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Short Communication

Molecular Diagnosis of Synovial Sarcoma and Characterization of a Variant *SYT-SSX2* Fusion Transcript

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The translocation t(X;18)(p11;q11) is seen in >80% of synovial sarcomas (SS) with informative karyotypes. The breakpoints of the t(X;18) have been cloned and shown to involve two novel genes, SSX (at Xp11) and SYT (at 18q11), which produce a chimeric SYT-SSX transcript as a result of the translocation. Recently, SSX has been shown to be duplicated, with both copies, SSX1 and SSX2, located within distinct subregions of Xp11. We performed a reverse transcriptase polymerase chain reaction (RT-PCR) assay for both chimeric SYT-SSX transcripts in a series of 35 SS (29 monophasic, 6 biphasic) to assess its usefulness in molecular diagnosis and to evaluate the incidence of molecular variants. Of the 35 cases, 29 (83%) showed a specific SYT-SSX RT-PCR product, using a consensus primer for SSX1 and SSX2. Upon excluding three negative cases that had poor quality RNA, the proportion of positives rose to 91% (29/32). The 29 positive cases were further studied using primers specific for either SSX1 or SSX2; 19 cases were positive for SYT-SSX1 and 10 for SYT-SSX2. The relationship of histological subtype (monophasic versus biphasic) to SSX1 or SSX2 involvement was not statistically significant. In a single histologically unremarkable monophasic SS, a slightly larger SYT-SSX2 RT-PCR product was observed. Sequencing of this novel variant showed a 129-bp segment inserted between the usual SYT and SSX2 fusion points, of which 126 bp were derived from a more proximal (5′) portion of SSX2. The 3 bp immediately 5′ to the fusion point could not be assigned to either SYT or SSX2 and may represent an insertion-deletion or a cryptic splicing event. This fragment maintains the reading frame of the chimeric product and encodes a predicted protein larger by 43 amino acids, which nevertheless replaces the region homologous to the transcriptional repression domain Kruppel-associated box, recently recognized in the 5′ portion of the SSX genes, with all but the 3′ end of the SYT transcript. Thus, a diagnosis of SS may be confirmed in >90% of cases using RT-PCR detection of the chimeric transcript resulting from the t(X;18), and the incidence of molecular variants appears low. (Am J Pathol 1995, 147:1592–1599)

Synovial sarcomas (SS) most commonly arise in the para-articular regions of adolescents and young adults. They are composed of spindle and epithelioid cells and, depending on the proportion of each, are classified as biphasic or monophasic spindle cell tumors. Separation of monophasic spindle cell SS from other spindle cell sarcomas may be difficult, sometimes requiring immunohistochemical demonstration of keratin or epithelial membrane antigen (EMA) expression, although these markers are not universally present.[1]

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The translocation t(X;18)(p11;q11) was first recognized in SS nearly 10 years ago.[2,3] Subsequent studies have found it in >80% of SS (for review, see ref. 4). The cloning of the breakpoints of this translocation identified novel genes, designated *SSX* (at Xp11), now known to be duplicated (see below); and *SYT* (at 18q11). *SYT* lacks significant sequence homology with any known gene.[5] The 5′ portion of the *SSX* genes encodes a region 40% identical to the consensus amino acid sequence of the Kruppel-associated box (KRAB), a transcriptional repression domain found in numerous zinc-finger transcription factors.[6] The function of this region of *SSX* has not yet been evaluated, and the remainder of the gene contains no other recognizable sequence elements, including DNA-binding domains such as zinc-finger motifs.[6] As in other sarcoma translocations, the t(X;18) leads to the formation of a chimeric transcript that can be detected by reverse transcriptase polymerase chain reaction (RT-PCR).[5] The chimeric transcript in SS replaces the 5′ portion of *SSX*, encoding the region of KRAB homology, with all but the 3′ end of *SYT*.[5,6]

This translocation has also been studied by fluorescent *in situ* hybridization (FISH) using cosmid probes flanking or spanning the breakpoint region on X in a variety of preparations, including metaphases, interphase nuclei, and even archival material.[7–10] FISH studies highlighted a peculiar feature of the t(X;18). The breakpoint at Xp11 was seen in one of two regions designated *OATL1* and *OATL2*, which contain ornithine aminotransferase-like (*OATL*) pseudogenes and are separated by at least two megabases.[7,8] Because an identical *SYT-SSX* chimeric transcript was observed in almost all cases, it was proposed that the *SSX* gene is duplicated and present in both regions.[5] Indeed, duplication of *SSX* was recently confirmed: *SSX1* and *SSX2* are located in the vicinity of *OATL1* and *OATL2*, respectively, and the predicted proteins are 85% identical.[6,11] A relationship between *OATL1 versus OATL2* breaks and histological subtype of SS has been proposed but remains controversial.[7–9] The identification of *SSX1* and *SSX2* now allows a more definitive evaluation of this question.

