Moderate nuclear staining	26-50%
3+	Strong nuclear staining	>50%
All cases with score of 1+ and above were considered positive for STAT6		

Table 2: Clinical, anatomical location and immunohistochemical properties of SFTs

SFT	Age	Gender	Location	Previous IHC
1	46	F	Brain	CD34+, CD99+, vimentin+, BCL-2+, SMA focal +, Desmin-, EMA-, CKAE1/AE3-, CD31-, S-100-
2	54	F	Right neck soft tissue	CD34+,Vimentin-,SMA-, S100-, CKAE1/AE3-
3	51	F	Left orbit	CD34-, CD99+, BCL-2+, SMA focal +, Vimentin+, S-100-, EMA-, Desmin-
4	44	F	Left submandibular	BCL-2+
5	48	F	Pelvis	CD34+, CD99+, BCL-2+, Desmin+, SMA-, myogenin-, CKAE1/AE3-
6	60	M	Spine	CD34+, CD99+, BCL-2+, Desmin+, SMA-, S100-, myogenin-, CKAE1/AE3-
7	60	F	Brain	CD34+, CD99+, GFAP-, CD31-, SMA-
8	63	F	Brain	CD34+, CD99+, GFAP-, CD31-, SMA-
9	88	M	Abdominal mass	CD 34+, SMA focal +, Desmin focal +, CKAE1/AE3-, CD 117-, EMA-, Calretinin -
10	73	F	Uterus	CD34+, BCL-2+, CD99-, Vimentin-, SMA-, Desmin-, CD10-
11	40	M	Kidney	CD34+, SMA-, S-100-, CKAE1/AE3-
12	55	M	Left preauricular mass	CD34+,Vimentin+, S100-,CKAE1/AE3-, LCA-
13	30	M	Small bowel	CD34+, CD99+, BCL-2+, Desmin-, SMA-, myogenin-
14	60	M	Spine	CD34-, CD99+, BCL-2+, Desmin+, SMA-, S-100-, myogenin-, CKAE1/AE3-

M: male; F: female; SMA: smooth muscle actin; GFAP: glial fibrillary acidic protein; EMA: epithelial membrane antigen

of the vessels were hyalinised. The nuclei are relatively uniform spindle cells or oval shape nuclei. Variable collagenised backgrounds were noted in all SFTs. Four of the cases were malignant and fulfilled the malignant criteria including hypercellularity, necrosis, and mitoses more than 4 per 10 HPF with variable nuclear atypia.

STAT6 Expression

Nuclear STAT6 staining was present in all solitary fibrous tumour cases (14/14) (100%) (Table 3). Majority of the benign cases (10/14, 71%) showed homogenous positivity 2+ with moderate intensity staining and 4 malignant cases (4/14, 29%) showed 3+ with strong intensity. Two of the

Table 3: STAT6 staining in solitary fibrous tumours

		SFT	Non SFT	Pearson Chi-Square
STAT6	Positive	14/14	0/16	$p < 0.001$
	Negative	0/14	16/16	$p < 0.001$

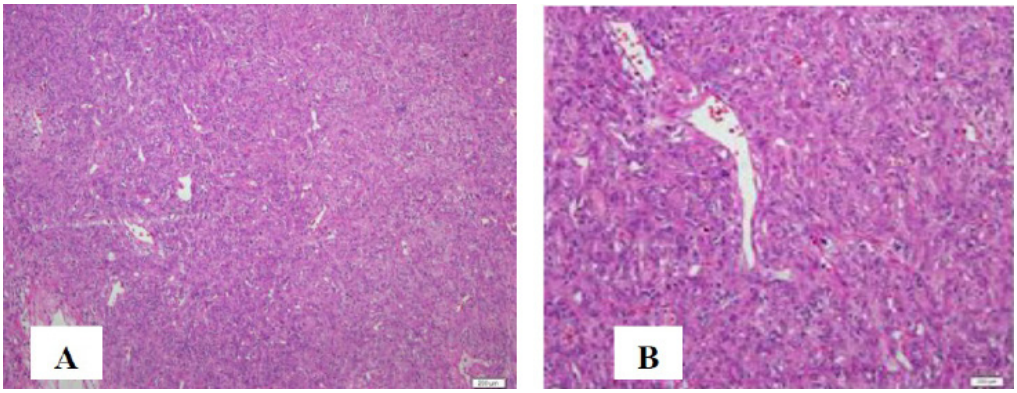

Figure 1: Images showing histologic features of solitaru fibrous tumour (A) with hypocellular and hypercellular pattern; (B) with haemangiopericytoma like vessels

cases were CD34 negative with SFT showing 2+ with moderate intensity staining. In our observation, the nuclear staining were confined within nucleus of cells with clean background. All other tumour types were negative for STAT6 (Table 4). One case was of dedifferentiated liposarcoma, another case of gastrointestinal stromal tumour while one case of spindle cell lipoma showing weak nonspecific cytoplasmic staining were seen, and therefore interpreted as negative (Figure 2).

