Recurrent and novel SS18-SSX fusion transcripts in synovial sarcoma: description of three new cases

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Abstract Synovial sarcoma (SS) is an aggressive type of tumor, comprising approximately 10 % of soft tissue sarcomas. Over 90 % of SS cases are characterized by the t(X;18)(p11.2;q11.2) translocation, which results mainly in the formation of oncogenic SS18-SSX1 or SS18-SSX2 fusions. In a typical SS18-SSX fusion transcript, exon 10 of SS18 is fused to exon 6 of SSX1/2. However, several variant fusion transcripts have been already described. In the present study, we examined the fusion transcript type in a series of 40 primary untreated SS tumor specimens using reverse transcription polymerase chain reaction and fluorescence in situ hybridization assay. We detected SS18-SSX1 transcript in 22 (55 %) patients and SS18-SSX2 transcript in 17 (42.5 %) patients, while in one patient, none of SS18-SSX1/2 fusion transcripts were identified. Among the cases under study, two tumors carried novel SS18-SSX1 and SS18-SSX2 variant translocations that were allegedly created by an alternative splicing, and in additional case, an unusual translocation variant previously described by other group was found. Our data suggest that alternative splicing may play an important role in novel fusion transcript formation, and additionally we show that it may be a recurrent event in SS. Furthermore, we describe the first case of a complex rearrangement possibly linking SS to REPS2 gene.

Keywords Synovial sarcoma · Molecular markers · Fusion genes · SS18-SSX fusion genes

Introduction

Synovial sarcoma (SS) accounts for approximately 10 % of soft tissue sarcomas. SS is an aggressive type of tumor which may arise in patients at any age but mainly develop in adolescents and young adults. SS originates principally in the extremities but may occur at any anatomic site. Metastases, which mainly affect the lungs, occur in approximately half of patients [1].

