Original Article

Diagnostic value of a monoclonal anti-STAT6 antibody for solitary fibrous tumors: immunohistochemical analyses

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Abstract: Solitary fibrous tumors (SFTs) have been shown to harbor the characteristic NAB2-STAT6 gene fusion. Some recent studies have suggested that STAT6 is a useful marker for the diagnosis of SFTs. The aim of this study was to evaluate the diagnostic value of monoclonal anti-STAT6 in the distinction of SFTs from histologic mimics. The expression of STAT6 was evaluated in tissue microarrays and in whole sections of 407 tumors (including 63 SFTs and 344 non-SFTs) using a monoclonal anti-STAT6 antibody (clone: YE361). Only STAT6 nuclear staining was recorded as positive. All cases of SFTs (100%) exhibited nuclear STAT6 expression, and 45 cases displayed 3+ and strong staining intensity (72%), 7 cases displayed 3+ and moderate staining intensity (11%), 4 displayed 2+ and strong staining intensity (6%), 3 displayed 2+ and moderate staining intensity (5%) and 4 displayed 3+ and weak staining intensity (6%). The vast majority of the non-SFTs (343/344, 99.7%) were negative for STAT6. Only 0.3% (1/344) of the non-SFTs (one case of synovial sarcoma) showed 1+ and weak staining intensity of nuclear STAT6 expression. The sensitivity and specificity of nuclear STAT6 staining were 100% and 99.7%, respectively. In conclusion, the monoclonal anti-STAT6 antibody used in this study is highly sensitive and specific for the differential diagnosis of SFTs. In small biopsy specimens, negativity for STAT6 cannot completely exclude the diagnosis of SFT. Similarly, focal positivity for STAT6 cannot completely exclude the possibility of non-SFTs.

Keywords: Solitary fibrous tumor, STAT6, immunohistochemistry, tissue microarray

Introduction

Solitary fibrous tumors (SFTs) are mesenchymal tumors that belong to the fibroblastic/myofibroblastic family of tumors with a spectrum that ranges from benign to malignant [1]. Some SFTs were previously termed hemangiopericytomas (HPCs), and HPC is often used a synonym of SFT [1, 2].

Histologically, typical SFTs usually show patternless architecture of spindle to ovoid tumor cells with indistinct cytoplasmic borders. In addition, the stroma is usually composed of thick bands of hyalinized collagen and branching hemangiopericytoma-like vessels. Moreover, SFTs may be classified into several subtypes, such as cellular variants, fat-forming variants and malignant SFTs [1-3]. Traditionally, a panel of immunohistochemical markers, including CD34, Bcl-2, and CD99, has been used to distinguish SFTs from their histologic mimics [4]. However, none of these markers is specific for the diagnosis of SFTs.

Recently, the NAB2-STAT6 fusion gene has been identified in the vast majority of SFTs and has been described as a distinct molecular feature of SFTs [5-7]. The NAB2-STAT6 fusion is due to an intra-chromosomal inversion that juxtaposes the 3′ region of NAB2 and the 5′ region of STAT6, which encodes a fusion protein [5-7]. Therefore, the identification of the NAB2-STAT6 fusion gene is helpful in the diagnosis of SFTs. However, molecular studies have not been widely used in all laboratories. Fortunately, Schweizer et al. [8] discovered that this fusion gene may be detected by immunohistochemistry for STAT6. Subsequently, some studies have detected the expression of STAT6 in SFTs using a polyclonal anti-STAT6 antibody (clone: S-20) [9-20]. A commercially available monoclonal
anti-STAT6 antibody (clone: YE361) has only recently emerged, and only 7 studies have used a monoclonal anti-STAT6 antibody to assess the diagnostic value of STAT6 in SFTs [21-27].

Here, we used the monoclonal anti-STAT6 antibody to investigate the expression of STAT6 in SFTs and in a large series of their histologic mimics to evaluate the diagnostic value of STAT6 in the diagnosis of SFTs.

