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Interphase Fluorescence *in Situ* Hybridization and Reverse Transcription Polymerase Chain Reaction as a Diagnostic Aid for Synovial Sarcoma

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Identification of the t(X;18)(p11.2;q11.2) that is associated with a high proportion of synovial sarcoma can be a useful diagnostic aid. The translocation results in fusion of the* SYT *gene on chromosome 18 to either the* SSX1 *or the* SSX2 *gene, two homologous genes within Xp11.2. Two-color interphase fluorescence* in situ *hybridization and reverse transcription polymerase chain reaction were assessed as approaches to identify the rearrangement in well characterized cases. The presence of the translocation, and the specific chromosome X gene disrupted, were inferred from the configuration of signals from chromosome-specific centromere probes, paints, and markers flanking each gene in preparations of interphase nuclei. Rearrangement was found in two cell lines and eight of nine tumor samples, including analysis of five touch imprints. This was consistent with cytogenetic data in four cases and reverse transcription polymerase chain reaction analysis using primers known to amplify both* SYT-SSX1 *and* SYT-SSX2 *transcripts. The transcripts were distinguished by restriction with* LspI *and* SmaI. *Contrary to previous suggestions, there was no obvious correlation between histological subtype and involvement of the* SSX1 *or* SSX2 *gene. These approaches could also be applied to the identification of tumor-free margins and metastatic disease. (Am J Pathol 1996, 148:559–567)

Synovial sarcoma, a tumor of unknown histogenesis, accounts for approximately 8% of soft tissue sarcomas and is most common in adolescents.[1,2] Continuity of the tumor with the synovium has not been demonstrated, and they usually present adjacent to joint capsules, tendon sheaths, or bursae. Occasionally these tumors present in areas where there are no obvious synovial structures, such as the abdominal wall or the head and neck region, but they are more usually associated with the extremities.[3] Two main histological subgroups are recognized. The biphasic form contains glandular epithelium and spindle cells, whereas the monophasic form consists of only one cell type.[4,5] The differential diagnosis of synovial sarcoma, particularly a spindle cell monophasic type, from other tumors such as malignant schwannoma, malignant hemangiopericytoma, malignant fibrous histiocytoma, and fibrosarcoma may be difficult. Synovial sarcoma exhibiting a predominantly epithelioid or glandular component may be confused with a metastatic adenocarcinoma or an adnexal carcinoma.[6] Cytogenetic studies have revealed that a specific chromosome translocation, t(X;18)(p11.2;q11.2), is associated with a high proportion of both subtypes of synovial sarcomas,[7–10] and this has been suggested as a diagnostic indicator.[11,12]

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Table 1. *Clinical Data and Cytogenetic Evidence for t(X;18)(p11.2;q11.2) in Synovial Sarcoma-Derived Samples*

Sample	Age/sex	Site	Diagnosis	Cytogenetics
STS416	13/M	Right hand	Biphasic	46,Y,t(X;18)(p11.2;q11.2)[1]/46,Y,t(X;18)(p11.2;q11.2), inv(7)(p13p22)[15]*
STS444	39/M	Right forearm	Monophasic	ND
STS450	44/F	Right foot	Biphasic	ND
STS493	57/M	Left calf	Biphasic	ND
STS518	43/F	Left knee	Monophasic	ND
STS519	28/F	Left thigh	Monophasic	ND
STS551	11/M	Left thigh	Biphasic	47,Y,t(X;18)(p11.2;q11.2),+der8[10]
STS552	46/F	Right thigh	Monophasic	45,X,t(X;18)(p11.2;q11.2),del(1)(p13p31),add (2)(q31),-5,der(6)t(5;6)(q11.2;q13),del(11)(q21)[10]
TS-I	60/M	Left thigh	Monophasic fibrous	53,Y,t(X;18)(p11.2;q11.2),+5,+7,+8,+12,+14,+15, +17[21]/46XY[1]

ND, not done.
*Reference 14.