DISCUSSION

In this study, we ascertained the STAT6 rabbit monoclonal antibody is expressed in SFT. It showed 100% expression with positive nuclear staining for STAT6 demonstrated in all 14 solitary fibrous tumours irrespective of histology and anatomical sites and CD34 status. Our results are in concordance with previously published study by Cheah et al., who reported 100% sensitivity and 100% specificity in 28 solitary fibrous tumours versus spindle cells histological mimics. In their study, the histological mimics including spindle cell lipoma, mammary-type myofibroblastoma, cellular angiofibroma, benign fibrous histiocytoma, dermatofibrosarcoma

Table 4: Negative STAT6 expression in histological mimics of solitary fibrous tumour

Tumour type	Total cases (n)
Monophasic synovial sarcoma	n : 4
Dedifferentiated liposarcoma	n : 2
Malignant peripheral nerve sheath tumor	n : 1
Desmoids fibromatosis	n : 1
Spindle cell lipoma	n : 1
Schwannoma	n : 2
Dermatofibrosarcoma protuberans	n : 3
Gastrointestinal stromal tumour	n : 2

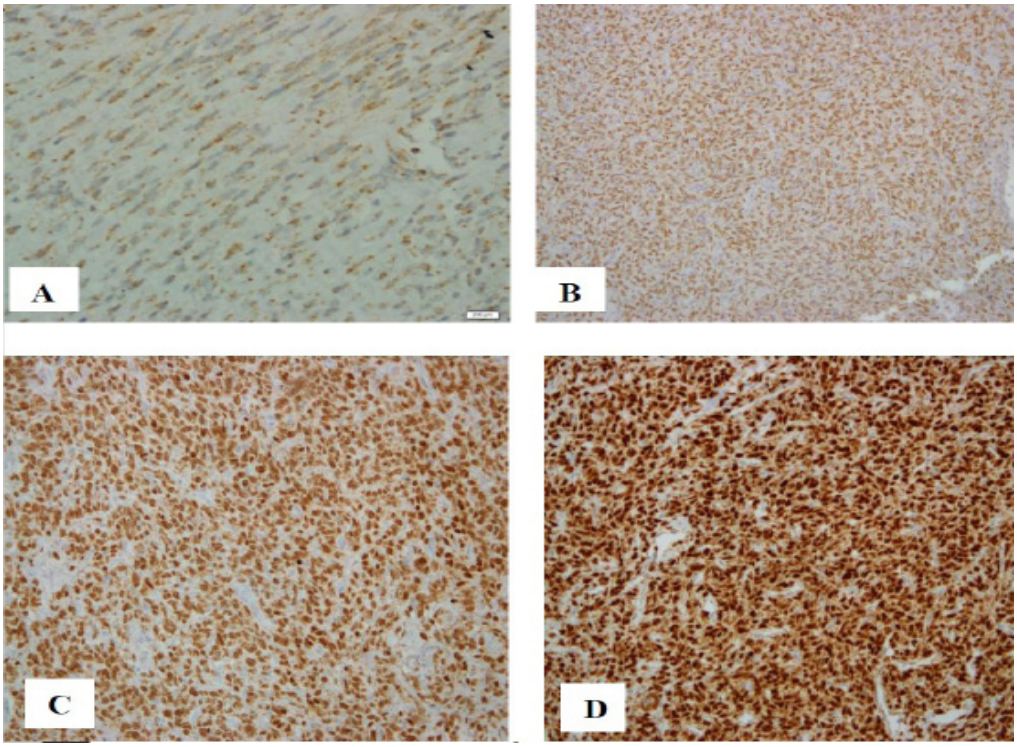

Figure 2: STAT6 expression of (A) 0 (negative); (B) 1+ (positive); (C) 2+ (positive); and (D) 3+ (positive)

protruberans, desmoid type fibromatosis, low-grade fibromyxoid sarcoma, schwannoma, malignant peripheral nerve sheath tumour, monophasic synovial sarcoma and mesenchymal chondrosarcoma were negative for STAT6 rabbit monoclonal antibody. A case of tumour arising from buccal region reported by Okui et al. (2019) was diagnosed as solitary fibrous tumour showing nuclear positivity to STAT6.