Cytogenetically, SS is characterized by the nonrandom presence of t(X;18)(p11.2;q11.2) [2, 3]. This translocation is detected in more than 90 % of SS cases and involves the
| No. | SS18 partner | Sex | Age | Primary site      | Mitotic index/10 HPF | Histosubtype | Necrosis (%) | PFS (days)/latest status | Karyotype                                                                 |
|-----|--------------|-----|-----|-------------------|----------------------|--------------|--------------|------------------------|---------------------------------------------------------------------------|
| 1   | SSX1         | F   | 74  | Wrist             | 35                   | Monophasic   | No           | 153/DOD               | 74-84,X(t;X:18)(p11;q11)x2[cp14]                                          |
| 2   | SSX1         | M   | 26  | Knee              | 20                   | Monophasic   | <50          | 591/DOD               | 42-44,Y(t;X:18)(p11;q11),der(9)(q10;p23; q21),-10,add(11)(p15)x2[cp16],del(14)(q10),-18, -20[cp4];83-84<4n;iden(q33)46,X,Y<3y1<4n,<3n,XXYY,inc[1] |
| 3   | SSX1         | F   | 85  | Shoulder          | 1                    | Biphasic     | <50          | 328/AWOD              | 46,X(del)(X)p11.2,der(18)ins(18;X)(q11; p11.22)[11]/46,XX[I]          |
| 4   | SSX2         | M   | 46  | Pharynx           | 36                   | Monophasic   | <50          | 1931/AWD              | 41-45,Y(t;X:18)(p11;q11),der(1;21)(q10;q10),add(14)(q32)add(17)(q25)+,-20[cp14] |
| 5   | SSX2         | F   | 28  | Thoracic wall     | 17                   | Monophasic   | NA           | 548/AWD               | 46,X(del)(X)(q23)(t;X:18)(p11.11)add(3)(p25);46,6q(config.46,XY[4]) |
| 6   | SSX1         | M   | 17  | Ankle             | 3                    | Biphasic     | No           | 1861/AWD              | 49-52,Y,del(X)(q21),+der(X)(t;X:18)(p11; q11)x2,t(1;2)(p22;q31),del(5)(q15),der(6)(q17)22;23(11)+,+12,add(14)(q11),del(17)(q21),-18,-20,+21/46,XY[4] |
| 7   | SSX1         | F   | 14  | Right lower leg   | 2                    | Monophasic/ biphasic | No       | 1276/AWD              | 46,X(t;X:18)(p11.11)[9]                                               |
| 8   | SSX2         | M   | 47  | Left kidney       | 54                   | Monophasic   | No           | 318/AWD               | 45,Y(t;X:18)(p11;q11),der(1;12)(p22;q13),dic(19;20)(q13;p13)[1]/46,XY[3] |
| 9   | SSX2         | M   | 63  | Right upper leg   | 98                   | Monophasic   | <50          | 323/AWD               | 73-78<3n+:XXYY,der(X)(t;X:18)(p11.11)x2, +2,-3,-4,7,+9,-10,+12,+12,+13,-16, der(18)(X:18),+21[cp5]46,XY[14] |
| 10  | SSX1         | M   | 25  | Right foot        | 4                    | Biphasic     | No           | 672/AWOD              | 46,Y(t;X:18)(p11.11)[18]                                               |
| 11  | SSX1         | M   | 44  | Left foot         | 1                    | Biphasic     | No           | 2573/AWOD             | 47,Y(t;X:18)(p11.11)x2;9(q23434)t(3;8)(q11.11)x2;8[12]46,XY[8]       |
| 12  | SSX1         | M   | 37  | Knee              | 23                   | Monophasic   | NA           | 160/DOR               | 38-41,Y,-X,-3,-11,-14,18,der(19)add(19)p13add(19)q13,add(22)q13,46,XY[12] |
| 13  | SSX1         | M   | 19  | Shoulder          | 11                   | Monophasic   | No           | 256/DOD               | 46,Y(t;X:18)(p11.11)[6]46,XY[14]                                       |
| 14  | SSX1         | F   | 39  | Upper leg         | 24                   | Biphasic     | No           | 661/DOR               | 44-45,Y(t;X:18)(p11.11)x3:12x27:q13,6,der(14)(q10)p10add(22)q13,2cp12 |
| 15  | SSX1         | F   | 47  | Left upper leg    | NA                   | Monophasic   | NA           | 123/DOD               | 57-60<2n:+XX,+X:+1,+2,+7,+9,+12,+12,+13,-16,+14,15,16,17,21[cp15] |
| 16  | SSX2         | M   | 56  | Stomach           | >15                  | Monophasic   | No           | 399/AWD               | NA                                                                       |
| 17  | SSX2         | M   | 55  | Left upper leg    | 14                   | Monophasic   | <50          | 172/DOD               | 35-38,Y,der(X)(t;X:18)(p11.11)-1,-2,add(2)x27;3,-4add(6)p23,-10,-11,add(12)x24;13x14,-14,15add(16)q24;18,-22,add(22)x13;14, -19mar,inc[19]/46,XY[1] |
| 18  | SSX1         | M   | 23  | Tibia             | 5                    | Monophasic   | <50          | 2566/AWOD             | 44,Y(t;X:18)(p11.11)x3,-14,22,add(10)46,XY[10]                          |
| 19  | SSX1         | F   | 58  | Stomach           | NA                   | Monophasic   | NA           | 257/AWOD              | 70<3n:+XX,X(t;X:18)(p11.2q11),-3,-6,+13,-14,+15,16[15]              |
