

False-negative fluorescence *in situ* hybridisation in synovial sarcoma: a case of missed *SS18::SSX2* fusion due to a rare genomic insertion



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To the Editor,

Synovial sarcoma is a malignant soft-tissue tumour characterised by variable epithelial differentiation and a distinctive genetic signature. Histologically, synovial sarcoma is classified into two main subtypes: monophasic and biphasic. Monophasic synovial sarcoma consists of spindle cells arranged in sheets or fascicles, while biphasic synovial sarcoma contains both spindle cell and epithelial components. Poorly differentiated areas with round or spindle cells, high nuclear atypia, high mitotic activity and necrosis may be present. Immunohistochemically, tumour cells often express cytokeratin, epithelial membrane antigen (EMA), CD56 and transducin-like enhancer of split 1 (TLE1).¹

Genomically, synovial sarcoma is defined by a translocation between chromosomes 18 and X, resulting in the formation of one of three fusion genes: *SS18::SSX1*, *SS18::SSX2* or *SS18::SSX4*.² In addition to these common fusions, rare variants have been reported, including *SS18L1::SSX1*, *SS18::NEDD4*, *EWSR1::SSX1* and *MN1::SSX1*.³ The *SS18::SSX* fusion protein drives synovial sarcoma transformation and proliferation by targeting the SWI/SNF (switch/sucrose non-fermentable) BAF (BRG1/BRM-associated factor) complex; this leads to loss of the tumour suppressor SMARCB1 (SWI/SNF-related BAF chromatin remodelling complex subunit B1) and disruption of chromatin remodelling.⁴

Synovial sarcoma can often be suspected based on morphological features and immunohistochemistry, but genomic confirmation is usually required for a definitive diagnosis. The fusion can be detected by fluorescence *in situ* hybridisation (FISH), reverse transcription polymerase chain reaction (RT-PCR) or next-generation sequencing (NGS). In addition to molecular assays, a recently developed *SS18*-*SSX* translocation-specific rabbit monoclonal antibody allows direct immunohistochemical demonstration of the fusion protein and provides a rapid ancillary diagnostic tool.⁵ FISH with the *SS18* break-apart probe has been widely used in clinical laboratories and is considered the gold standard. However, the sensitivity of FISH is reported to be less than 90%,⁶ and it can yield false-negative results in cases with atypical fusions or complex genomic rearrangements. According to the 2020 World Health Organization classification of soft-tissue tumours, the diagnosis of synovial sarcoma can be established on the basis of characteristic histologic and immunohistochemical features alone; molecular confirmation is considered a desirable, but not mandatory criterion, that is

most useful in selected ambiguous cases. The present report illustrates the critical value of molecular testing when routine morphology and immunoprofile are inconclusive.

We report a case of poorly differentiated synovial sarcoma with an *SS18::SSX2* fusion that was not detected by FISH but was identified by targeted RNA NGS. Whole-genome sequencing (WGS) revealed a complex insertion rearrangement between *SS18* and *SSX2*, highlighting the limitations of FISH in detecting such genomic events. The findings emphasise the need for complementary molecular techniques to ensure accurate diagnosis and appropriate management of synovial sarcoma.

A 2-year-old female patient presented with a papillary mass on the dorsum of her tongue. The surgically removed tumour measured 1.3×0.9×0.1 cm; it appeared greyish white to slightly yellow and had soft to firm texture. Microscopic examination revealed a tumour located in the connective tissue adjacent to the basement membrane, predominantly composed of spindle-shaped cells with focal areas of round cells. The spindle-shaped tumour cells were arranged in bundles and intertwined, with a fascicular and herringbone pattern. The cells exhibited moderately eosinophilic to clear cytoplasm, finely granular or glassy chromatin, and a small nucleolus. Mitotic figures were easily observed, along with areas of haemorrhage and vascular proliferation (Fig. 1A–D).

Immunohistochemistry demonstrated diffuse positivity for *SSX* and CD56, with additional diffuse TLE1 expression. Scattered positivity for smooth muscle actin and EMA was also observed, while S100 and SOX10 showed focal positivity. Control skeletal muscle tissue showed positive MyoD1, myogenin and desmin staining (Fig. 1E–I). Immunohistochemistry for the *SS18*-*SSX* fusion protein [using E9X9V rabbit monoclonal antibody 72364 (Cell Signaling Technology)] was additionally performed and showed completely negative staining in the tumour cells.

FISH analysis was performed on formalin-fixed paraffin-embedded (FFPE) tumour tissue sections using break-apart probe sets targeting the 5'-end (red) and 3'-end (green) of the *SS18* gene, spanning a 75-kb genomic region (Fig. 2A). The results showed no split signals in the tumour cells, indicating a negative result for *SS18* rearrangement (Fig. 2B).