We have assessed RT-PCR detection of the *SYT-SSX* fusion transcript in the largest series of clinical samples of SS studied to date. Furthermore, we describe a novel molecular variant of this translocation, which involves *SYT* and *SSX2*, but produces a fusion gene slightly larger than previously reported, because of the inclusion of additional *SSX2* sequences. We have also reexamined the proposed association of histological subtype with X chromosome breakpoint location.

Materials and Methods

Cases seen at Memorial Sloan-Kettering Cancer Center (MSKCC) were collected prospectively and retrospectively on the basis of a pathological diagnosis of SS, abundant tumor within the surgical specimen, and availability of frozen tissue for RNA extraction. A total of 35 specimens of SS dating from 1985 to 1995 were obtained from 35 patients (Table 1). They included 17 males and 18 females, and the mean age was 34 years (range 14 to 70 years). The most common primary sites included the thigh (11), popliteal fossa and knee (6), and foot (4). The primary tumor was studied in 23 cases, metastatic tumor in 11 cases and a local recurrence in one case. The histopathology and immunohistochemistry on all cases were reviewed by two of the authors (J. W. and F. L.) blinded to the RT-PCR results. All cases had morphological features and, in most cases, immunohistochemical studies consistent with SS.

Total RNA was isolated from snap-frozen tumor samples using acid guanidinium thiocyanate (Tel-Test, Friendswood, TX) followed by phenol-chloroform extraction and isopropanol precipitation. The RNA was quantitated spectrophotometrically, and its integrity was further assessed by RT-PCR for a 343-bp portion of the β-actin gene transcript or a 741-bp fragment of the *EWS* gene transcript, also ubiquitously expressed.[12] Oligonucleotides were synthesized at the MSKCC Core Microchemistry Facility. The sequences of the oligonucleotides used are shown in Table 2.

RT-PCR was performed with 2 μg of total RNA, on an automated thermal cycler (Omnigene, Hybaid, UK). Reverse transcription was performed in a total volume of 20 μl with 100 units of Superscript II reverse transcriptase (GIBCO BRL, Gaithersburg, MD) in the presence of 20 units of ribonuclease inhibitor (Boehringer Mannhein, Indianapolis, IN) for 30 minutes at 42°C using random hexamers. The reverse transcriptase was then inactivated at 99°C for 5 minutes and on ice for 5 additional minutes. The PCR reagents, including AmpliTaq DNA polymerase (Perkin-Elmer Cetus, Norwalk, CT) and 30 pmol of each primer, were added up to a final volume of 100 μl. The cycling parameters were: 45 cycles of 95°C for 1 minute, 60°C for 1 minute, and 72°C for 1 minute with a final extension at 72°C for 5 minutes. The final concentration of $MgCl_2$ was 2.0 mmol/L. The RT-PCR products were electrophoresed on agarose gels

Table 1. *Clinical, Histological, and Molecular Data*

Unique tumor number	Age	Sex	Primary site	Primary (P) or Metastasis (M)	Subtype	*SYT-SSX*	*SSX1 versus SSX2*
SS01	58	M	Foot	M	M	+	1
SS02	29	M	Popliteal	M	M	+	2
SS03	19	M	Foot	P	M	+	1
SS04	46	M	Lower leg	P	M	+	1
SS05	28	F	Popliteal	M	M	+	1
SS06	38	M	Popliteal	M	M	−*	ND
SS07	32	F	Shoulder	P	M	+	2
SS08	21	F	Groin	P	M	+	2
SS09	40	F	Pelvis	M	M	+	1
SS10	14	F	Thigh	P	B	+	1
SS11	42	F	Thigh	P	B	−†	ND
SS12	38	F	Thigh	P	M	+	2
SS13	36	F	Thigh	P	M	−†	ND
SS14	17	M	Neck	P	M	+	1
SS15	25	M	Thigh	M	M	+	1
SS16	13	F	Chest wall	P	M	+	2
SS17	35	M	Knee	P	B	+	1
SS18	18	M	Buttock	P	M	+	1
SS20	31	M	Abdominal wall	P	M	+	1
SS25	34	M	Thigh	P	B	+	1
SS26	28	F	Thigh	P	M	+	1
SS29	51	F	Foot	P	M	+	1
SS30	35	F	Groin	P	M	+	2
SS31	58	F	Foot	M	M	+	1
SS36	70	F	Popliteal	P	M	+	1
SS37	52	M	Lower leg	M	B	−*	ND
SS38	26	F	Chest wall	R‡	M	+§	2
SS39	39	M	Thigh	M	M	+	2
SS41	32	F	Thigh	P	M	+	1
SS42	22	M	Groin	P	M	+	2
SS43	39	M	Thigh	P	B	+	1
SS44	35	M	Popliteal	P	M	−*	ND
SS47	29	F	Pelvis	M	M	−†	ND
SS49	25	M	Breast	M	M	+	1
SS50	29	F	Thigh	P	M	+	2

*Good RNA quality (see Results); †poor RNA quality, §variant fusion product; ‡Local recurrence. Subtypes: M, monophasic; B, biphasic. ND, not determined.