Cytogenetic analysis of solid tumor material is technically demanding and requires the culture of fresh tumor cells. The alternative approaches of interphase fluorescence *in situ* hybridization (FISH) and reverse transcription polymerase chain reaction (RT-PCR) have been used to detect diagnostically relevant translocations in both hematological malignancies and sarcomas. Molecular cytogenetic characterization of the t(X;18)(p11.2;q11.2) has revealed two different breakpoints on the X chromosome in different tumor-derived samples.[13,14] We have shown that the rearrangement corresponds to disruption of the *SSX1* and *SSX2* genes on chromosome X, either of which becomes fused to the *SYT* gene on chromosome 18, resulting in the production of an aberrant fusion transcript.[15,16] The analysis of complex rearrangements and the occasional loss of the derivative 18 chromosome indicate that it is the formation of the *SYT-SSX* fusion gene on the derivative X chromosome that is of pathogenetic significance.[9,10,17] It has been suggested that the involvement of either the *SSX1* or the *SSX2* gene is associated with the histological subtype of the tumor.[13,18,19]

In this study, two-color interphase FISH using differentially labeled chromosome-specific paints and centromere probes were hybridized to disaggregated fixed material and tumor touch imprints to infer the presence of the reciprocal translocation. The breakpoint region on both the X and 18 chromosomes lies near the centromere, and therefore the juxtaposition of a centromere-specific signal from one chromosome with that of a specific paint from the other chromosome was indicative of the rearrangement. It was also possible to compare the number of signals from the centromere probe with that of the chromosome paint. An excess of the latter was indicative of a translocation and has previously been used in paraffin-embedded material.[20,21] Cosmid probes flanking each of these genes were identified and hybridized to nuclei to infer disruption of either the *SSX1* or the *SSX2* gene. Aberrant *SYT-SSX* fusion transcripts have been previously shown to be identified by a single set of primers due to the sequence homology between the *SSX1* and *SSX2* genes, and analysis of the nucleotide sequence of the *SYT-SSX1* and *SYT-SSX2* transcripts has shown that they can be distinguished by digestion with restriction enzymes.[16]

These approaches have been assessed for their ability to identify the reciprocal translocation between chromosomes X and 18 and the presence of aberrant transcripts in well characterized cases of synovial sarcoma. In addition, it has been possible to determine the involvement of either the *SSX1* or the *SSX2* genes to ascertain whether the samples were consistent with the suggested correlation between histological subtype and the chromosome X gene involved.

Materials and Methods

Cell Lines and Tumors

Two synovial sarcoma cell lines were analyzed. A2243 was a cell line provided by Aaronson and co-workers (Rockefeller Institute, New York) and the origin of the cell line HS-SY-II has been previously described.[22] These cell lines have a t(X;18)(p11.2;q11.2) amidst a complex karyotype. The patient details and final diagnosis of the nine synovial sarcoma samples studied, five of which were included in our previous molecular analysis, are indicated in Table 1.[16] Three synovial tumor samples were obtained fresh for cytogenetic studies: STS551, STS552, and TS-I. The karyotype of STS416 has been previously described.[14] Part of the tumors, with the exception of TS-I, were snap frozen as soon as possible after surgery and stored in liquid nitrogen. The frozen

samples were used to make tumor touch preparations and RNA.