SFT is a fibrotic neoplasm with variable clinical behaviour. Most SFTs follow a benign clinical course. However, the recurrence and metastasis develops in 5-10% of cases (Fletcher et al. 2013; Hornick 2013). The specific symptoms depend on location of the tumour. Those with somatic soft tissue present as painless slow growing mass, whereas in those within abdominal cavity presented with impingement of specific organs. Solitary fibrous tumour of pleura is asymptomatic and diagnosed by incidental findings. About 5% of the patients develop hypoglycaemic symptoms (Zafar et al. 2003).

Grossly, the tumours were well circumscribed and located at deep soft tissue. The diameter of the tumor usually 5-10cm with grey-white to red-brown colour on cut surfaces. Histological features of the tumour exhibit proliferation of spindle cells in various patterns including 'patternless' pattern with a prominent branching vessels or staghorn vessels (Goldblum et al. 2013). The histological criteria of malignancy

comprise of mitoses more than 4 per 10HPF, necrosis, hypercellularity with nuclear atypia. Some of the tumours displayed variable amount of fat and can be misdiagnosed as liposarcoma. The other unusual features include presence of pseudovascular spaces, cystic changed and tumour giant cells. Most of the solitary fibrous tumour is benign tumour but a malignant case has been reported based on histologic criteria of diameter, mitotic rate, highly cellular and presence of haemorrhage and necrosis. The neoplastic cells express CD34, CD99, and BCL-2. The tumour showed CD34, positivity. However, it was less specific as many lesions which include in the differential diagnoses positive for CD34. About 5-10% of solitary fibrous tumour was negative for CD34 and makes it more challenging in making the diagnosis (Goldblum et al. 2013). Yokoi et al. (1998) studied on CD34 reactivity in malignant solitary fibrous tumour. Ten cases were analysed and it revealed that CD34 was positive in 7 cases, with low and intermediate grade but negative in 3 high grade tumours. These studies suggest expression of CD34 can be lost in high grade solitary fibrous tumour with malignant transformation.

STAT6 is a protein, function as a signal tranducers and activators of transcription (STAT) family. STAT6 families are involved in immune function and cell growth. It also has been found activated in various malignancies as tumorigenesis such as lymphomas, leukaemia, rhabdomyosarcoma and prostatic adenocarcinoma. STAT6 is activated by cytokines, IL-13, and IL-4 via thyrosine phosphorylation. The first definitively oncogenic alteration of STAT6 was identified as a specific driver of tumoregenesis of solitary fibrous tumour (Chmielecki et al. 2013; Robinson et al. 2013). The pathogenesis of solitary fibrous tumour was found due to recurrent gene fusion NAB2-STAT6 involving chromosome 12q13 which encodes a chimeric protein (Chmielecki et al. 2013; Robinson et al. 2013). This chimeric protein combined the EGR-binding domain of NAB2 forming a repressor of early growth response (EGR) transcription factors which regulate differentiation and proliferation by transactivation of the STAT6 as a transcription factor mediates cytokines signalling. In normal cells, early growth response 1 (EGR1) promotes NAB2 expression which in turn repress EGR1 in a tightly regulated by feedback loop. When NAB2-STAT6 fusion gene occurs, it causes overexpression of chimeric transactivation factor to increase tumour proliferation (Chmielecki et al. 2013).

In the present study, we found the nuclear staining were distinct with lack of background staining. There were 2 cases which were CD34 negative, and SFTs were homogenously positive for nuclear staining STAT6. Regardless of the primary tumour sites, recurrent, malignant or benign, the nuclear expressions of the STAT6 were mutually expressive. There were many studies validating nuclear STAT6 expression of highly specific and sensitive in SFTs. Therefore, STAT6 expression can be used to help in the diagnosis of solitary fibrous tumour. The nuclear reactivity likely revealed the NAB2/STAT6 fusion

protein as proven by many studies previously.

CONCLUSION

Distinguishing SFTs and histological mimics have an important clinical implication as warranted different clinical management. Nuclear STAT6 immunoreactivity is reliable in the diagnosis of SFTs especially in difficult cases or small biopsies. It can be applied as routine diagnostic test for SFTs. However, research studies were performed on limited cases due to rarity of the tumour. A larger study is needed to validate these findings.