Materials and methods

Study cases and tumor specimens

This study was approved by the West China Hospital Institutional Review Board. Formalin-fixed, paraffin-embedded (FFPE) tissue samples of SFTs and non-SFTs from January 2006 to January 2016 were retrieved from the archives of the Department of Pathology of the West China Hospital of Sichuan University. Pathology reports, histology sections, and immunohistochemically stained slides were reviewed by 3 experienced soft tissue pathologists (H.Z., H.C. and Z.Z.) and 1 general surgical pathologist (X.H.) according to the criteria of the fourth edition of World Health Organization Classification of Tumors of Soft Tissue and Bone and the sixth edition of Enzinger and Weiss’s Soft Tissue Tumors [1, 3].

Tissue microarray (TMA) blocks were constructed. Briefly, routine hematoxylin- and eosin-stained sections were evaluated, and representative areas of the tissues were marked using a felt-tip pen for easy detection. One tissue cylinder with a diameter of 1.2 mm was punched from each morphologically representative area of each tissue block and placed in a recipient paraffin block (40 mm×30 mm×10 mm) using a homemade semiautomated tissue arrayer. To exclude bias due to possible tumor heterogeneity, 3 to 5 punches were obtained from each tumor specimen. A total of 375 tissue samples were selected from the TMA blocks including the following: 49 SFTs, 64 synovial sarcomas, 39 schwannomas, 35 neurofibromas, 32 malignant peripheral nerve sheath tumors (MPNSTs), 19 dermatofibrosarcoma protubersans (DFSPs), 18 rhabdomyosarcomas, 17 Ewing sarcomas, 15 desmoid-type fibromatoses, 15 epithelioid sarcomas, 14 clear cell sarcomas of the soft tissue, 12 leiomyosarcomas, 10 mesenchymal chondrosarcomas, 10 spindle cell carcinomas, 10 myxofibrosarcomas, 9 fibrosarcomas, 7 myofibrosarcomas, 2 dedifferentiated liposarcomas, one spindle cell lipoma, one spindle cell liposarcoma, one cellular fibrous histiocytoma, and one undifferentiated pleomorphic sarcoma.

In summary, 407 cases were included in the present study: 63 SFTs and 344 histological mimics (68 synovial sarcomas, 41 schwannomas, 35 neurofibromas, 34 MPNSTs, 21 DFSPs, 18 rhabdomyosarcomas, 17 Ewing sarcomas, 17 desmoid-type fibromatoses, 15 epithelioid sarcomas, 14 clear cell sarcomas of the soft tissue, 12 leiomyosarcomas, 10 mesenchymal chondrosarcomas, 10 spindle cell carcinomas, 10 myxofibrosarcomas, 9 fibrosarcomas, 7 myofibrosarcomas, 2 dedifferentiated liposarcomas, one spindle cell lipoma, one spindle cell liposarcoma, one cellular fibrous histiocytoma, and one undifferentiated pleomorphic sarcoma). All SFTs were previously assessed via immunohistochemistry with CD34, Bcl-2 and CD99 antibodies. All synovial sarcomas were previously confirmed by fluorescence in situ hybridization (FISH) for t(X;18)(SS18;SSX).

Immunohistochemistry

Four-micrometer-thick sections were cut from TMA blocks and from conventional paraffin tissue blocks and were autoclaved in citrate buffer (pH 6.0) for antigen retrieval. Immunohistochemical staining for STAT6 was performed using a monoclonal antibody (YE361, 1:400 dilution; Abcam, Cambridge, UK) and the EnVision detection system (Dako, Carpinteria, CA, USA). 3,3'-diaminobenzidine (Dako, Carpinteria, CA, USA) was used as the chromogen, and the sections were counterstained with hematoxylin. Both positive and negative control sections were included.

In regards to the STAT6 staining, only nuclear staining was recorded as positive. The results of the STAT6 immunohistochemistry were semi-quantitatively scored using a previously reported scoring system [21]. The extent of staining was graded as follows: 0 (no staining), 1+
(1-25% staining), 2+ (26-50% staining), and 3+ (>50% staining). The staining intensity was graded as negative, weak, moderate, or strong.