Chromosome and Nuclei Preparation

A piece of fresh tumor was disagreggated using collagenase and harvested for chromosomes and nuclei by standard procedures after short-term culture, as previously described.[23] Tumor touch imprints were prepared for cases STS518, STS519, STS444, STS450, and STS493 by lightly touching a piece of frozen tumor against a slide preheated to 50°C. These were incubated in a 0.01% solution of collagenase type H (Sigma Chemical Co., St. Louis, MO) in L15 media (GIBCO BRL, Gaithersburg, MD) for 5 minutes at 37°C and rinsed twice for 10 minutes in Dulbecco's phosphate-buffered saline with 50 mmol/L $MgCl_2$ (pH 7.3) before FISH was performed.[24]

Fluorescence in Situ *Hybridization*

Chromosome X- and 18-specific paints were hybridized according to the manufacturer's instructions (Cambio, Cambridge, UK) and co-hybridized with 20 ng of either an X or 18 centromere-specific probe (pSVX5 and LI84, respectively) that was labeled by nick translation with either biotin or digoxigenin dUTP (Boehringer, Mannheim, Germany). Cosmids in the region of the *SSX1* and *SSX2* genes had been isolated in a previous mapping study.[14] Cosmid C7 was mapped telomeric to the *SSX1* locus, cosmid F17 between the *SSX1* and *SSX2* loci, and cosmid 110 centromeric to the *SSX2* locus. Cosmids C7 and F17 were therefore markers flanking the *SSX1* gene and cosmids 110 and F17 were markers flanking the *SSX2* gene. 1 μg of DNA from each cosmid was labeled with either biotin-11 dATP or digoxigenin dUTP by nick translation. 40 ng of differentially labeled pairs of DNA probes flanking each locus were mixed with 5 μg of Cot 1 DNA and co-hybridized to nuclei to determine which *SSX* gene was disrupted. The slides were incubated and washed, and the probes were detected as previously described.[25] Slides were counterstained with diamidino-2-phenylindole dihydrochloride (DAPI) and viewed using a Zeiss Axioplan microscope with appropriate filters, including a dual bandpass filter for fluorescein isothiocyanate and Texas Red for visual analysis. Images were captured using a cooled CCD camera (Photometrics, Tucson, AZ) coupled to a Macintosh computer with software from Digital Scientific (Cambridge, UK).

Only non-overlapping nuclei were included in the analysis, which was done blind. In the experiments to determine whether a reciprocal translocation was present between chromosomes X and 18, it was noted whether the signal from the paint and centromere for chromosome X and 18 were adjacent in at least one area of the nucleus. Only female nuclei with at least two areas of chromosome X or 18 paint and two signals from the centromere probes and male nuclei that had at least one area of X paint or two areas of 18 paint and one signal from the X centromere or two signals from the 18 centromere were included in this analysis, unless there was an obvious deviation from the expected copy number. In addition, the number of areas of signal from the paint and the centromere-specific probe were counted in nuclei showing at least one signal. In experiments to determine which *SSX* locus was involved, the signals from each cosmid were viewed separately before merging images of these to note whether the signals from the different cosmids were adjacent or not in one of the homologues. For each pair of probes, 20 nuclei were scored.

RT-PCR

RT-PCR to detect *SYT-SSX* transcripts was carried out as described by Clark et al.[15] RNA was extracted from cell lines using a cell lysate method and from frozen tumors using an acid guanidinium thiocyanate-phenol-chloroform extraction.[26,27] An additional precipitation step was performed before reverse transcription of 1 μg of RNA using SuperscriptII reverse transcriptase (GIBCO BRL) and random primers (6-mer primers, Pharmacia, Uppsala, Sweden) in a total volume of 25 μl according to the manufacturer's instructions. To determine that each RNA sample could yield RT-PCR products, amplification was performed using two actin primers: 5′-GAGCGGGAATCGTGCGTGACATT-3′ and 5′-GATGGAGTTGAAGGTAGTTTCGTG-3′, which are from two separate exons. Amplification conditions were 93°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute for 30 cycles in a 25-μl volume. The primers used to amplify *SYT-SSX* cDNA transcripts involving breakpoints in either the *SSX1* and *SSX2* regions on chromosome X were 5′-CAACAGCAAGATGCATACCA-3′ and 5′-CACTTGCTATGCACCTGATG-3′.[15,16] Amplification was carried out in a final volume of 25 μl for 35 cycles at 93°C for 1 minute, 59°C for 1 minute, and 72°C for 1 minute. To determine whether the transcript involved *SSX1* or *SSX2*, the RT-PCR products were precipitated with isopropanol and digested with the restriction enzymes *Lsp*I and *Sma*I. The enzyme *Lsp*I cuts only in *SSX1* RT-PCR products