| 20  | SSX1         | M   | 49  | Right groin       | 21                   | Monophasic   | <50          | 511/DOD               |                                                                           |
| No. | SS18 partner | Sex | Age | Primary site       | Mitotic index/10 HPF | Histosubtype | Necrosis (%) | PFS (days)/latest status | Karyotype                                                                 |
|-----|--------------|-----|-----|--------------------|----------------------|--------------|--------------|--------------------------|---------------------------------------------------------------------------|
| 21  | Negative     | F   | 14  | Left upper leg     | 27                   | Biphasic      | <50          | 4245/AWD                 | 54,Y(X;18)(p11;q11),+8,+8,der(10;13)(q10; q10),+12,+12,-15,+19,+21,+21[20] |
| 22  | SSX2         | F   | 25  | Right groin        | 33                   | Monophasic    | <50          | 123/AWD                  | 46,X(X;18)(p11q11)[15]                                                   |
| 23  | SSX2         | F   | 28  | Shoulder           | 7                    | Monophasic    | No           | 2255/AWD                 | 45,-X,der(X)(q26q27q28q26q13),add (16q22)[cp5],46,XX[8]                |
| 24  | SSX2         | M   | 40  | Lung               | 2                    | Monophasic    | No           | 449/AWD                  | 46,Y(X;18)(p11.2q11)[2][47],idem, +8[11]/46,XY[2]                      |
| 25  | SSX1         | M   | 41  | Right upper leg    | 22                   | Biphasic      | <50          | 403/DOD                  | 45,Y(X;18)(p11q11),der(13;14)(q10; q10)c? [cp20]                       |
| 26  | SSX1         | F   | 41  | Left inguinal region | 3                   | Monophasic    | No           | 3729/AWD                 | 46,X,der(X)(q18)(p11q11),der(12q27q15),det(11q11p11q11p13-24), del(12q15),del(18q11)[20] |
| 27  | SSX1         | M   | 62  | Left ankle         | NA                   | Monophasic    | NA           | 1981/AWD                 | 46,Y(X;18)(p11q11)[cp19]/46,XY[1]                                     |
| 28  | SSX2         | M   | 19  | Paravertebral      | 28                   | Biphasic      | NA           | 594/DOD                  | 46,Y(X;18)(p11q11),det(11q13q21)[10]/46,XY[10]                         |
| 29  | SSX1         | M   | 33  | Right knee         | 2                    | Biphasic      | <50          | 1275/AWD                 | 46-52,Y,XX;18(p11q11),add(5q26),+5,+8,+9,+14,+17,+19,(21)q10,+mar [cp15]/46,XY[6] |
| 30  | SSX2         | F   | 20  | Left rib           | 17                   | Monophasic    | No           | 3572/AWD                 | 45-46,X,XX;18(p11q11)[cp19]                                            |
| 31  | SSX1         | F   | 26  | Right upper leg    | 8                    | Monophasic    | No           | 611/AWD                  | 46,X(X;18)(p11q11),det(11q25),add (8p23),add(9q34)[15]                |
| 32  | SSX2         | M   | 52  | Right upper leg    | 14                   | Biphasic      | No           | 267/AWD                  | 46,Y,der(X)(X;18)(p11q11),det(5q5q20q11p11), det(12q22)-18,der(20q10p11p11), +mar[20] |
| 33  | SSX2         | F   | 27  | Right upper leg    | 8                    | Monophasic    | No           | 2031/AWD                 | 46,X(X;18)(p11q11)[17]/55,idem, +der(X),t (X;18)(p11q11),+2,+4,+8,+9,+12,+19,+20,+21[3] |
| 34  | SSX1         | F   | 15  | Left upper leg     | 18                   | Monophasic    | <50          | 351/AWD                  | 46,X(X;18)[9]                                                          |
| 35  | SSX2         | M   | 16  | Right ankle        | 2                    | Monophasic    | No           | 1717/AWD                 | 46,Y(X;18)(p11q11)[cp10]/46,XY[10]                                     |
| 36  | SSX2         | M   | 57  | Right upper leg    | 1                    | Monophasic    | No           | 272/AWD                  | 46,XY[19]                                                              |
| 37  | SSX2         | F   | 33  | Mediastinum/retrotracheal | 3                   | Biphasic      | <50          | 101/AWD                  | NA                                                                      |
| 38  | SSX1         | M   | 77  | Left upper leg     | 25                   | Monophasic    | NA           | 243/AWD                  | 46,Y,der(X)(X;23)(p11p21),t(20;20q11q12),add(9p22q24q17r (10;21)q22q21)[20] |
| 39  | SSX2         | M   | 49  | Left lower leg     | 28                   | Monophasic    | No           | 551/DOD                  | NA                                                                      |
| 40  | SSX1         | M   | 5   | Pharynx            | 50                   | Monophasic    | <50          | 630/AWD                  | 45-47,Y,XX;18(p11q11),t(7;12)(q2; q24)[cp20]                            |