RNA NGS targeting 118 genes commonly involved in solid tumours was performed on total RNA isolated from FFPE tumour tissue sections. The sequence reads were visualised using Integrative Genomics Viewer, which showed an *SS18::SSX2* fusion transcript, including the first 10 exons of *SS18* and the last four exons of *SSX2*, consistent with the classic *SS18::SSX2* fusion pattern (Fig. 2C).

WGS was performed on DNA isolated from FFPE tumour tissue sections, which identified a complex insertion rearrangement between *SS18* and *SSX2*. An 87.5-kb fragment from chromosome X (chrX: 51856187–52731050), containing the last four exons of *SSX2*, replaced a 12.8-kb region on chromosome 18 (chr18: 23599342–23612131), resulting in the formation of a fusion containing the first nine exons of *SS18* and the last four exons of *SSX2*. Identical microhomology

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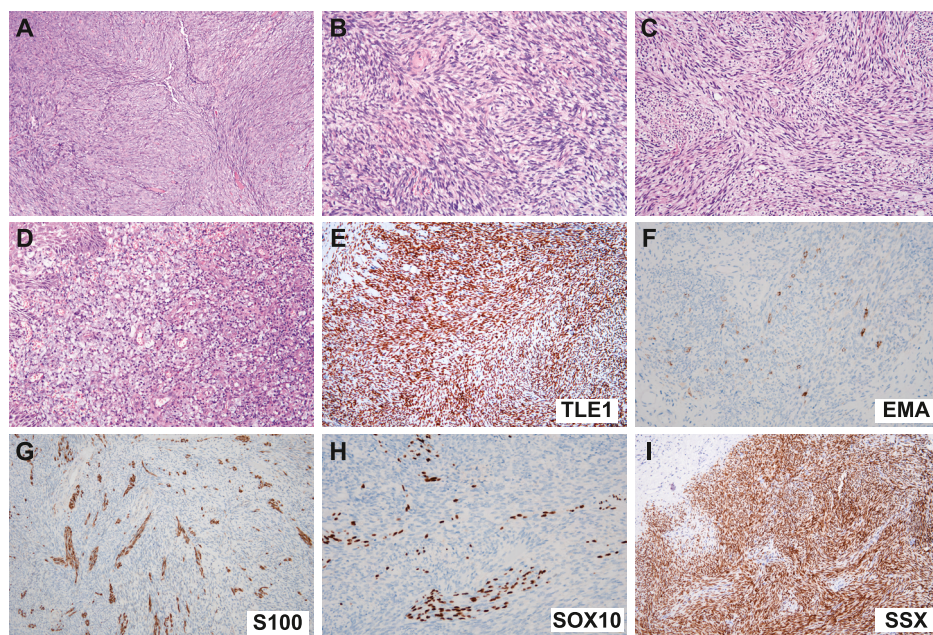


Fig. 1. Histopathological and immunohistochemical features of the tumour. (A) Magnification of $\times 100$, the tumour tissue is slightly lobulated and consists of spindle cells, and a small amount of residual skeletal muscle tissue can be seen between the tumour tissues. (B,C) Magnification $\times 200$, the spindle-shaped tumour cells are arranged in bundles and intertwined. (D) Magnification of $\times 200$, the tumour tissue is in the subepithelial stroma and composed of epithelioid cells, the mesenchyme is rich in blood vessels, the cytoplasm is eosinophilic or translucent, and the nucleus is vacuolated and has a small nucleolus visible. (E) Immunohistochemistry shows that the tumour tissue diffusely expresses TLE1. (F) Immunohistochemistry shows that the tumour tissue sporadically expresses EMA. (G,H) The tumour tissue sporadically expresses S100 and SOX10 in small amounts. (I) Immunohistochemistry shows that the tumour tissue diffusely expresses SSX. EMA, epithelial membrane antigen; SSX, synovial sarcoma X chromosome breakpoint; TLE1, transducin-like enhancer of split-1.

sequences, including 'CA' at the 5'-end and 'TTTT' at the 3'-end, were present at both breakpoint junctions, suggesting a mechanism involving homologous recombination (Fig. 2D,E). Based on the histopathological, immunohistochemical and molecular evidence of *SS18::SSX2*, a final diagnosis of poorly differentiated synovial sarcoma was established.