**Results**

The 63 SFT specimens were acquired from 31 males and 32 females, and no gender predilection was observed. The age of the patients ranged from 18 to 78 years (mean: 41 years, median: 48 years). This series of SFTs arose from a wide variety of anatomical sites including the lung and pleura (32, 51%), head and neck (8, 13%), mediastinum (6, 9%), pelvis cavity (5, 8%), extremities (4, 6%), abdominal cavity (3, 5%), retroperitoneum (2, 3%), trunk (2, 3%), and inguinal region (1, 2%). The tumor size ranged from 2 to 23 cm (mean: 8.6 cm, median: 6.5 cm). The histological subtypes of the 63 SFTs included 37 (59%) conventional SFTs, 12 (19%) cellular SFTs, one (1%) fat-forming SFT and 13 (21%) malignant SFTs. CD34 immunohistochemical staining was performed in all cases of SFTs at the time of diagnosis. Sixty-two cases of SFTs (98%) were positive for CD34, whereas 1 malignant tumor was negative for this marker. Fifty-four cases were diffusely and strongly positive for CD34, and eight SFTs (seven conventional and one cellular) exhibited focal staining patterns of CD34 expression.

A summary of the STAT6 immunohistochemical staining results is provided in Table 1. All cases of SFTs (100%) exhibited nuclear positivity, regardless of the anatomic site and histological subtype (Figure 1). Among this series of SFTs, the rates of STAT6 positivity in TMAs and whole sections were 94% (46/49) and 100% (14/14), respectively. The three cases of STAT6-negative SFTs (two conventional and one cellular) from the TMAs were again subjected to STAT6 immunohistochemistry but as whole-sections. The results showed that one conventional SFT and one cellular SFT displayed 3+ and strong staining intensity, while one conventional SFT displayed 3+ and weak staining intensity. In summary, the distribution and the staining intensity were as follows: 45 cases (72%) displayed 3+ and strong staining intensity, 7 (11%) displayed 3+ and moderate staining intensity, 4 (6%) displayed 2+ and strong staining intensity, 3 (5%) displayed 2+ and moderate staining intensity and 4 (6%) displayed 3+ and weak staining intensity. The majority of SFTs (52/63, 83%) displayed diffuse staining (3+) and a pattern of moderate to strong staining intensity.

The vast majority of the nonSFTs (343/344, 99.7%) were negative for STAT6 expression (Figure 2). Only a single case of the nonSFTs (1/344, 0.3%) (one typical monophasic synovial sarcoma), showed 1+ and a weak intensity with respect to nuclear reactivity. However, this...
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monophasic synovial sarcoma was positive for the expression of Transducin-like enhancer of split 1 (TLE1), epithelial membrane antigen, AE1/AE3, and CK7. More importantly, this tumor was found to harbor t(X; 18) (Figure 3).

In addition, 16% (55/344) of the nonSFTs, including 24 (35%) synovial sarcomas, 21 (51%) schwannomas, 5 (14%) neurofibromas, 3 (14%) DFSPs and 2 (29%) myofibrosarcomas, exhibited cytoplasmic STAT6 staining to various degrees and all of them exhibited focal or patchy distribution patterns with weak to moderate staining intensity (Figure 4).

In regards to the diagnosis of SFTs, the sensitivity and specificity of nuclear STAT6 staining were 100% and 99.7%, respectively.

**Discussion**

Most conventional SFTs can be diagnosed directly by a combination of morphologic features and the expression of traditional immunohistochemical markers including CD34, CD99 and Bcl-2. However, SFTs are sometimes difficult to distinguish from nonSFTs, especially when the tumors exhibit peculiar clinical findings or unusual morphologies. For example, SFT can be confused with other CD34-positive spindle cell neoplasms. Furthermore, negativity for CD34 cannot rule out the possibility of SFT. The NAB2-STAT6 fusion gene is a novel molecular genetic discovery in SFTs [5-7]; this fusion gene may exist as several variants such as NAB2ex4-STAT6ex2/3 and NAB2ex6-STAT6ex16/17 [9, 17, 20, 22-24, 26, 28]. However, the C-terminus of STAT6 is present in all fusion proteins [5-7]. Therefore, an antibody that recognizes the C-terminus of STAT6 has been proposed as a surrogate marker for the NAB2-STAT6 fusion gene in the diagnosis of SFTs [8]. Thus far, the majority of the previous studies used a polyclonal anti-STAT6 antibody [9-20], and the rates of STAT6 reactivity in SFTs ranged from 86% to 100% [9-20].