Abbreviations: 
- **DOD** died of disease;  
- **AWOD** alive without evidence of disease;  
- **AWD** alive with disease;  
- **DOR** died of other reason. Cases described in the present study are marked in bold.
SS18 (previously known as SYT) gene on chromosome 18 and one of the SSX genes on the X chromosome [4–6]. In a typical SS18-SSX fusion transcript, exon 10 of SS18 is fused to exon 6 of SSX1/2 [7]. Approximately two-thirds of tumors carry SS18-SSX1 translocation, and the SS18-SSX2 variant is found in one-third of cases [5, 8, 9]. Moreover, rare cases of SS18-SSX4 chimeric variants in SS have been described. However, SS18-SSX4 fusions have been characterized by high breakpoint variability, resulting in functional unpredictability [10–12]. The rare SS cases which lack the classical SS18-SSX fusion gene may represent tumors with unusual variant transcripts, which failed to be detected using conventional approaches [13]. Studies investigating the prognostic value of the different fusion types provide contradictory results. Several studies reported a more favorable outcome in patients carrying SS18-SSX2 fusions [5, 11, 14–17], and others failed to find any significant correlation between fusion type and clinical outcome [9, 18, 19].

In the present study, we examined fusion transcript type using reverse transcription polymerase chain reaction (RT-PCR) in a series of 40 SS patients. We report two novel SS18-SSX1 and SS18-SSX2 variant translocations, and in one patient we detected unusual SS18-SSX1 translocation variant previously described by other group [13].

**Materials and methods**

**Patients**

Forty fresh frozen surgical biopsies of primary tumors were obtained from the University Hospital Leuven, Belgium (n = 39) and the Maria Sklodowska-Curie Memorial Cancer Centre and Institute of Oncology, Warsaw, Poland (n = 1). The specimens originated from 24 males and 16 females with the mean age at the diagnosis of 38 years (range from 5 to 85 years). All tumors in our cohort were diagnosed as conventional synovial sarcomas by means of morphology and routine immunohistochemistry, with 26 tumors being classified as monophasic and 13 as biphasic. One specimen presented features of both mono- and biphasic histosubtypes. All specimens were collected with informed consent, according to the protocol approved by the local ethical committee. The clinicopathologic data of this series are shown in Table 1.

**Table 2** Primers used for PCR and sequencing

| Designation | Sequence | Direction | Position | NCBI reference sequence |
|-------------|----------|-----------|----------|-------------------------|
| SS18        | 5′ AGGATATAAACGAGCCACAGCC 3′ | Forward | 1242-1262 | NM_001007559.1 |
| SSX1        | 5′ GGTCAGTGGTTCCCATCG 3′ | Reverse | 493-512  | NM_005635.2 |
| SSX2        | 5′ GGCAACGTCTTTCCCATCA 3′ | Reverse | 510-529  | NM_175698.1 |
| SSX4        | 5′ GGCAACGCTTTCCCATCA 3′ | Reverse | 460-479  | NM_005636.3 |