Table 2. *Percentage of Synovial Sarcoma-Derived Nuclei with Four, Three, Two, and One Area(s) of Signal from Chromosome-Specific Paints and Centromere Probes*

Chromosome specificity of probe	X paint	18 paint	X paint	18 paint	X centromere	18 centromere	X paint	18 paint	X centromere	18 centromere	X paint	18 paint	X centromere	18 centromere
Number of areas of signal	4		3		3		2		2		1		1	
Normal/F	0%	**0%**	1%	**0%**	0%	**0%**	66%	**65%**	92%	**97%**	33%	**35%**	8%	**3%**
A2243/F*	0%	0%	47%	51%	0%	0%	39%	44%	88%	92%	14%	5%	12%	8%
STS450/F†	0%	0%	5%	2%	0%	0%	45%	66%	97%	96%	40%	32%	3%	4%
STS518/F†	0%	0%	32%	34%	0%	0%	41%	36%	85%	82%	27%	30%	15%	18%
STS519/F†	0%	0%	34%	42%	1%	0%	38%	34%	75%	84%	28%	24%	24%	16%
STS552/F	0%	0%	59%	55%	0%	2%	30%	40%	94%	95%	11%	5%	6%	3%
Normal/M	0%	0%	0%	2%	0%	0%	10%	76%	1%	99%	90%	22%	99%	1%
STS416/M	0%	0%	0%	53%	0%	0%	80%	34%	0%	98%	20%	13%	100%	2%
STS444/M†	0%	0%	0%	65%	0%	0%	76%	27%	0%	95%	24%	8%	100%	5%
STS493/M†	0%	38%	64%	32%	0%	0%	30%	30%	94%	98%	6%	0%	6%	2%
STS551/M	0%	0%	0%	48%	0%	3%	70%	38%	1%	90%	30%	14%	99%	7%
TS-I/M	0%	0%	0%	50%	0%	1%	68%	40%	2%	87%	32%	10%	98%	12%

An average of 110 nuclei (between 102 and 137) were scored.
*Cell line.
†Tumor touch imprint.

whereas *Sma*I cuts only in products involving *SSX2*.[16] The RT-PCR products and their restriction digests were separated by gel electrophoresis and visualized by staining with ethidium bromide.

Results

In those samples in which fresh tumor was available for culture, cytogenetic analysis demonstrated a t(X;18)(p11.2;q11.2) amidst a more complex karyotype (Table 1).

Interphase FISH Analysis

The centromere probes hybridized to the normal and tumor nuclei with greater than 90% efficiency (Table 2). Detection of the paints was less efficient than the centromeres, and overlapping areas of signal are likely to have contributed to this (Table 2). It was noted whether the signal from the paint and centromere for chromosome X and 18 were adjacent in at least one area of the nucleus in an average of 88 nuclei (range, 74 to 103). Greater than 75% of the nuclei had separated signals in the controls whereas the situation was reversed in the tumor-derived samples (Figures 1, A–C, and 2). This juxtaposition of signals was indicative of the t(X;18). In sample STS493, greater than 75% of the nuclei showed the juxtaposition of signal from the 18 paint and X centromere in two regions, indicating two copies of the derivative X chromosome (Figure 1D). In addition, an average of 110 nuclei (range, 102 to 137) were scored for the number of signals from both the paint and centromere probes (Table 2). There was no significant difference in the number of centromere signals found in the controls *versus* the synovial sarcoma-derived material (two-way contingency table analysis; $P < 0.0001$) with the exception of the X centromere and STS493, which was deduced to have an additional copy of the derivative X chromosome. However, a significant difference was found comparing the number of areas of paint in the normals with the number found in all of the tumor samples except for STS450 ($P < 0.0001$). The more areas of signal scored from the chromosome-specific paints compared with the number of centromere signals is indicative of a translocation. As this was seen for both the chromosome X and 18 probes, it was also indicative of a reciprocal translocation.