Synovial sarcoma is a rare and aggressive soft-tissue tumour that primarily affects young adults. However, approximately 30% of cases occur in individuals younger than 20 years, with rare instances of congenital synovial sarcoma reported.⁷ Owing to its non-specific symptoms—often presenting as a slow-growing, painless lump—early diagnosis can be challenging. The prognosis varies, with better outcomes typically observed in younger patients, those with smaller tumours (<5 cm) and those with localised tumours. Treatment generally involves a combination of surgery, radiation therapy and chemotherapy. In recent years, targeted therapies have shown promise, including pazopanib, a multikinase inhibitor, and tazemetostat, an inhibitor of enhancer of zeste homologue 2 (EZH2).^{8,9} Pazopanib targets several key pathways involved in cancer cell growth, survival and angiogenesis.⁸ The *SS18-SSX* fusion protein directly interacts with and deregulates polycomb repressive complex 2 (PRC2), which contains EZH2.¹⁰ As a result, tazemetostat has shown efficacy in treating metastatic synovial sarcoma.¹⁰ Additionally, afamitresgene autoleucel, an engineered T-cell therapy targeting melanoma-associated antigen A4 (MAGE-A4), is being investigated for treating solid tumours, including synovial sarcoma. Early clinical trial results for afamitresgene autoleucel have shown promising signs of tumour shrinkage and stable disease in some synovial sarcomas, with evidence of T-cell expansion and an active immune response.¹¹

Despite these advances, diagnosing synovial sarcoma can still be complex, particularly in clinical settings with limited access to advanced molecular testing. FISH using an *SS18* break-apart probe is a standard laboratory assay for synovial sarcoma, although it can be non-informative in certain cases. Several translocation variants that do not involve *SS18* on chromosome 18 have been reported. One example is *SS18L1*, a gene on chromosome 20 that shares functional domains with *SS18*, including an N-terminal SYT homology domain and a C-terminal QPGY domain.¹² In these cases, a t(X;20) (p11;q13.3) translocation, rather than the usual t(X;18), was detected. This results in the formation of *SS18L1::SSX1*, with exon 10 of *SS18L1* fused to exon 6 of *SSX1*.¹³ Other

non-*SS18* rearrangements include *EWSR1::SSX1* and *MN1::SSX1*. These non-*SS18* variants constitute less than 5% of all synovial sarcoma cases.

In the case that we have reported, poorly differentiated synovial sarcoma with an *SS18::SSX2* fusion was not detected by FISH but was identified through targeted RNA NGS. Molecular analysis revealed a small insertion, where a segment of *SSX2* from chromosome X was inserted into intron 10 of *SS18* on chromosome 18, replacing a 12.8 kb DNA fragment within *SS18*. Due to the small size of the insertion (87.5 kb), the FISH signals remained closely spaced and were not visibly separated at the microscopic resolution, leading to a false-negative result. Interestingly, the first two nucleotides and the last four nucleotides at both breakpoints on chromosomes 18 and X were identical, suggesting that homologous recombination mediated this rearrangement.¹⁴ Microhomology refers to short stretches of sequence similarity (typically 2–6 base pairs) between two DNA regions. Increasing evidence suggests that microhomology can facilitate chromosomal translocations and other genomic alterations by serving as a substrate for DNA repair mechanisms such as non-homologous end joining or microhomology-mediated end joining.¹⁵ Whether this represents a recurrent event in synovial sarcoma remains to be investigated.

Despite its widespread use, FISH has limitations in detecting complex genomic rearrangements, as demonstrated in this case. The false-negative result highlights the importance of considering alternative molecular techniques when faced with challenging cases. Whilst WGS is not practical for routine diagnostics due to its high cost and time-consuming nature, it serves as a powerful tool for uncovering complex genomic rearrangements that may be missed by standard analyses. In this case, WGS was crucial for identifying the rare insertion event, but targeted RNA NGS or RT-PCR would be more practical for routine diagnostic purposes. Each diagnostic technique has its strengths and limitations, and the choice of method should be tailored to the clinical context.

Immunohistochemical results, such as the diffuse positivity for SSX, TLE1 and CD56 in this case, can support the diagnosis of synovial sarcoma. However, molecular confirmation is essential for accurate diagnosis. Immunohistochemistry plays a significant role in the diagnosis of synovial sarcoma by providing supportive evidence, but molecular validation is necessary, especially when the results are atypical or uncertain.