Fig. 1 Detection of novel fusion transcripts by electrophoresis in 2% agarose gel stained with ethidium bromide. a Detection of unusual SS18-SSX1 fusion transcripts. M — 1-kb Plus DNA ladder (Invitrogen); 1 — 344-bp PCR product obtained from primary tumor specimen derived from patient #3; 2 — 344-bp PCR product obtained from metastatic tumor specimen derived from patient #3; 3 — 194-bp PCR product obtained from primary tumor specimen derived from patient #29; 4 — negative control, no cDNA added; 5 — positive control, PCR product obtained from patient with the SS18-SSX1 fusion confirmed by sequencing. b Detection of novel SS18-SSX2 fusion transcript. M — 1-kb Plus DNA ladder (Invitrogen); 1 — 263-bp PCR product obtained from primary tumor specimen derived from patient #16; 2 — negative control, no cDNA added; 3 — positive control, PCR product obtained from 1273/99 cell line with confirmed SS18-SSX2 fusion transcript.
RNA extraction and reverse transcription

Total RNA was extracted from fresh frozen tumor specimens characterized as SS using miRNeasy kit according to the manufacturer’s protocol (Qiagen). RNA quality was assessed using BIO-RAD Experion RNA StdSens Analysis system (BIO-RAD). One microgram of total RNA was reverse transcribed with oligo(dT)\textsubscript{12-18} primers and random hexamers using SuperScript III Reverse Transcriptase (Invitrogen).

Fusion transcript detection by PCR

We performed SS18-SSX1 and SS18-SSX2 detection in all of examined cases, and additionally SS18-SSX4 detection in the case negative for SS18-SSX1/2 fusions. PCR was performed with two microliters of cDNA using AmpliTaq Gold DNA Polymerase (Invitrogen). Amplification was performed at +94 °C for 30 s, +58 °C for 60 s, and +72 °C for 60 s for 35 cycles, and the final extension was performed for 10 min. Only the annealing temperature for SS18-SSX4 detection was +57 °C. Primers used for amplification are listed in Table 2 [20, 21]. cDNA from synovial sarcoma 1273/99 cell line (kindly provided by Professor Fredrik Mertens, Skone University Hospital, Lund University, Sweden) was used as a positive control of SS18-SSX2 amplification, and cDNA from patient with known SS18-SSX1, confirmed by direct sequencing, was used as a positive control of SS18-SSX1 amplification. Negative controls were also included in every step of the procedure. PCR products were analyzed by electrophoresis in 2 % agarose gels stained with ethidium bromide and photographed.

Sequence analysis

PCR products were purified using QIAquick PCR Purification Kit (Qiagen). Direct sequencing of both strands of PCR products was performed using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and analyzed on the 3130xL Genetic Analyzer (Applied Biosystems). Both strands of the PCR products were sequenced at least twice. Chromas
Lite 2.01 software (Technelysium Pty Ltd) and BLAST software (http://blast.ncbi.nlm.nih.gov/Blast.cgi) were used for the computer analysis of obtained sequence data.

Fusion type detection by fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) assay was performed in selected cases to confirm the type of SS18-SSX1/2 translocations, according to the protocol described by Suret and coworkers [22].

Results

We detected SS18-SSX1 transcripts in 55% (n=22) and SS18-SSX2 transcripts in 42.5% (n=17) of patients. In one tumor, we did not detect any fusion transcript using RT-PCR; although FISH was not performed due to insufficient material, the karyotype of this specimen was available in the hospital database, revealing a t(X;18). Among analyzed cases, we noticed larger than expected 158-bp PCR products in three patients (Fig. 1). These samples were subjected to sequence analysis. Obtained sequence data were aligned to reference fusion transcript sequences (SS18-SSX1—GenBank S79325, SS18-SSX2—GenBank S79332) and analyzed using the BLAST software. Two patients carried novel SS18-SSX variant translocations, and in one patient we detected atypical variant that was previously described by Amary et al. in 2007 [13].