(FMC Bioproducts, Rockland, MN), visualized by ethidium bromide staining, and transferred onto nylon membranes (Oncor, Gaithersburg, MD) for subsequent hybridization with ^{32}P-end-labeled oligonucleotides. Negative controls for RT-PCR were devoid of RNA or contained RNA from unrelated cell lines, and positive controls were known cases of previously detected chimeric product.

Table 2. *Oligonucleotides*

CAACAGCAAGATGCATACCA	SYT (5)
CACTTGCTATGCACCTGATG	SSX* (5)
GGATATGACCAGATCATCATGCCCAAG	SYT-SSX* (5)
TATCCACCCCAGCAGCAGCAGTAC	SYT OLIGO
AGAAAACAGCTGGTGATTTATGAA	SSX OLIGO†
GGTGCAGTTGTTTCCCATCG	SSX1 (11)
GGGACAGCTCTTTCCCATCA	SSX2 (11)
AGACCAACACAGCCTGGACCA	SYT-SEQ
TTCTGGCACTTCCTCCGAATCA	SSX2-SEQ
CAGGGAGGTGTCCACAGT	SYT-REV
AGGCCAACCGCGAGAAGATGACC	Actin primer A
GAAGTCCAGGGCGACGTAGCAC	Actin primer B
AGCCTAGGATATGGACAGA	EWS-A (16)
CTTTCCTGTTTCCTTGTCC	EWS-B (16)

All sequences 5′ to 3′. *No mismatch with *SSX1* or *SSX2*. †One mismatch with *SSX1* near 5′ end.

Direct sequencing of both strands of the RT-PCR products was performed using Sequenase and dideoxy chain termination (Sequenase PCR Product Sequencing Kit; U.S. Biochemical, Cleveland, OH), with the same SYT and SSX primers used for RT-PCR, as well as the SYT-SEQ and SSX2-SEQ primers, which flank more closely the usual *SYT-SSX* fusion point (Table 2). The sequencing reactions were run on 6% Long Ranger polyacrylamide gels (FMC Bioproducts).

To examine the normal *SYT* and *SSX* transcripts, conventional PCR was performed on purified cDNA with AmpliTaq DNA polymerase (Perkin-Elmer Cetus), according to the manufacturer's protocol. The cycling parameters were: 36 cycles of 94°C for 45 seconds, 54°C for 1 minute, and 72°C for 30 seconds with a final extension at 72°C for 5 minutes. The

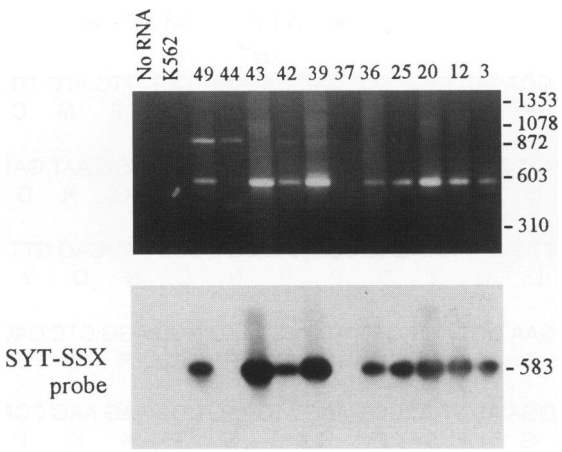

Figure 1. *RT-PCR detection of the SYT-SSX chimeric transcripts resulting from the t(X;18) in SS.* (Top) *Agarose gel of RT-PCR products in 11 SS samples. All but two SS (nos. 37 and 44) show the 583-bp product expected with the consensus SSX primer. Nonspecific bands above 872 bp are seen in a few lanes; these did not hybridize with the internal oligonucleotides SYT OLIGO and SSX OLIGO. The negative control lane (K562 leukemia cell line) and the no-RNA lane show no product. The positions of selected size marker bands (in bp) are indicated on the right. The specificity of the products was confirmed by transfer and hybridization with an oligonucleotide probe spanning the chimeric junction, SYT-SSX* (Bottom).

final concentrations of $MgCl_2$ and of each primer were 1.5 mmol/L and 30 pmol/reaction, respectively. The products were electrophoresed as described above.

Results

Of the 35 cases, 29 (83%) showed a specific RT-PCR product using the SYT primer and the SSX consensus primer (Table 1, Figure 1). The specificity of the RT-PCR product was confirmed by hybridization with the *SYT-SSX* oligonucleotide (Figure 1), which spans the common junction point of both chimeric mRNAs. In all but one case (see below), the product was of the expected size, 583 bp.

The study group included 28 monophasic and 7 biphasic tumors. Among the 6 negative cases there were 4 monophasic and 2 biphasic SS (Table 1). There was thus no clear relationship between the incidence of the translocation and the subtype of SS. Two cases showed unusual histological patterns, rhabdoid appearance (SS-47) and ribbon growth of epithelioid cells (SS-43). In the latter case, an *SYT-SSX* fusion transcript was detected, but SS-47 was one of three negative cases with poor RNA (see below).