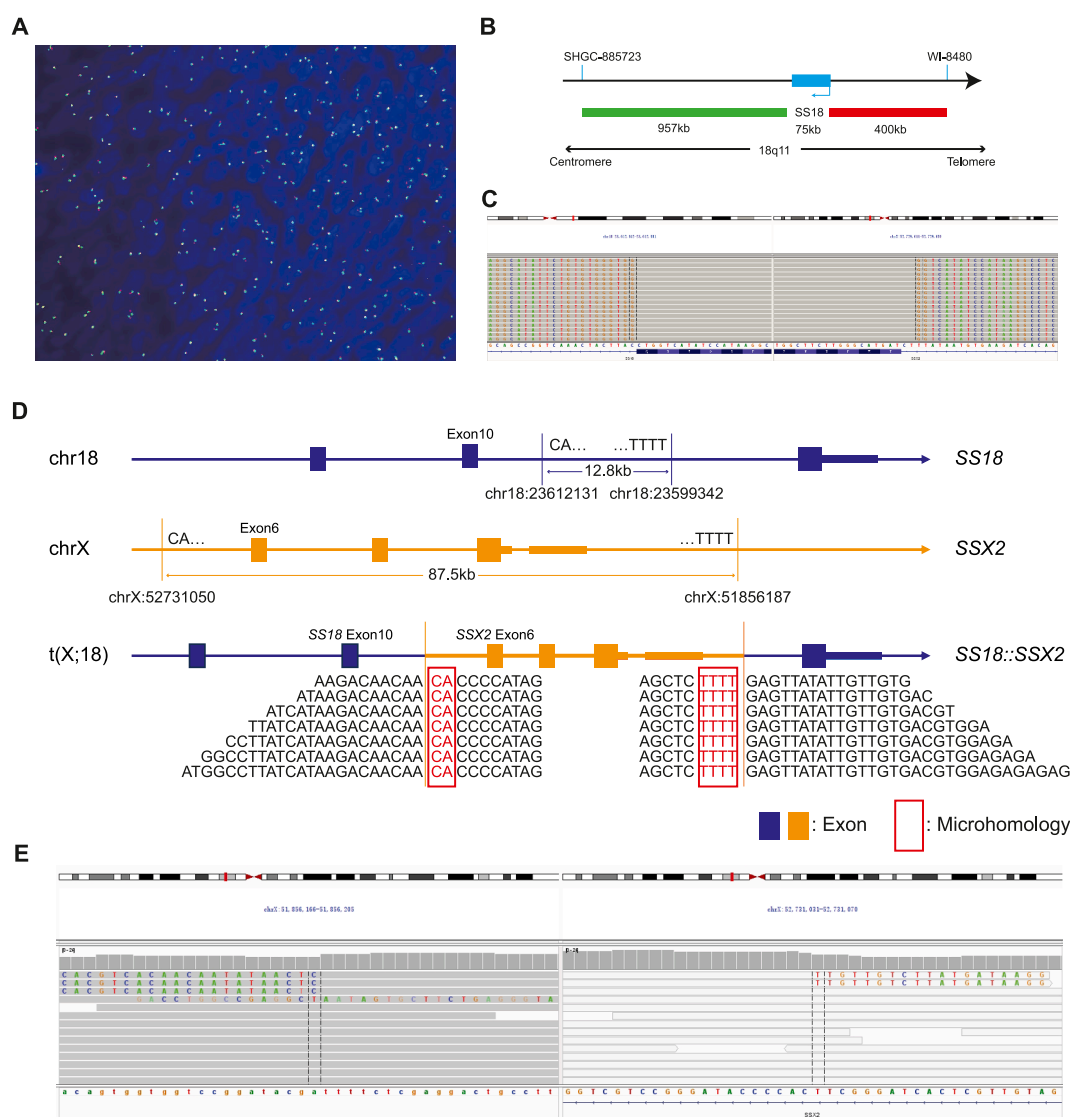


Fig. 2. Results of FISH, targeted RNA NGS and WGS. (A) FISH analysis using 5' *SS18* (red) and 3' *SS18* (green) probes showing no split signal, indicating a negative result for *SS18* rearrangement. The absence of split signals indicates an intact *SS18* locus, which is inconsistent with the expected rearrangement in synovial sarcoma. (B) Schematic diagram illustrating the location and length of the *SS18* FISH probes. The probes span a 75-kb genomic region on chromosome 18 (18q11), with the centromere and telomere indicated for orientation. (C) Targeted RNA NGS revealing an *SS18::SSX2* fusion. The fusion reads are visualised using Integrative Genomics Viewer. This result confirms the presence of a fusion transcript, which was missed by FISH. (D) WGS showing an insertion rearrangement between *SS18* exon 10 and *SSX2* exon 6, resulting from a complex rearrangement between chromosome 18 and chromosome X. The breakpoints are marked by vertical lines, and two microhomology sequences (CA ... TTTT) are identified at the breakpoint junctions. This complex genomic event explains the false-negative FISH result and highlights the limitations of FISH in detecting small insertion-based rearrangements. (E) The fusion reads are visualised using Integrative Genomics Viewer, illustrating the insertion of an 87.5-kb fragment from chromosome X (chrX: 51856187-52731050) containing the last four exons of *SSX2* into intron 10 of *SS18* on chromosome 18 (chr18: 23599342-23612131). The presence of microhomology sequences suggests a mechanism involving homologous recombination. FISH, fluorescence *in situ* hybridisation; NGS, next-generation sequencing; WGS, whole-genome sequencing.