Interphase FISH was also used to determine whether the *SSX1* or *SSX2* region was involved in the cell lines and tumor samples. Cosmids C7 and F17, which flank the *SSX1* region, were co-hybridized to look for disruption of this locus, and cosmids 110 and F17, which flank the *SSX2*, were co-hybridized to look for disruption of the *SSX2* region. A pair of non-adjacent cosmid signals was indicative of disruption. In normal nuclei, two sets of adjacent signals were seen in the majority of nuclei with both pairs of flanking cosmids. In all of the synovial sarcoma-derived samples except STS450, one set of signals was disrupted with one of the pairs of flanking cosmids (Table 3 and Figure 1D). One or two false positive or false negative configurations were scored and attributed to either

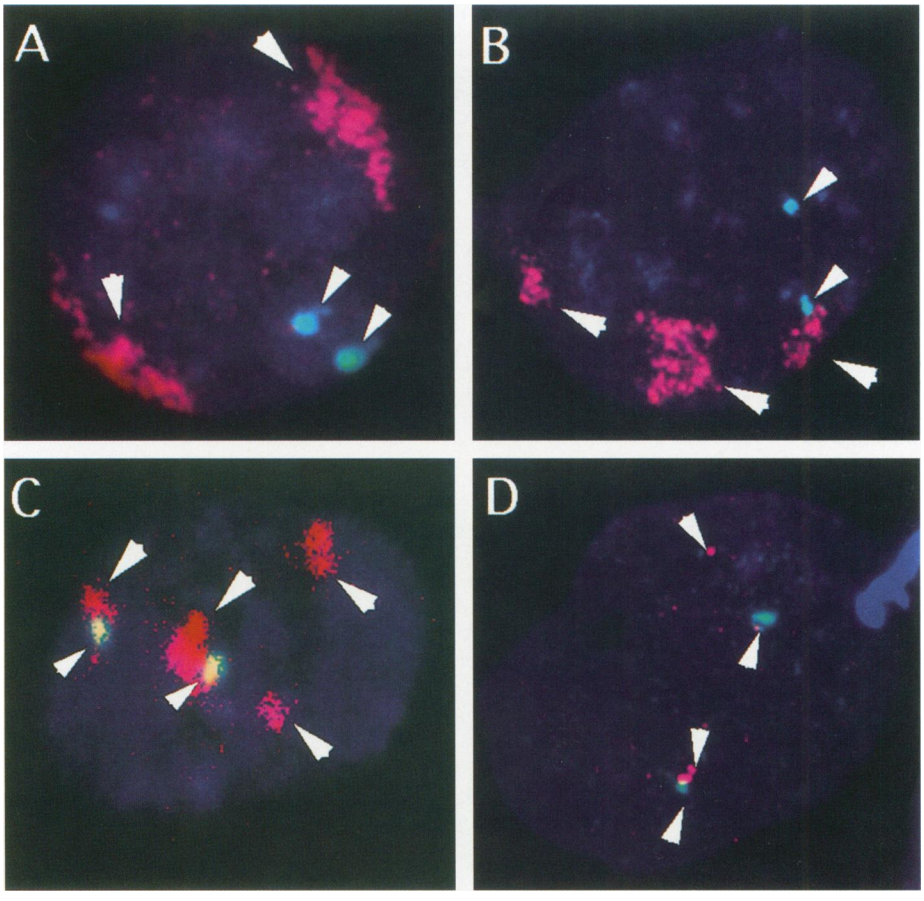

Figure 1. *Chromosome 18-specific paint, biotinylated and detected with Texas Red* (small arrow) *and a chromosome X centromere-specific probe labeled with digoxigenin and detected with FITC* (larger arrow) *hybridized to the following.* A: *Normal female nucleus showing the non-adjacent signals from the paint and centromere.* B: *Nucleus from STS552 showing signal from the paint and centromere adjacent in one region.* C: *Nucleus from STS493 demonstrating two areas with adjacent signal from the paint and centromere indicative of two derivative X chromosomes. Cosmid probes F17 (green) and 110 (red), which flank the* SSX2 *gene were hybridized to female nuclei from STS519* (D). *The pair of red/green signals correspond to the normal homologue, whereas the other pair of signals are separated by the translocation. This indicates disruption of the* SSX2 *gene.*