Three of the six negative cases, SS-11, SS-13, and SS-47, showed poor quality RNA as indicated by little or no amplification of the ubiquitous transcripts, β-actin and *EWS*. In the other three cases, SS-6, SS-37, and SS-44, the 743-bp *EWS* product was readily amplified, but the typical 583-bp *SYT-SSX* product was nevertheless undetectable. SS-37 was a typical biphasic SS (Figure 2). SS-44 was a monophasic SS positive for EMA, but negative for keratins by immunohistochemistry. SS-6 was a monophasic spindle cell tumor consistent with SS, but negative for both keratins and EMA. Notably, some monophasic cases positive by the RT-PCR assay also showed a negative immunophenotype. Thus, three cases in our series appear to represent examples of SS with adequate RNA that were negative for the t(X;18) by this RT-PCR assay, which detects both *SYT-SSX1* and *SYT-SSX2*. Additional studies are underway to further characterize these negative cases.

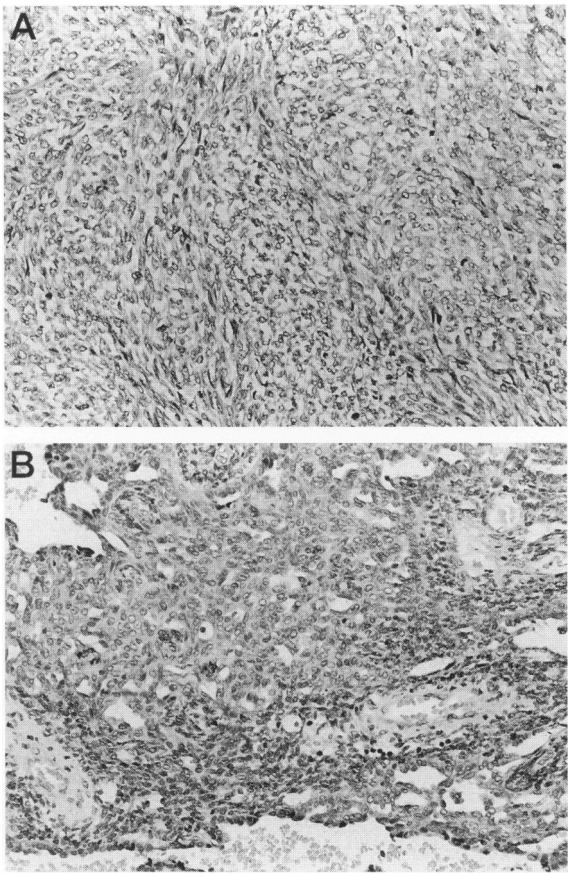

Figure 2. *Case SS-37. Biphasic SS negative for the t(X;18) by this SYT-SSX RT-PCR assay. Spindle cell* (A) *and glandlike areas* (B) *are shown. This case was positive for EMA in the epithelioid cells (not illustrated).*

Selected examples of several other tumor types, including a leiomyosarcoma, a chordoma, a low grade chondrosarcoma, a Ewing's sarcoma, a fibrosarcoma, a blastic chronic myelocytic leukemia cell line (K562), a fibrosarcoma cell line (HT1080; American Type Culture Collection, Rockville, MD), and a

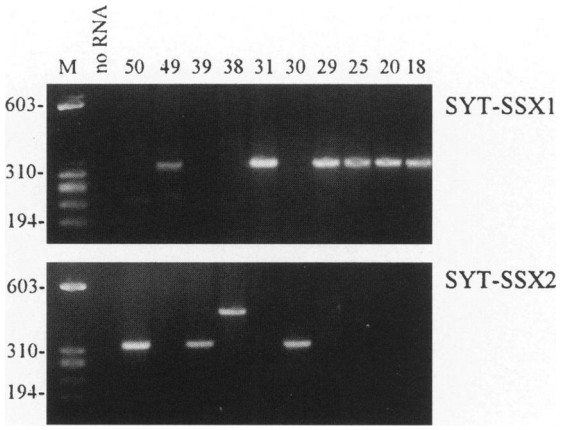

Figure 3. *RT-PCR detection of either SYT-SSX1 or SYT-SSX2 using specific SSX1 and SSX2 primers. The expected product size is 331 bp for both. The reciprocal involvement in 10 cases of SS of either SSX1 or SSX2 is apparent. Case SS-38 shows a slightly larger variant SYT-SSX2 product (see Figure 4). The positions of selected size marker bands (in bp) are indicated on the left.*