Although *SS18*-*SSX* fusion-specific immunohistochemistry is highly sensitive for canonical translocation-type synovial sarcoma,⁵ negativity in the current case suggests that insertional rearrangements can distort the antibody-binding epitope or destabilise the fusion protein. This observation underscores (1) the possibility that complex genomic events compromise the performance of routine immunohistochemistry, mandating cautious interpretation of negative results, and (2) the continued primacy of molecular assays (RNA-NGS and WGS) as the diagnostic gold standard, especially when FISH or immunohistochemistry findings are discordant. We now explicitly recommend when both FISH and immunohistochemistry findings are negative, yet morphology and ancillary markers (TLE1 and *SSX*) strongly favour synovial sarcoma, RNA-NGS or WGS should be pursued without delay to avoid misdiagnosis. Larger cohort studies are also warranted to define the frequency of

insertional rearrangements among *SS18*-*SSX* fusion sarcomas and to systematically assess their impact on immunohistochemistry.

This case adds to the growing body of evidence that complex genomic rearrangements can confound standard diagnostic procedures in the clinical management of tumours. Future studies should explore the prevalence of insertion-based rearrangements in FISH-negative synovial sarcomas and refine FISH probe designs or diagnostic algorithms to account for such events. For now, integrating NGS-based technologies into the diagnostic workflow appears essential for resolving challenging synovial sarcoma cases, ensuring accurate diagnosis and timely management.

This case report highlights the diagnostic challenges associated with synovial sarcoma, particularly when relying solely on traditional methods such as FISH. The false-negative FISH result in this poorly

differentiated synovial sarcoma case underscored the limitations of this technique in detecting complex genomic rearrangements, such as the rare insertion event identified through RNA NGS and WGS. The presence of microhomology sequences at the breakpoints suggests a potential role of homologous recombination in the formation of this unique rearrangement, further complicating detection by conventional means. The integration of advanced molecular techniques, including RNA NGS and WGS, is crucial for accurate diagnosis and comprehensive understanding of the underlying genetic alterations in synovial sarcoma. These technologies not only provide a more detailed genomic landscape but also help in identifying novel and complex rearrangements that may be missed by standard assays. Future studies should focus on exploring the prevalence of such insertion-based rearrangements in FISH-negative cases and on refining diagnostic approaches to account for these complexities. This case emphasises the importance of a multimodal diagnostic strategy—combining histopathological, immunohistochemical and advanced molecular analyses—to ensure accurate diagnosis and optimal management of disease.

Data availability statement

The data and materials from this case are available from the authors on reasonable request.

Consent for publication

Informed consent for publication was obtained from the patient's parents/guardians.

Standard of reporting

The CARE guidelines (for CAse REports) were followed.

Author contributions

SX, RM: conceptualisation. RM: funding acquisition. CZ, SC: data curation. CZ: validation. DL: writing—(original draft). All authors: writing—(review and editing).

Conflicts of interest and sources of funding

The authors state that there are no conflicts of interest to disclose. This work was supported by the Foshan 14th Five-Year Medical Key Specialty Construction Project (FSZD145010).

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Mutation-tissue specificity in *DICER1* syndrome: insights from familial pleuropulmonary blastoma and a pan-tumour hotspot analysis



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To the Editor,

DICER1 syndrome is an autosomal-dominant tumour predisposition disorder caused by germline mutations in the *DICER1* gene, which

confers an increased risk for a spectrum of neoplasms including pleuropulmonary blastoma (PPB). Type I PPB, characterised by cystic lung lesions, may be clinically or radiologically misinterpreted as congenital pulmonary airway malformation (CPAM) due to overlapping clinical and radiological features.

We report two sisters who presented with cystic lung lesions initially diagnosed as CPAM, but were subsequently confirmed as having type I PPB based on molecular testing. Family screening revealed that the mother carried the identical *DICER1* germline mutation and had thyroid nodules, whereas another sister was an asymptomatic carrier. Both affected siblings harboured a second-hit mutation in their lung tumours

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