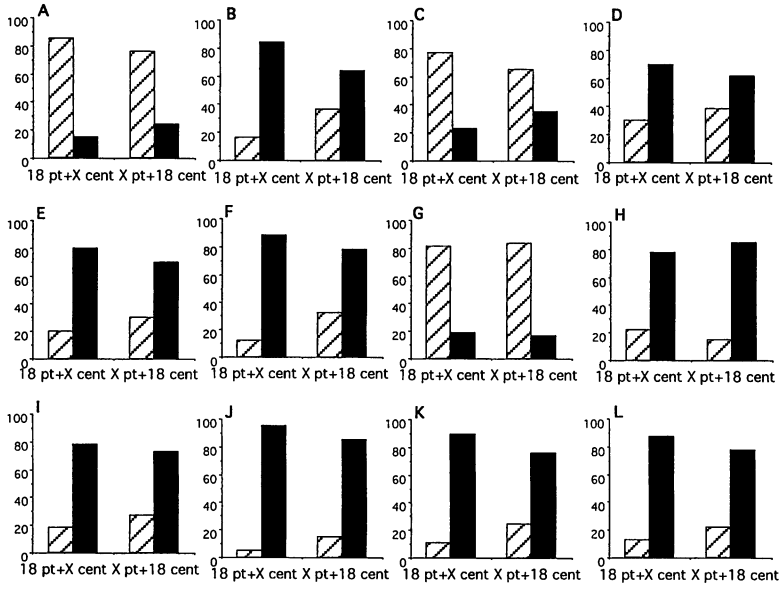

Figure 2. *Histograms representing the percentage of nuclei in which the centromere (cent) and paint (pt) for the X and 18 chromosomes were separate* (hatched bar) *or adjacent* (solid bar) *in at least one area of a nucleus after co-hybridization and differential detection of the probes. Only female nuclei with at least two signals from each probe were included in the analysis and male cells with at least one signal from the X probe and two signals from the 18 probe. Nuclei from the following samples are represented:* A, *Normal female;* B, *A2243;* C, *STS450;* D, *STS518;* E, *STS519;* F, *STS552;* G, *normal male;* H, *STS416;* I, *STS444;* J, *STS493;* K, *STS551; and* L, *TS-1.*

Table 3. *Interphase FISH and RT-PCR Analysis to Determine Whether the* SSX1 *or the* SSX2 *Gene was Involved*

		Interphase FISH analysis				RT-PCR analysis, *SYT-SSX*			
		Probes flanking *SSX1* gene		Probes flanking *SSX2* gene					
Sample	Subtype	Separate	Adjacent	Separate	Adjacent	+/−	*Lsp*I site	*Sma*I site	Confirmed position of breakpoint
Normal		0	20	1	19	−			−
HS-SY-II*	Monophasic‡	20	0	1	19	+	+	−	*SSX1*
A2243*	Biphasic	2	18	19	1	+	−	+	*SSX2*
STS416	Biphasic	19	1	2	18	+	+	−	*SSX1*
STS444†	Monophasic	2	18	19	1	+	−	+	*SSX2*
STS450†	Biphasic	0	20	0	19	−			−
STS493†	Biphasic	17	3	1	19	+	+	−	*SSX1*
STS518†	Monophasic	20	0	1	19	+§	+	−	*SSX1*
STS519†	Monophasic	0	20	20	0	+§	−	+	*SSX2*
STS551	Biphasic	0	20	19	1	+§	−	+	*SSX2*
STS552	Monophasic	18	2	0	20	+§	−	+	*SSX1*
TS-I	Monophasic	0	20	18	2				*SSX1 (by FISH only)*