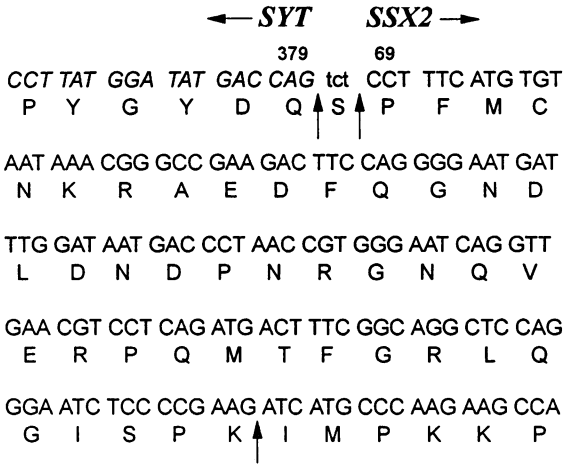

Figure 4. *Partial cDNA sequence of variant SYT-SSX2 fusion transcript from case SS-38 and predicted amino acid sequence. The SYT-derived portion* (italics) *ends at codon 379. The SSX2-derived portion begins at codon 69. The intervening three bases* (lower case, *between first two arrows*) *cannot be assigned to either gene. The site of usual SSX2 fusion point is indicated by the third arrow.*

large cell lymphoma cell line (SU-DHL-4) were negative by RT-PCR for the *SYT-SSX* chimeric mRNA (results not shown).

The 29 positive cases were further studied using primers specific for either *SSX1* or *SSX2*; 19 cases were positive for *SYT-SSX1* and 10 for *SYT-SSX2* in a mutually exclusive fashion (Table 1 and Figure 3). In selected cases, breakpoint assignment to *SSX1* or *SSX2* was further confirmed by digestion of the 583-bp consensus *SYT-SSX* product with *Alu*I, which cleaves *SYT-SSX1* once and *SYT-SSX2* twice[11] (results not shown). The *SYT-SSX1*-positive group included 15 monophasic and 4 biphasic SS, whereas the *SYT-SSX2*-positive group consisted of 10 monophasic and no biphasic cases. The relationship of histological subtype to *SSX1* or *SSX2* involvement was not statistically significant.

In a single case, SS-38, a larger RT-PCR product was observed, slightly >700 bp with the SYT and SSX primers (results not shown), and ~460 bp with the SYT and SSX2 primers (Figure 3). This novel product did not hybridize with the SYT-SSX oligonucleotide, but did hybridize with the SYT-OLIGO and SSX-OLIGO probes, which are internal to the SYT and consensus SSX primers (results not shown). This suggested that this novel product was indeed an *SYT-SSX2* chimera, but that the fusion point did not correspond to that of the common *SYT-SSX2* RT-PCR product.

Direct sequencing of both strands of this product showed a novel segment of 129 bases inserted between the previously reported *SYT* and *SSX2* fusion points (Figure 4). It corresponds in 126 bases to the portion of *SSX2* immediately 5′ to the typical *SYT-SSX2* fusion point.[6] The three bases at the novel fusion point, TCT, encode a serine and cannot be assigned to either *SYT* or *SSX2*. They may be the result of a small insertion-deletion or a cryptic splicing event. Nonetheless, this 129-bp fragment preserves the predicted reading frame of the chimeric product.

Performing PCR on isolated cDNA, we also examined the expression of the *SYT* and *SSX* genes in small bowel, placenta, testis (Clontech, Palo Alto, CA), and in the K562 leukemia and HT1080 fibrosarcoma cell lines. As expected from previous studies,[5] the fairly ubiquitous *SYT* transcript was detected in all samples using the SYT and SYT-REV primers (product size: 342 bp) (Table 2). Using primers detecting both *SSX1* and *SSX2* (SSX-OLIGO and SSX; product size, 165 bp), *SSX* expression was only found in K562, HT1080, and testis. Expression of the *SSX* genes in testis and HT1080 fibrosarcoma cells has also been documented by Crew et al.[6]

Discussion

Various types of sarcomas harbor specific chromosomal translocations.[4] In many cases these present as the sole cytogenetic abnormality and are thus likely to be etiologically significant. In recent years, the molecular structure of several of these translocations has been determined.[13] All translocations analyzed so far appear to result in the production of novel, tumor-specific chimeric transcription factors, a biologically fascinating observation with implications for studies of gene expression and tissue dif-

ferentiation. Clinically, the cloning of these translocation breakpoints provides new assays for molecular diagnosis and new targets for investigative therapeutics.

The translocation t(X;18)(p11.2;q11.2) was first identified in 1986, and its association with SS was recognized soon thereafter.[2] A recent review of the cytogenetic literature found the t(X;18)(p11.2;q11.2) in 90% of SS, including biphasic and monophasic types, and confirmed its specificity for SS.[4] In several instances, the translocation has been reported as the sole abnormality, implicating it as the pivotal genetic change in the pathogenesis of SS. In 1994, two independent groups, respectively, isolated the genomic junction fragment[14] and the chimeric transcript of this translocation.[5] The translocation involves *SYT* (at 18q11) and a duplicated gene, *SSX* (at Xp11).[5,6] The predicted protein encoded by the *SYT-SSX* chimeric transcripts consists of the 396 amino terminal amino acids of *SYT* fused to the 78 carboxy terminal amino acids of either *SSX1* or *SSX2*.[5,6] *SYT* contains a predicted glutamine-proline-glycine-rich region suggestive of a transcriptional activation domain, which replaces a region of KRAB homology within the 5′ portion of the *SSX* genes.[5,6] The substitution of a putative transcriptional repression domain (ie, the KRAB-homologous region) by a putative transactivation domain suggests that, like the products of other sarcoma translocations, the chimeric product in SS may be involved in transcriptional events. However, no DNA-binding domains have so far been identified in either *SYT*, *SSX1*, or *SSX2*.

