

# Poorly differentiated pulmonary synovial sarcoma with SYT gene amplification: A case report

XIAOYAN LI\*, DAN WU\*, YUHUI ZHENG, HUANXING YANG and YINGHONG YANG

Department of Pathology, Fujian Medical University Union Hospital, Fuzhou, Fujian 350001, P.R. China

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**Abstract.** Fluorescence *in situ* hybridization (FISH) and reverse-transcription polymerase chain reaction (RT-PCR) analysis may be used for the diagnosis of synovial sarcoma (SS), particularly of the poorly differentiated type. While the majority of the studies report that the SYT FISH probe is considered to be break-apart in SS, with two orange and two green signals, the SYT probe in the present case of a 52-year-old male patient with pulmonary SS displayed orange and green signal separation, along with SYT orange signal amplification. RT-PCR was used to verify that the SYT gene amplification was another form of expression of SYT-SSX gene fusion t(X; 18)(p11; q11). In this case, the tumour sample obtained by biopsy was small; therefore, the definitive diagnosis of poorly differentiated SS originating from the lung with SYT gene amplification was confirmed by FISH and RT-PCR. Therefore, these mature biomarkers, which are available as immunohistochemical stains in the molecular pathology laboratory, may help pathologists to diagnose intractable soft tissue tumours based only on small cytological specimens.

## Introduction

Synovial sarcoma (SS) may originate from pluripotent stem cells of unknown origin. SS is a malignant tumour bearing the characteristics of diphasic epithelial and mesenchymal cell differentiation. SS has three histological types, including monophasic, diphasic and poorly differentiated. The monophasic and poorly differentiated types are difficult to diagnose based on histomorphology alone, and molecular detection methods play an important accessory diagnostic role in such cases. SS has a characteristic chromosome translocation t(X;18)(p11;q11),

resulting in SYT-SSX gene fusion. This genovariation may be detected by fluorescence *in situ* hybridization (FISH) and reverse transcription-polymerase chain reaction (RT-PCR) analysis. The majority of studies have reported that the SYT FISH probe was considered to be break-apart when a pair of orange and green signals was separated by a distance greater than the size of one hybridization signal in the SS cell nucleus (1). In the present case report, the SYT FISH probe displayed orange and green signal separation along with the SYT orange signal amplification in a poorly differentiated pulmonary SS.

## Case report

A 52-year-old male patient with a smoking history of 2 packs/day for >30 years presented with left-sided chest pain for 2 months. The patient had no cough, sputum, dyspnoea or palpitations, but reported a 5-kg weight loss since the appearance of the symptoms. Thoracic pre- and post-contrast enhanced computed tomography (CT) scans revealed a solid mass with rough edges in the upper lobe of the left lung, 8.1x8 cm in size. In addition, another small nodule with greatest diameter 0.5 cm displaying the same characteristics was identified in the inferior lobe of the right lung, and multiple enlarged lymph nodes in the left pulmonary hilum and mediastinum were also observed. Magnetic resonance imaging examination revealed multiple disseminated nodules in the bilateral frontal and parietal lobes, and color Doppler flow imaging also demonstrated bilateral supraclavicular lymph node enlargement. The patient underwent CT-guided percutaneous transthoracic needle aspiration biopsy of the mass in order to determine the clinicopathological diagnosis.

The tissue sample was fixed in formalin and embedded in paraffin, then processed according to the standard procedure including dehydration, clearing, wax embedding and sectioning, and stained with hematoxylin and eosin. Immunohistochemical (IHC) staining for vimentin, epithelial membrane antigen (EMA), CD34, thyroid transcription factor 1 (TTF-1), cytokeratin (CK), calretinin, Wilms' tumor-1 (WT-1), mesothelial cell (MC), napsin A, surfactant protein (SP)-B, P40, P63, CK5/6, CD56, synaptophysin (Syn), chromogranin A (CgA), desmin, CD99, S-100, CD45, CDX2, placental alkaline phosphatase (PLAP), CK19, glypican-3 and Ki-67 (Fuzhou Maixin Biotech Co., Ltd., Fuzhou, China) was performed in the Lumatas automated immunostainer (Fuzhou Maixin Biotech Co., Ltd.) with appropriate controls.