After co-hybridization of the probes flanking the SSX genes to interphase nuclei their position was determined to be either separate or adjacent in one homologue.
ND, not done.
*Control cell line.
†Tumor touch preparations.
‡Subtype revised to biphasic in reference 19.
§Reference 16.

background signals or normal cell contamination in the tumor samples.

RT-PCR Analysis

All of the samples yielded 234-bp RT-PCR products with the actin primers, indicating that the RNA was sufficiently intact. The primers yielded an identical sized product of 585-bp in all of the synovial sarcoma-derived samples, with the exception of STS450 and control RNA (Table 3). To determine whether the *SSX1* or the *SSX2* loci were involved, the 585-bp products were restricted with *Lsp*I and *Sma*I (Figure 3 and Table 3). Sequence analysis of the RT-PCR products from samples STS416, STS518, STS519, STS551, STS552, and the cell lines has previously validated the involvement of both the *SYT* and either the *SSX1* or the *SSX2* gene.[16]

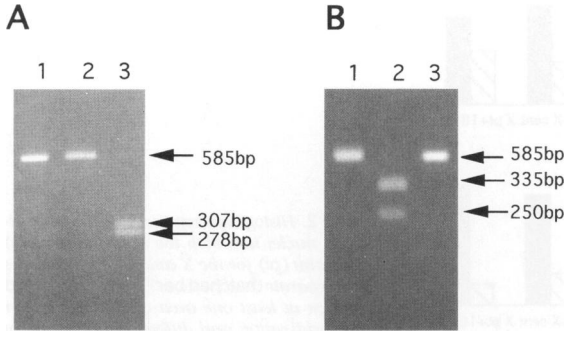

Figure 3. *Detection of* SYT-SSX1 *and* SYT-SSX2 *fusion transcripts by RT-PCR in the synovial sarcoma samples.* **A:** *STS493.* **B:** *STS444.* **Lanes 1** *contain the 585-bp products after RT-PCR amplification for the* SYT-SSX *transcript. A sample of these products was restricted with* Sma*I in* **lanes 2** *and* Lsp*I in* **lanes 3**. *The RT-PCR product from STS493 was cleaved by* Lsp*I* (**A, lane 3**) *but not by* Sma*I, indicating involvement of the* SSX1 *gene, whereas the product from STS444 was cleaved with* Sma*I* (**B, lane 2**) *and not* Lsp*I, indicating involvement of the* SSX2 *gene.*

Discussion

The monophasic and undifferentiated forms of synovial sarcoma can be difficult to distinguish from other tumors such as fibrosarcomas, malignant fibrous histiocytomas, malignant schwannomas, and, in rare instances, carcinomas such as adenocarcinomas.[3,4,11] Correct diagnosis in such cases may impact on treatment. Using two-color FISH, the configuration of the signals from a centromere-specific probe and a chromosome-specific paint in nuclei, including those prepared from tumor touch imprints, has been shown to be an effective way to identify the t(X;18) associated with synovial sarcoma. Hybridization of the X centromere probe with a chromosome 18-specific paint to indicate the presence of the derivative X chromosome is regarded as the most effective test because formation of this chromosome is thought to be the key event in tumorigenesis. The derivative X, rather than the derivative 18, is found in complex rearrangements and loss of the derivative 18 has been noted in some tumors and cell lines, suggesting that it is not required in the maintenance of the tumor's phenotype.[10,17] Comparing the number of signals from the chromosome-specific paint

with the number of centromere signals was also used here and previously to infer the presence of the t(X;18).[20]

In case STS493 in this study, it was deduced that there were two copies of a derivative X chromosome analogous to duplication of the Philadelphia chromosome found in chronic myelogenous leukemia during the clonal evolution of the disease.[28] Duplication of a derivative chromosome may have a gene dosage effect with advantageous results on cellular proliferation.