In the present series, 83% of cases were positive for this translocation by RT-PCR. If the three negative cases with poor RNA quality are excluded, the proportion of positives rises to >90% (29/32). In the only other comparable series, Crew et al[6] studied 32 tumors, including 28 clinical tumor samples and 4 SS cell lines, and found 29 positive. Thus the detection of this translocation by RT-PCR provides a highly sensitive diagnostic marker for SS, comparable with the t(11;22)(q24;q12) of Ewing's sarcoma and its resulting *EWS-FLI1* chimeric transcript.[15,16] The clinical importance of this finding is stressed by the fact that the differential diagnosis of SS is broad and often problematic, and may include various sarcomas, carcinomas, and carcinosarcomas.[1] Given the technical advantages of RT-PCR over conventional cytogenetic analysis, and the high sensitivity and specificity of the t(X;18) for SS, RT-PCR detection of this translocation is likely to become an extremely useful confirmatory test for this entity.

The specificity of the t(X;18) for SS has been well established in the cytogenetic literature.[4] Among hundreds of sarcomas successfully karyotyped, only two non-SS tumors with a t(X;18) have been reported, a fibrosarcoma[17] and a tumor originally called malignant fibrous histiocytoma but reclassified as a spindle cell sarcoma on review.[3,18] Both tumors were negative for cytokeratins. These high grade spindle cell sarcomas are in the differential diagnosis of SS, and a clear distinction might have been difficult. It is worthwhile to note that certain RT-PCR-positive monophasic SS in this series did not show immunostaining for cytokeratins.

Three cases of SS were negative in the *SYT-SSX* RT-PCR assay, in spite of apparently adequate RNA, as evidenced by ready amplification of the ubiquitous transcripts, β-actin and *EWS*. There are three possible explanations for these negative cases. First, it is possible that neither *SSX* nor *SYT* is involved in these two cases. A second possibility is that these cases may contain variant translocations that recombine *SYT* or *SSX* with other as yet undescribed genes. Cytogenetically, one such variant translocation has been reported in an SS, t(5;18)(q11;q11).[19] Experience in Ewing's sarcoma and alveolar rhabdomyosarcoma[20] suggests that variant translocations involve homologues of the genes rearranged in the classic form of the translocation (for review, see ref. 13). For instance, the cloning of variant translocation breakpoints in Ewing's sarcoma has identified two homologues of *FLI1*, *ERG* and *ETV1*, which can form analogous chimeric transcripts with *EWS* .[21–23] A third possible explanation is that these negative cases represent molecular variants of the t(X;18), which exclude one (or both) of the primer binding sites due to variation in the location of the fusion point or alternative splicing of the chimeric transcript. Molecular heterogeneity is well described in other sarcoma translocations, eg, the t(11;22) of Ewing's sarcoma, or the t(12;16) of myxoid liposarcoma.[21,24] Additional studies are in progress to further characterize these negative cases. Three cases of SS negative by RT-PCR have also been reported by Crew et al.[6]

Our analysis of case SS-38 demonstrated a molecular variant of the t(X;18) containing an additional 129-bp segment inserted at the previously described fusion point between *SYT* and *SSX2*. This fragment maintained the predicted reading frame of the chimeric product and contained no stop codons. Sequence analysis and RT-PCR using primers discriminating between *SSX1* and *SSX2* confirmed that this was an *SYT-SSX2* fusion. This variant chimeric transcript encodes a predicted protein of 500 amino acids, compared with 457 amino acids for the classic form. The 129 additional bases include 126

bases of *SSX2* immediately 5′ to the usual *SYT-SSX2* fusion point.[6] The three bases at the novel fusion point, TCT, encoding a serine, are not present in the known normal transcripts of either *SYT* or *SSX2* (or *SSX1*). The role of small insertion-deletions or cryptic splicing events in preserving the reading frame of variant chimeric transcripts has been described in other translocations.[21,25] For instance, we have recently characterized a molecular variant of the *EWS-WT1* rearrangement in desmoplastic small round cell tumor in which a deletion of four bases and an insertion of six bases had occurred at the fusion point.[26]

Among 3 of 29 positive cases reported by Crew et al,[6] there were two other molecular variants of *SYT-SSX*. In one case, the fusion point of the variant chimeric transcript was 132 and 144 bp 5′ to the typical fusion points of *SYT* and *SSX1*, respectively, resulting in a transcript containing 12 additional bases. In two other cases, a segment of 87 bases that did not match either *SYT* or the *SSX* genes was inserted between the usual fusion points of *SYT* and *SSX1*. The elucidation of molecular variants of the Ewing's sarcoma t(11;22) has provided valuable data regarding the exon structure of the *EWS* and *FLI1* genes.[21] Likewise, the analysis of molecular variants of the *SYT-SSX* transcripts should assist in the delineation of the exon structure of these three genes.