*Correspondence to:* Dr YingHong Yang, Department of Pathology, Fujian Medical University Union Hospital, 29th Xinquan Road, Fuzhou, Fujian 350001, P.R. China  
E-mail: yyh1555@163.com

\*Contributed equally

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For FISH, 2.5- $\mu$ m paraffin-embedded sections were transferred to APES and baked for 2 h at 65°C. The slides were deparaffinized by xylene and washed in 100% ethanol. Following pretreatment in boiling water for 20 min, the slides were incubated in 0.5 mg/ml pepsin solution (in 0.01N HCl, pH 2.0) at 37°C for 7 min. Then, the SYT break-apart probe mixture (probe:hybridization buffer:purified H<sub>2</sub>O=1:7:2, GP Medical Technologies Co., Ltd., Beijing, China) was added to the slides in the dark. The slides were then incubated at 83°C for 5 min to co-denature, followed by hybridization with the probes in StatSpin ThermoBrite (Abbott Pharmaceutical Co. Ltd., Lake Bluff, IL, USA) at 42°C overnight. Following hybridization, the slides were counterstained with 10  $\mu$ l DAPI reagent (Vysis, Abbott Laboratories, Abbott Park, IL, USA) and coverslipped. Fluorescence signals were observed by a fluorescence microscope (Olympus BX51; Olympus Corporation, Tokyo, Japan).

For RT-PCR analysis, total RNA was isolated from paraffin-embedded tumour tissue sections according to the nucleic acid extraction kit protocol (Amoydx Biomedical Technology, Xiamen, China) and reverse-transcribed with Prime Script™ RT reagent kit (Takara Biotechnology Co., Ltd., Dalian, China). The tumour cDNA was amplified with primers for the SYT-SSX gene (forward primer for SYT: 5'-CCAGCAGAGGCCTTATGGATA-3'; reverse primer for SXX1: 5'-GTGCAGTTGTTTCCCATCG-3' 118 bp, and for SXX2: 5'-GCACAGCTCTTCCCATCA-3', 118 bp). The h-actin gene (forward primer: 5'-TGGGTGTGAACCATGAGAAGT-3' and reverse primer: 5'-AAGGCCATGCCAGTAGCTT-3' 310 bp) was used as an internal control for PCR and for quality assessment of the tumour cDNA. The amplification conditions included an initial denaturation step at 95°C for 5 min, followed by denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 30 sec for 40 cycles, with a final extension step at 72°C for 10 min. The PCR products were separated by electrophoresis in agarose gels and visualized with ethidium bromide. The amplified fragments were identified according to their size on gels.

On histological examination of the tissue sections under an optical microscope, the boundary between the tumour and the surrounding lung tissue was unclear. The tumour cells around the thin-walled vessels in the stroma appeared to be loosely attached to each other and they were small and round, with scant cytoplasm and round or oval cell nuclei with homogeneous granulated chromatin. A small nucleolus and pathological mitoses were readily identified (Fig. 1A).

On IHC examination, the tumour cells were diffusely positive for vimentin (Fig. 1B) and EMA (Fig. 1C), focally positive for CD34 and TTF-1, and ~80% were Ki-67-positive (Fig. 1D). The tumour cells were negative for CK, calretinin, WT-1, MC, napsin A, SP-B, P40, P63, CK5/6, CD56, Syn, CgA, desmin, CD99, S-100, CD45, CDX2, PLAP, CK19 and glypican-3 expression.

As regards FISH, the SYT probe was considered to be break-apart when a pair of orange and green signals was separated by a distance greater than the diameter of one hybridization signal. Tumour cells positive for SYT exhibited at least one pair of break-apart signals per nucleus. In this case, five different tumour areas were observed under the fluorescence microscope with clear DAPI counterstaining and fluorescence signals. In each

tumour zone, 20 non-overlapping cell nuclei with SYT signal were continuously counted. The total ratio of SYT-positive cells was calculated as 67% in 100 cells. Interestingly, 60% of the cell nuclei presented as clusters of orange signals (~10-12 signals per nucleus) against the background of separated signals, while the green signals were ~1-3 per nucleus (Fig. 1E).

In addition, RT-PCR was applied to confirm the FISH results and SYT-SSX2 gene fusion transcripts were detected by electrophoresis in agarose gels (Fig. 1F).