ACKNOWLEDGEMENT

This research study was funded by Universiti Kebangsaan Malaysia (Grant number: FF-2016-051). This study was presented as a poster in the 4th Annual Scientific Meeting 2017 of the International Academy of Pathology Malaysian division in Kelantan Malaysia in (9-11th September 2017). The poster abstract was published in the Malays J Pathol 2017; 39(3): 331-349.

REFERENCES

Cheah, A.L., Billings, S.D., Goldblum, J.R., Carver, P., Tanas, M.Z., Rubin, B.P. 2014. STAT6 rabbit monoclonal antibody is a robust diagnostic tool for the distinction of solitary fibrous tumour from its mimics. *Pathology* **46**(5): 389-95.

Chmielecki, J., Crago, A.M., Rosenberg, M., O'Connor, R., Walker, S.R., Ambrogio, L., Auclair, D., McKenna, A., Heinrich, M.C., Frank, D.A., Meyerson, M. 2013. Whole-exome sequencing identifies a recurrent NAB2-STAT6 fusion in solitary fibrous tumors. *Nat Genet* **45**(2): 131-2.

Demicco, E.G., Harms, P.W., Patel, R.M., Smith, S.C., Ingram, D., Torres, K., Carskadon, S.L., Camelo-Piragua, S., McHugh, J.B., Siddiqui, J., Palanisamy, N., Lucas, D.R., Lazar, A.J., Wang, W.L. 2015. Extensive survey of STAT6 expression in a large series of mesenchymal tumors. *Am J Clin Pathol* **143**(5): 672-82.

Doyle, L.A., Vivero, M., Fletcher, C.D., Mertens, F., Hornick, J.L. 2014. Nuclear expression of STAT6 distinguishes solitary fibrous tumor from histologic mimics. *Mod Pathol* **27**(3): 390-5.

Fletcher, C.D.M., Bridge, J.A., Hogendoorn, P., Mertens, F. 2013. World Health Organization Classification of tumours of soft tissue and bone. 4th. IARC, Lyon

Goldblum, J.R., Weiss, S.W., Folpe, A.L. 2013. *Enzinger and Weiss's Soft Tissue Tumors E-Book*. Elsevier Health Sciences.

Hornick, J.L. 2013. Practical Soft Tissue Pathology: A diagnostic approach. 1st Edition. Elsevier

Okui, T., Ibaragi, S., Kawai, H., Sasaki, A. 2019. Solitary fibrous tumor arising in the buccal space. *Case Rep Med* **2019:** Article ID 9459837.

Robinson, D.R., Wu, Y.M., Kalyana-Sundaram, S., Cao, X., Lonigro, R.J., Sung, Y.S., Chen, C.L., Zhang, L., Wang, R., Su, F., Iyer, M.K. 2013. Identification of recurrent NAB2-STAT6 gene fusions in solitary fibrous tumor by integrative sequencing. *Nat Genet* **45**(2): 180-5.

Stout, A.P., Murray, M.R. 1942. Hemangiopericytoma: a vascular tumor featuring Zimmermann's pericytes. *Ann Surg* **116**(1): 26-33

Vogels, R.J., Vlenterie, M., Versleijen-Jonkers, Y.M., Ruijter, E., Bekers, E.M., Verdijk, M.A., Link, M.M., Bonenkamp, J.J., van der Graaf, W.T., Slootweg, P.J., Suurmeijer, A.J., Groenen, P.J., Flucke, U. 2014. Solitary fibrous tumor–clinicopathologic, immunohistochemical and molecular analysis of 28 cases. *Diagn Pathol* **9**: 224.

Yokoi, T., Tsuzuki, T., Yatabe, Y., Suzuki, M., Kurumaya, H., Koshikawa, T., Kuhara, H., Kuroda, M., Nakamura, N., Nakatani, Y. and Kakudo, K. 1998. Solitary fibrous tumour: significance of p53 and CD34 immunoreactivity in its malignant transformation. *Histopathology* **32**(5): 423-32.

Zafar, H., Takimoto, C.H., Weiss, G. 2003. Doege-potter syndrome: hypoglycemia associated with malignant solitary fibrous tumor. *Med Oncol* **20** (4): 403-8

Received: 31 Jul 2018
Accepted: 5 Dec 2019