Earlier FISH studies detected heterogeneity of the X chromosome breakpoint in SS.[7,8] Rearrangements were seen either in the vicinity of *OATL1* or *OATL2*. An intriguing finding in these studies was an apparent correlation between X chromosome breakpoint location and histological subtype. In aggregate, these studies found that 7 of 8 biphasic SS had *OATL1* breaks, whereas 12 of 15 monophasic SS had *OATL2* breaks.[7–9] We now know that breaks within the *OATL1* and *OATL2* regions correspond to *SSX1* and *SSX2* rearrangements, respectively.[6,11] In the present series, 10 of 25 monophasic SS showed *SSX2* involvement, whereas all 4 biphasic SS showed *SSX1* involvement. The difference here was not statistically significant. Moreover, Crew et al[6] analyzed their data in the same fashion and also found no significant relationship. It is nonetheless tempting to speculate about the possible role of minor amino acid differences between *SSX1* and *SSX2* in differential chimeric protein function and hence in other aspects of the biology of SS.

Finally, the availability of a sensitive and specific RT-PCR assay for SS should also allow molecular staging of this aggressive sarcoma. Stage is presently the most important prognostic factor in SS.[27] As in most sarcomas, hematogenous metastases predominate in SS, and bone marrow is involved in up to 20% of patients with metastatic disease (for review, see ref. 1). Furthermore, the incidence of late metastases is relatively high. Thus, this type of RT-PCR assay could be used to detect occult tumor cells in the circulation or in the marrow, which should lead to more accurate assignment of pathological stage and hence more aggressive treatment of the appropriate patients from the outset.

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References

1. Enzinger FM, Weiss SW: Soft Tissue Tumors, ed 3. St. Louis, Mosby, 1995
2. Turc-Carel C, Dal Cin P, Limon J, Li F, Sandberg AA: Translocation X;18 in synovial sarcoma. Cancer Genet Cytogenet 1986, 23:93
3. Turc-Carel C, Dal Cin P, Limon J, Rao U, Li FP, Corson JM, Zimmerman R, Parry DM, Cowan JM, Sandberg AA: Involvement of chromosome X in primary cytogenetic change in human neoplasia: nonrandom translocation in synovial sarcoma. Proc Natl Acad Sci USA 1987, 84:1981–1985
4. Sreekantaiah C, Ladanyi M, Rodriguez E, Chaganti RSK: Chromosomal aberrations in soft tissue tumors. Relevance to diagnosis, classification, and molecular mechanisms. Am J Pathol 1994, 144:1121–1134
5. Clark J, Rocques PJ, Crew AJ, Gill S, Shipley J, Chan AML, Gusterson BA, Cooper CS: Identification of novel genes, SYT and SSX, involved in t(X;18)(p11.2;q11.2) translocation found in human synovial sarcoma. Nature Genet 1994, 7:502–508
6. Crew AJ, Clark J, Fisher C, Gill S, Grimer R, Chand A, Shipley J, Gusterson BA, Cooper CS: Fusion of SYT to two genes, *SSX1* and *SSX2*, encoding proteins with homology to the Kruppel-associated box in human synovial sarcoma. EMBO J 1995, 14:2333–2340
7. de Leeuw B, Suijkerbuijk RF, Olde Weghuis D, Meloni AM, Stenman G, Kindblom LG, Balemans M, van den Berg E, Molenaar WM, Sandberg AA: Geurts van Kessel A: Distinct Xp11.2 breakpoint regions in synovial sarcoma revealed by metaphase and interphase FISH: relationship to histologic subtypes. Cancer Genet Cytogenet 1994, 73:89–94
8. Shipley JM, Clark J, Crew AJ, Birdsall S, Rocques PJ, Gill S, Chelly J, Monaco AP, Abe S, Gusterson BA, Cooper CS: The t(X;18)(p11.2;q11.2) translocation found in human synovial sarcomas involves two distinct loci on the X chromosome. Oncogene 1994, 9:1447–1453
9. Janz M, De Leeuw B, Olde Weghuis D, Werner M, Nolte

M: Geurts van Kessel A, Nordheim A, Hipskind RA: Interphase cytogenetic analysis of distinct X-chromosomal translocation breakpoints in synovial sarcoma. J Pathol 1995, 175:391–396