The final diagnosis was 'small round-cell malignant nodules in the upper lobe of the left lung, with molecular pathological characteristics consistent with poorly differentiated SS'.

## Discussion

In the present case, the examination of the lung mass biopsy specimens under the microscope revealed small round-cell malignant nodules. The histological and immunohistochemical findings did not include diagnostic pathological characteristics. Lung adenocarcinoma, squamous cell carcinoma, small-cell carcinoma, as well as mesothelioma, lymphoma, and other common types of poorly differentiated carcinomas that commonly metastasize to the lung should be ruled out. However, depending on the above-mentioned evidence, which should always lead to diagnostic hesitancy, it is important to make the differential diagnosis clearly because the presence of overlapping categories which have significant prognostic and therapeutic implications respectively. In the present case, the tumour did not display specific epithelial and/or mesenchymal morphological characteristics; therefore, it was difficult to diagnose poorly differentiated SS based only on a small number of tumour cells. The morphological characteristic of SS, consisting of with small round cells, markedly overlapped with other sarcomas, including Ewing's sarcoma/peripheral neuroectodermal tumour (ES/PNET) family, neuroblastoma, rhabdomyosarcoma, malignant hemangiopericytoma and mesenchymal chondrosarcoma. The immunohistochemical phenotype was not helpful for the diagnosis of SS, with vimentin- and EMA-positive results.

The chromosomal translocation t(X;18)(p11.2;q11.2) is both sensitive and specific for SS. Use of this chromosomal anomaly as a diagnostic biomarker may enable accurate diagnosis of SS, particularly when the available specimens are small. In our case, the balanced chromosomal translocation was detected by both FISH and RT-PCR. In several laboratories, FISH is performed to confirm the diagnosis of SS as the preferred molecular test; it allows the detection of molecular characteristics together with histomorphology, and non-neoplastic areas can be excluded (2). Under the fluorescence microscope, 5 tumour areas and a total of 100 tumour nuclei were counted, and the positive ratio of SYT gene break-apart was found to be 67%. Surprisingly, 60% tumour nuclei presented SYT C'-terminal fragment amplification (orange spectrum 800 kb, green spectrum 1,000 kb) in the genetic disruption background. In order to explore whether the SYT amplification was another expression form of the SYT-SSX gene fusion, RT-PCR was used to verify the results, and SYT-SSX2 gene fusion transcripts were identified in the agarose gels. Combined with the FISH and PCR results, there was sufficient evidence to support the diagnosis of poorly differentiated SS in the upper lobe of the left lung.