Prior studies on the use of monoclonal anti-STAT6 antibodies for SFTs are relatively scarce. In the present study, we used a monoclonal anti-STAT6 antibody to investigate the expression of STAT6 in SFTs and in a large series of histologic mimics. Nuclear STAT6 positivity was observed in 100% of the SFTs regardless of the anatomic site and histological subtype, and no cytoplasmic staining was observed; moreover, the sensitivity of this antibody was 100% for the diagnosis of SFTs. In addition, we noticed that STAT6 usually (83%) displays a diffuse staining pattern and moderate to strong staining intensity in the series of SFTs. In previous series, when monoclonal antibodies were used, the rates of STAT6 positivity in SFTs ranged from 72% to 100% [21-27].

Based on previous research and the present study, STAT6 is generally highly sensitive for SFTs, irrespective of whether a monoclonal or a polyclonal anti-STAT6 antibody is used. However, in the study by Macagno et al. [27], two negative cases that were stained with a monoclonal anti-STAT6 antibody exhibited a nuclear signal with a polyclonal anti-STAT6 antibody. Their results may indicate greater sensitivity of the polyclonal antibody for degraded antigen in older samples.

As is well known, CD34 seems to be one of the most important diagnostic markers for SFTs in clinical practice [1, 3]. However, approximately 5%-10% of SFTs are negative for CD34 [1], and CD34 expression is also common in spindle cell lipomas, DFSPs, epithelioid sarcomas and MPNSTs [29-32], which may be easily mistaken for SFTs, especially in small biopsy specimens. In our study, all SFTs were subjected to immunohistochemistry for CD34 at the time of diagnosis. One malignant SFT was negative for CD34, and 8 SFTs (seven conventional and one cellular) exhibited focal staining patterns of CD34 expression in whole sections; however, all of them exhibited STAT6 3+ and strong staining intensity in TMAs. Among the samples in
In this series, the rate of STAT6 positivity in TMAs was 94%. Interestingly, the tissue volumes used in TMAs are very small and are thus similar to biopsy specimens to some extent. These findings indicate that STAT6 is a sensitive and useful marker for the diagnosis of SFTs when the biopsy specimens are small. Cheah et al. [21] also suggested that STAT6 immunohistochemistry may be a valuable diagnostic adjunct in diagnostically challenging cases, particularly those where core needle biopsies were obtained. However, it should be noted that, in our study, the three cases of STAT6-negative SFTs in the TMAs were positive for STAT6 in whole sections. This may imply that in small biopsy specimens, STAT6-negativity does not completely rule out the possibility of SFTs. In such situations, traditional immunohistochemical markers may be complementary, and surgically resected specimens are necessary for a definitive diagnosis.

In previous studies, when a monoclonal anti-STAT6 antibody was used, none of the nonSFTs showed nuclear positivity for STAT6 [21, 27]. In the present study, the specificity of this biomarker for SFTs was 99.7%, which is similar to that in previous studies. However, in the pres-
ent study, nuclear STAT6 reactivity was observed in single case of monophasic synovial sarcoma, which demonstrated 1+ and weak staining intensity. Of course, further investigation is warranted to explain this phenomenon. This indicates that focal and weak nuclear STAT6 staining may not lead to a definitive diagnosis of SFTs. In contrast, diffuse and strong nuclear STAT6 staining with a monoclonal antibody may be highly suggestive of SFTs.

In previous studies, which used polyclonal anti-STAT6 antibodies, the positive rate of non-SFTs ranged from 1% to 4% [8, 11-15]. The non-SFTs comprised a wide variety of tumor types as follows: dedifferentiated liposarcoma (1.4%~14%) [11, 12, 14, 15, 33, 34], meningioma (3/90) [8], deep fibrous histiocytoma (1/10) [11], undifferentiated pleomorphic sarcoma (2/130, 2/173) [12, 14], nodular fascitis (1/63) [12], low-grade fibromyxoid sarcoma (2/7), myxoid/round cell liposarcoma (1/9), ovarian fibroma (1/2) [13], desmoid-type fibromatosis (14/184), unclassified spindle cell/epithelioid sarcoma (8/65) [14], well-differentiated liposarcoma (2/75) and synovial sarcoma (1/15) [15]. This may suggest that the specificity of polyclonal anti-STAT6 antibodies is slightly lower than that of monoclonal anti-STAT6 antibodies.