SS18-SSX1 variant described by Amary et al. (2007)

An unexpected PCR product of 194 bp was detected in SS of patient #29. Direct sequencing indicated the identical SS18-SSX1 variant transcript that was previously described by Amary and

Fig. 3 Identification of novel fusion transcript SS18-SSX2. a Schematic representation of the expected PCR product and novel SS18-SSX2 fusion transcript. In the upper part of the picture, the nucleotide positions derived from the original sequences of SS18 and SSX2 mRNA are indicated. b Nucleotide sequence of the 263-bp PCR product with predicted amino acid sequence of the chimeric protein.
coworkers [13]. This variant includes additional 12 codons derived from exon 5 of SSX1, which results in the fusion of codon 410 of SS18 to codon 99 of SSX1 without reading frame shift. Amary et al. described this transcript variant in a 24-year-old male with a 30-mm tumor located in the right triceps. The tumor was classified as monophasic with 50% of necrosis and 19 mitotic figures per 10 high-power fields. Patient #29 in our study was a 33-year-old male presenting biphasic a 30-mm tumor in the right knee, with less than 50% of necrosis and two mitotic figures per 10 high-power fields. The patient underwent tumor resection directly after first diagnosis, and he was alive without disease 42 months after surgery.

Novel SS18-SSX1 variant

Tumor specimen #3 was obtained from an 85-year-old female with a tumor of 105-mm maximal diameter located in the shoulder. Additionally, a 30-mm metastatic tumor specimen from deep tissues of the shoulder was available from the same patient (metastasis developed 11 months after initial diagnosis). FISH analysis indicated SS18-SSX1 fusion transcript in both of these specimens.

PCR with primers specific for the SS18-SSX1 fusion transcript presented larger than expected products in both of the specimens. The nucleotide sequences of amplified products were identical. BLAST analysis showed an insertion of 186 bp in the site of usual SS18-SSX1 junction. The additional nucleotides corresponded to 136 bp of complete exon 6 of RALBP1 associated Eps domain containing 2 (REPS2) (NM_004726.2) and 50 bp of complete exon 5 of SSX1, indicating a complex rearrangement in this tumor (Fig. 2). REPS2-SSX1 insert was fused to the exon 410 of SS18 at the 5′ end and to the exon 111 of SSX1 at the 3′ end. This rearrangement maintained the original reading frame. The predicted chimeric protein contains 410 N-terminal codons of SS18, followed by 62 codons of REPS2-SSX1 fragment (codon 46 for valine situated at the junction site of these two genes) and 78 C-terminal codons of SSX1.

Novel SS18-SSX2 variant

We detected the third unusual fusion variant in a specimen #16, derived from a 56-year-old male with a 80-mm monophasic SS primary tumor located in the stomach. Patient underwent tumor resection directly after diagnosis. He developed metastasis after 13 months and was alive with disease after 42 months. FISH analysis indicated SS18-SSX2 fusion transcript in this specimen. PCR with primers specific for SS18-SSX2 amplified larger than expected product of 263 bp. The insertion of additional 105 bp was fused in the position of usual SS18-SSX2 junction point. Codon 410 of SS18 at the 5′ portion of fusion was followed by 29 bp of intron 10 of SS18, 14 bp of exon 5 of SSX2, 62 bp of intron 5 of SSX2, and 91 bp of exon 6 of SSX2, which is usually present in the 3′ end of the fusion transcript (Fig. 3). This insertion conserved the original reading frame.

Discussion

The most common SS18-SSX fusion transcripts involve 410 N-terminal codons of SS18 and 78 C-terminal codons

A

![Typical SS18-SSX1 fusion junction](image)

B

![Typical SS18-SSX2 fusion junction](image)

C

![Typical SS18-SSX4 fusion junction](image)

**Fig. 4** Schematic representation of novel fusion sites in the unusual transcript variants of SS18-SSX1 (a), SS18-SSX2 (b), and SS18-SSX4 (c) described in the literature
of either SSX1 or SSX2. To the best of our knowledge, there are 17 reports describing atypical variants of SS-associated translocations, which are mostly caused by small insertions [4, 7, 11–14, 23–32]. Novel SS18-SSX transcript variants are frequently created not only by the unusual junction of SS18 and one of SSX genes but also by the mutations within SS18 or SSX genes which occur apart from the fusion site, e.g., deletion or truncation of SSX1 [26, 32] or insertion in SS18 sequence [12]. Figure 4 presents previously reported fusion variants characterized by different SS18-SSX junction sites. Our examination of fusion transcript type in a series of 40 SS primary tumor specimens revealed three unusual transcript variants, two of which were not published before.