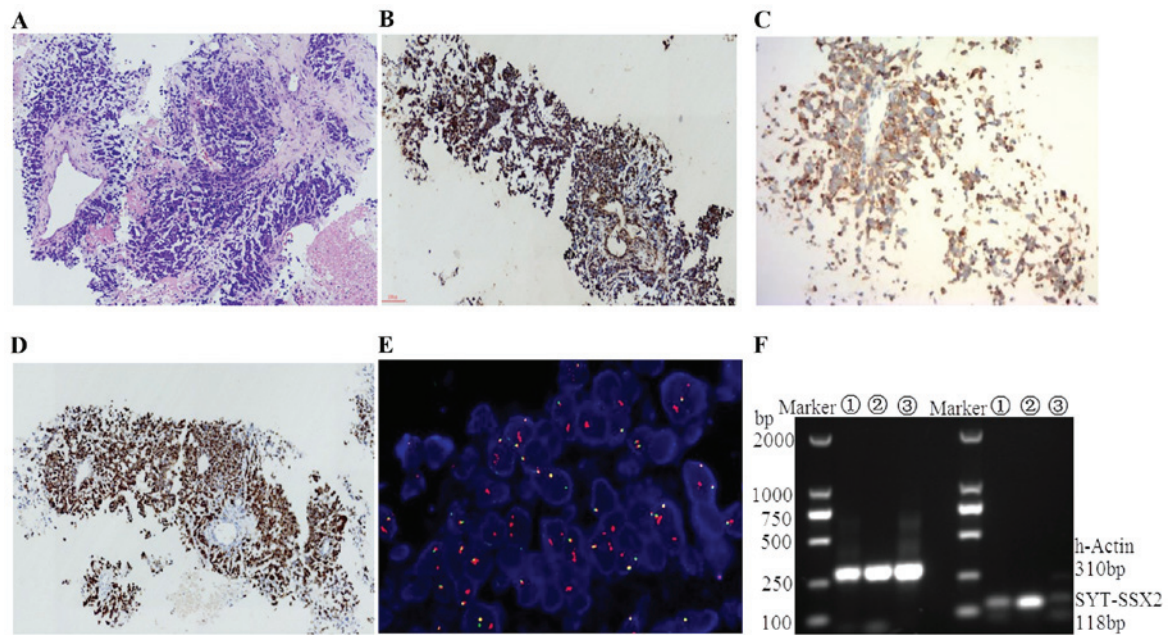


Figure 1. Histomorphology, immunochemistry, FISH and RT-PCR results of poorly differentiated synovial sarcoma. (A) The tumour cells around the thin-walled vessels were small and round, with scant cytoplasm and round or oval nuclei; haematoxylin and eosin stain, magnification, x100. (B) Immunostaining for vimentin was diffusely positive; magnification, x100. (C) Immunostaining for epithelial membrane antigen was diffusely positive; magnification, x200. (D) Immunostaining for Ki-67 was positive (80%); magnification, x100. (E) The FISH images revealed 60% of tumour cell nuclei forming clusters of orange signals gathered against the background of the orange and green signal break-apart. (F) The RT-PCR result revealed the SYT-SSX2 fusion transcripts in agarose gels: ①③ present case, ② positive control. FISH, fluorescence *in situ* hybridization; RT-PCR, reverse transcription-polymerase chain reaction.

According to the FISH product specification, the C'-terminal of the SYT gene (orange fluorescence) is the transcriptional activation domain, which is rich in glutamine, proline, glycine and tyrosine. In the present case, multiple orange signals in clumps were detected in over half of the tumour cells. In view of this observation, it was hypothesized that translocation may activate the SYT transcriptional activation domain resulting in the amplification of the functional gene fragment. Then, the SYT-SSX fusion protein may cause a disorder of gene expression in tumour cells (3). Some studies have confirmed that the genes involved in the Wnt signalling pathway, including TLE1, were upregulated by the SYT-SSX fusion proteins (4,5), promoting the malignant progression of SS.

Some researchers have reported that the SYT-SSX fusion transcripts are not only a definitive diagnostic marker of SS, but also yield important independent prognostic information (6). It was reported that a patient with a SS bearing the SYT-SSX2 translocation had a better prognosis compared with a patient bearing the SYT-SSX1 translocation (7,8). This conclusion was supported by a multi-institutional retrospective study of 243 SS patients by Ladanyi *et al* (9). The patient in the present case harboured the SYT-SSX2 fusion transcript, as shown by RT-PCR, and exhibited no other symptoms apart from emaciation during the 6-month interval from the date of symptom onset to the date of death in August, 2017, although multiple lesions were detected in the lungs, brain and lymph nodes of the mediastinum, left pulmonary hilum and supraclavicular regions when the patient first visited a doctor on February 2017. During those 6 months, the patient had been taking traditional Chinese medicines at home, having declined radiotherapy and chemotherapy.

In summary, this case of poorly differentiated SS originating from the lung with SYT gene amplification was confirmed by

FISH and RT-PCR. These mature biomarkers, available as immunohistochemical stains in the molecular pathology laboratory, may help pathologists to diagnose intractable soft tissue tumours based only on small cytological specimens (10).

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#### Availability of data and materials

The data and materials are available from the corresponding author on reasonable request.

#### Authors' contributions

XL is the major contributor to the writing of the manuscript. DW collected all the patient's clinical and follow-up visit data. XL and DW are co-first authors. HY performed the histological examination. YZ performed the molecular examination. YY provided the tissue materials and pathological data. All the authors have approved the final version of the manuscript.

#### Ethics approval and consent to participate

The study was approved by the Institute Research Ethics Committee of Fujian Medical University Union Hospital.

### Patient consent for publication

Informed consent for publication of the case details and any associated images was obtained from the patient's family according to the regulations of Institute Research Ethics Committee of Fujian Medical University Union Hospital.

### Competing interests

The authors declare that they have no competing interests.

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