It should be noted that reactivity for polyclonal anti-STAT6 antibodies in a small subset of non-SFTs can pose potential pitfalls in the differential diagnosis of SFTs, especially for the variant subtype. Among the aforementioned non-SFTs, the most remarkable is dedifferentiated liposarcoma [11, 12, 14, 15, 33, 34]. It is difficult to distinguish dedifferentiated liposarcoma from fat-forming SFTs, especially when small biopsy samples are obtained. However, the majority of these dedifferentiated liposarcomas displayed weak to moderate, focal to mul-

Figure 4. STAT6 immunohistochemistry in non-SFTs. A. Antoni A areas with short fascicles and focal nuclear palisading in a schwannoma. B. Weak to moderate granular cytoplasmic staining pattern of STAT6 in a schwannoma. C. A neurofibroma contains elongated cells with wavy and darkly stained nuclei. D. A small subset of tumor cells exhibits weak cytoplasmic staining for STAT6 in a neurofibroma (original magnification 400×).
tifocal nuclear STAT6 staining, but the STAT6 expression in SFTs is usually diffuse and strong [33]. Immunohistochemistry for MDM2 or CDK4 [35], especially FISH for MDM2 amplification [36], is an invaluable diagnostic tool for dedifferentiated liposarcomas. Doyle et al. [33] demonstrated STAT6 amplification by FISH in 4 cases that were positive for STAT6 by immunohistochemistry. STAT6 (on 12q13) is close to MDM2 (on 12q14.3-q15) and CDK4 (on 12q14) on chromosome 12q. Therefore, the 12q amplification that includes the STAT6 locus might be one of the reasons for STAT6 amplification in a small subset of dedifferentiated liposarcomas [11, 12, 33]. However, the amplifications of 12q13-15 are frequent in dedifferentiated liposarcomas [37], whereas STAT6 amplification is only a low-frequency event in dedifferentiated liposarcomas. Therefore, further investigation is warranted to explain this phenomenon. Synovial sarcomas may share some morphologic features with SFT and seem to be one of the most challenging tumor types in terms of the differential diagnosis of SFTs. However, synovial sarcomas usually show strong positivity for TLE1 [38-41] and do not show diffuse reactivity for STAT6 or CD34. The definitive diagnosis should be aided by molecular tests in extremely difficult cases.

STAT6 is a transcription factor that is involved in interleukin 4 signaling [42]. Normally, wild-type NAB2 protein localizes to the nucleus [43], whereas wild-type STAT6 protein is primarily localized to the cytoplasm and phosphorylated STAT6 translocates to the nucleus [42]. Therefore, the STAT6 positive signal is located in the nucleus due to the formation of NAB2-STAT6 fusion protein. In the present study, 16% (55/344) of the nonSFTs, including synovial sarcomas, schwannomas, neurofibromas, DFSPs and myofibrosarcomas, exhibited cytoplasmic staining. This result was similar to that of previous studies, especially those that used polyclonal anti-STAT6 antibodies [8, 13]. However, pathologists should consider that cytoplasmic staining must not be interpreted as positive in the differential diagnosis of SFTs. Therefore, the correct interpretation of the results of STAT6 immunohistochemistry is very important.

In summary, this study is a single-center, large-scale analysis of STAT6 immunohistochemical staining in SFTs and their histological mimics in Chinese patients using a monoclonal anti-STAT6 antibody. This antibody is highly sensitive and specific for the differential diagnosis of SFTs. It should be noted that negativity for STAT6 cannot completely rule out the diagnosis of SFTs in small biopsy specimens. Similarly, focal positivity for STAT6 cannot completely exclude the possibility of nonSFTs.

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Disclosure of conflict of interest

None.

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