The first unusual SS18-SSX1 transcript variant detected in our study was earlier described by Amary et al. [13]. Our detection of the identical fusion transcript variant confirms that the rare variants can be recurrent in SS patients and they are probably not caused by unique nor random events. Similar observations were already reported in several publications. Figure 4 indicates whether the atypical fusion variant was described in more than a single case.

The novel SS18-SSX1 transcript variant was detected both in primary and metastatic specimens derived from the same patient. This translocation involved another gene, i.e., REPS2, also known as POBI, located on the X chromosome at Xp22. The product of this gene is involved in the growth factor signaling and control of cell proliferation [33–36]. The expression of this gene might serve as a biomarker of favorable outcome in breast and prostate cancers; conversely, REPS2 downregulation has been demonstrated in the progression of prostate cancer [34, 37–39]. The fusion site of REPS2 and SSX1 involves amino acids encoded across a splice junction—codon 303 for glycine on the border of exons 6 and 7 of REPS2 and codon 94 for valine at the junction of exons 4 and 5 of SSX1. Predicted chimeric protein sequence preserves valine in this position. Noteworthy, the junction between REPS2 and SSX1 was formed at the overlapping nucleotides “GGT” which are present at the 3’ end portion of REPS2 and 5’ end portion of SSX1. Possibly, these overlapping nucleotides created a preferential site for the fusion of these genes. REPS2 exon 6, which is involved in this unusual fusion, encodes a portion of the Eps15 homology domain (residues 282–373). This domain has been described to interact with Epsin, Eps15, and p65 subunit of NFκB [37, 39]. However, gene expression profiles of synovial sarcoma cases described in the present study did not show any significant differences or correlations concerning REPS2 and its interactors (unpublished data). Therefore, the possible impact of REPS2 gene involvement in SS-associated translocation remains unclear. Apparently, this variant preserved the transforming function of the usual SS18-SSX1 fusion oncogene, which indicates that the mechanism of tumorigenesis in this case might have been similar as in other synovial sarcoma cases.

In addition, we have also detected novel SS18-SSX2 fusion transcript variant that consists of exonic and intronic regions of both SS18 and SSX2 genes. This transcript was also presumably created by the alternative splicing mechanism since there are two cryptic “AG” acceptor sites and one cryptic “GT” donor site in the direct proximity of the fusion points. First, there is an AG signal immediately before the junction point of SS18 exon 10 and intron 10 in position 12615–12616 of original intron 10. Second, the cryptic acceptor splice site is present immediately before the fusion point of SS18 intron 10 and SSX2 exon 5, involving codons 104 and 105 of SSX2 exon 5. Finally, directly after the SSX2 intron 5 portion involved in the fusion, there is a cryptic donor splice signal GT, which was presumably used with the authentic acceptor splice site in the 3’ end of this intron, resulting in the junction of SSX2 intron 5 and SSX2 exon 6.

Cryptic splicing mechanisms have previously been reported in the unusual fusion transcript formation [28]. Our data confirm that alternative splicing may play an important role in novel fusion transcript formation and that it may be a recurrent event in SS.

In our series, 64 % of the patients carrying SS18-SSX1 translocation and 71 % of the patients with SS18-SSX2 fusion transcript developed local recurrence or metastasis. This finding supports previous reports [9, 18, 19] showing no significant correlation between fusion type and clinical outcome.

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Conflicts of interest None

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