10. Lee W, Han K, Harris CP, Shim S, Kim S, Meisner LF: Use of FISH to detect chromosomal translocations and deletions. Analysis of chromosome rearrangement in synovial sarcoma cells from paraffin-embedded specimens. Am J Pathol 1993, 143:15–19
11. de Leeuw B, Balemans M, Olde Weghuis D, Geurts van Kessel A: Identification of two alternative fusion genes, *SYT-SSX1* and *SYT-SSX2*, in t(X;18)(p11.2;q11.2)-positive synovial sarcomas. Hum Mol Genet 1995, 4:1097–1099
12. Delattre O, Zucman J, Plougastel B, Desmaze C, Melot T, Peter M, Kovar H, Joubert I, de Jong P, Rouleau G, Aurias A, Thomas G: Gene fusion with an ETS DNA-binding domain caused by chromosome translocation in human tumours. Nature 1992, 359:162–165
13. Ladanyi M: The emerging molecular genetics of sarcoma translocations. Diagn Mol Pathol 1995,4:162–173
14. de Leeuw B, Balemans M, Weghuis DO, Seruca R, Janz M, Geraghty MT, Gilgenkrantz S, Ropers HH, Geurts van Kessel A: Molecular cloning of the synovial sarcoma-specific translocation (X;18)(p11.2;q11.2) breakpoint. Hum Mol Genet 1994, 3:745–749
15. Delattre O, Zucman J, Melot T, Garau XS, Zucker JM, Lenoir GM, Ambros PF, Sheer D, Turc-Carel C, Triche TJ, Aurias A, Thomas G: The Ewing family of tumors: a subgroup of small-round-cell tumors defined by specific chimeric transcripts. N Engl J Med 1994, 331: 294–299
16. Ladanyi M, Lewis R, Garin-Chesa P, Rettig WJ, Huvos AG, Healey JH, Jhanwar SC: EWS rearrangement in Ewing's sarcoma and peripheral neuroectodermal tumor. Molecular detection and correlation with cytogenetic analysis and MIC2 expression. Diagn Mol Pathol 1993, 2:141–146
17. Mandahl N, Heim S, Arheden K, Rydholm A, Willen H, Mitelman F: Multiple karyotypic rearrangements, including t(X;18)(p11;q11), in a fibrosarcoma. Cancer Genet Cytogenet 1988, 30:323–327
18. Limon J, Dal Cin P, Sandberg AA: Translocations involving the X chromosome in solid tumors: presentation of two sarcomas with t(X;18)(q13;p11). Cancer Genet Cytogenet 1986, 23:87–91
19. Limon J, Mrozek K, Nedoszytko B, Babinska M, Jaskiewicz J, Kopacz A, Zoltowska A, Borowska-Lehman J: Cytogenetic findings in two synovial sarcomas. Cancer Genet Cytogenet 1989, 38:215–222
20. Davis RJ, D'Cruz CM, Lovell MA, Biegel JA, Barr FG: Fusion of PAX7 to FKHR by the variant t(1;13)(p36;q14) translocation in alveolar rhabdomyosarcoma. Cancer Res 1994, 54:2869–2872
21. Zucman J, Melot T, Desmaze C, Ghysdael J, Plougastel B, Peter M, Zucker JM, Triche TJ, Sheer D, Turc-Carel C, Ambros P, Combaret V, Lenoir G, Aurias A, Thomas G, Delattre O: Combinatorial generation of variable fusion proteins in the Ewing family of tumours. EMBO J 1993, 12:4481–4487
22. Sorensen PHB, Lessnick SL, Lopez-Terrada D, Liu XF, Triche TJ, Denny CT: A second Ewing's sarcoma translocation, t(21;22), fuses the *EWS* gene to another ETS-family transcription factor, ERG. Nature Genet 1994, 6:146–151
23. Jeon I-S, Davis JN, Braun BS, Sublett JE, Roussel MF, Denny CT, Shapiro DN: A variant Ewing's sarcoma translocation (7;22) fuses the *EWS* gene to the ETS gene *ETV1*. Oncogene 1995, 10:1229–1234
24. Panagopoulos I, Mandahl N, Ron D, Hoglund M, Nilbert M, Mertens F, Mitelman F, Aman P: Characterization of the CHOP breakpoints and fusion transcripts in myxoid liposarcomas with the 12;16 translocation. Cancer Res 1994, 54:6500–6503
25. Hunger SP, Devaraj PE, Foroni L, Secker-Walker LM, Cleary ML: Two types of genomic rearrangements create alternative E2A-HLF fusion proteins in t(17;19)-ALL. Blood 1994, 83:2970–2977
26. de Alava E, Ladanyi M, Rosai J, Gerald WL: Detection of chimeric transcripts in desmoplastic small round cell tumor and related developmental tumors by RT-PCR. A specific diagnostic assay. Am J Pathol 1995 (In press)
27. Oda Y, Hashimoto H, Tsuneyoshi M, Takeshita S: Survival in synovial sarcoma. A multivariate study of prognostic factors with special emphasis on the comparison between early death and long-term survival. Am J Surg Pathol 1993, 17:35–44