RT-PCR for the *SYT-SSX* fusion transcripts, demontrated here and previously, were consistent with the interphase FISH results.[15] This excluded the possibility that the negative FISH results in case STS450 were due to sampling normal rather than tumor cells. By designing primers that can amplify a smaller sized product from the fusion transcript, it should also be possible to detect an aberrant transcript in RNA isolated from paraffin-embedded samples in addition to the fresh and frozen samples used here. The most appropriate approach for a laboratory can be chosen as an adjunct to diagnosis. The approaches could also be used to determine tumor-free margins to ensure complete resection of the sarcoma and to identify patients with metastatic disease who should be treated more aggressively.

Previous studies have suggested a correlation between the histological subtype and the position of the chromosome X breakpoint; biphasic tumors were associated with rearrangement of the *SSX1* locus and monophasic tumors were associated predominantly, but not exclusively, with rearrangement of the *SSX2* locus.[13,18,19] This relationship was not found in a larger series of 32 samples[16] or in the new patient samples presented here. Pertinent to examining the possible correlation is the potential misclassification of the histological subtype. This may be possible for the monophasic subtype in which the second cellular component is not apparent. However, in the biphasic tumors both epithelial and spindle cells have been identified, and in this study biphasic samples have been shown by two approaches to have disruption in both the *SSX1* and the *SSX2* genes (Table 3). The 585-bp fusion transcripts identified for *SYT-SSX1* and *SYT-SSX2* are highly homologous but have minor differences in sequence, which result in 13 amino acid changes.[16,29] A total of four variant transcripts have been identified, and it is interesting to speculate how these differences and those between *SYT-SSX1* and *SYT-SSX2* might affect protein folding and function and whether this could alter the phenotype of the tumors.[16,29]

Inferring disruption of the genes involved by cohybridizing flanking probes to interphase nuclei and RT-PCR to amplify the *SYT-SSX* fusion transcripts may identify cases that do not have a t(X;18) at the cytogenetic level but nonetheless involve the molecular rearrangement associated with the translocation. This situation has been found in other tumors associated with specific translocations; for example, in acute promyeloctyic leukemia, cryptic involvement of the *RARA* gene was demonstrated despite the intact cytogenetic appearance of two number 17 chromosomes, which normally harbor this gene.[30] There is also the possibility of other genes becoming fused to either the *SYT* gene or the *SSX* genes, analogous to the promiscuous nature of the *EWS* and *FKHR* genes rearranged in the Ewing family of tumors and alveolar rhabdomyosarcoma, respectively.[31–34] The RT-PCR-negative synovial sarcoma case presented here was typically biphasic and does not appear to involve the *SSX* genes or a reciprocal translocation of chromosome 18. Three other RT-PCR-negative cases (out of a total of thirty-two cases) were previously identified that did not show involvement of either the *SSX* or the *SYT* genes.[16] Additional molecular characterization of such cases is required.

Recently, interphase FISH and RT-PCR have been used as an effective diagnostic aid in distinguishing members of the Ewing family of tumors from other small round cell tumors.[24,35–37] Detection of the fusion gene or aberrant transcripts that result from the specific translocations associated with this group of tumors are suggested to be pathognomonic.[31,37] A similar rationale for classifying synovial sarcomas based on the presence of fusion transcripts detected by RT-PCR and/or FISH and cytogenetic data may now be valid. Investigation of additional cases to determine the frequency and molecular nature of rearrangements found in synovial sarcoma is warranted to validate this suggestion.

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