

SYT-SSX GENE FUSION AS A DETERMINANT OF MORPHOLOGY AND PROGNOSIS IN SYNOVIAL SARCOMA

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ABSTRACT

Background Synovial sarcomas account for up to 10 percent of soft-tissue sarcomas and include two major histologic subtypes, biphasic and monophasic, defined respectively by the presence and absence of glandular epithelial differentiation in a background of spindle tumor cells. A characteristic SYT-SSX fusion gene resulting from the chromosomal translocation t(X;18)(p11;q11) is detectable in almost all synovial sarcomas. The translocation fuses the SYT gene from chromosome 18 to either of two highly homologous genes at Xp11, SSX1 or SSX2. SYT-SSX1 and SYT-SSX2 are thought to function as aberrant transcriptional regulators. We attempted to determine the influence of the two alternative forms of the SYT-SSX fusion gene on tumor morphology and clinical outcome in patients with this sarcoma.

Methods We analyzed SYT-SSX fusion transcripts in 45 synovial sarcomas (33 monophasic and 12 biphasic) by the reverse-transcriptase polymerase chain reaction and compared the results with relevant clinical and pathological data.

Results The SYT-SSX1 and SYT-SSX2 fusion transcripts were detected in 29 (64 percent) and 16 (36 percent) of the tumors, respectively. There was a significant relation ($P=0.003$) between histologic subtype (monophasic vs. biphasic) and SSX1 or SSX2 involvement in the fusion transcript: all 12 biphasic synovial sarcomas had an SYT-SSX1 fusion transcript, and all 16 tumors that were positive for SYT-SSX2 were monophasic. Kaplan-Meier analysis of 39 patients with localized tumors showed that the 15 patients with SYT-SSX2 had significantly better metastasis-free survival than the 24 patients with SYT-SSX1 ($P=0.03$ by multivariate analysis; relative risk, 3.0). There were no significant correlations between the type of SYT-SSX transcript and age, sex, tumor location and size, whether there were metastases at diagnosis, or whether patients underwent chemotherapy. Histologic subtype alone was not prognostically important.

Conclusions The type of SYT-SSX fusion transcript correlates with both the histologic subtype and the clinical behavior of synovial sarcoma. SYT-SSX fusion transcripts are a defining diagnostic marker of synovial sarcomas and may also yield important independent prognostic information. (N Engl J Med 1998;338:153-60.)

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SYNOVIAL sarcomas, which account for 5 to 10 percent of soft-tissue sarcomas, typically arise in the para-articular regions in adolescents and young adults. These tumors occur in two major forms, biphasic and monophasic.¹ Biphasic synovial sarcomas contain both epithelial cells arranged in glandular structures and spindle cells, whereas monophasic types are entirely composed of spindle cells.

Cytogenetic studies of synovial sarcomas have revealed a characteristic chromosomal translocation, t(X;18)(p11;q11), in more than 90 percent of both biphasic and monophasic tumors.² The presence of this translocation as the sole cytogenetic abnormality in at least some tumors suggests that it is the primary causal event in synovial sarcoma. Cloning of the translocation breakpoints showed that t(X;18) results in the fusion of two novel genes, designated SYT (at 18q11) and SSX (at Xp11).³ It soon became apparent that the Xp11 breakpoint actually involves either of two closely related genes, SSX1 and SSX2,^{4,5} located in the vicinity of ornithine aminotransferase-like (OATL) pseudogenes 1 and 2, respectively. The SSX1 and SSX2 genes are presumably derived from a relatively recent duplication event and encode proteins with considerable homology (81 percent). Recently, additional related SSX genes, apparently not involved by t(X;18), have been identified in Xp11.^{6,7}

Like other chromosomal translocations in sarcomas, t(X;18) results in the formation of a chimeric protein that probably deregulates the transcription and, hence, the expression of specific target genes.^{8,9} Consistent with the intracellular site of transcriptional regulators, SYT, SSX, and SYT-SSX are nuclear proteins.^{10,11} Moreover, the amino-terminal regions of both SSX1 and SSX2 contain a repressor domain that inhibits transcription.¹⁰ In the chimeric transcript of synovial sarcoma this repressor domain, encoded by the 5' portion of SSX1 and SSX2, is replaced by all but the 3' end of SYT, a ubiquitously expressed gene encoding a region that can function as a transcriptional activation domain.¹⁰ SYT and the

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SSX proteins probably regulate transcription primarily through interactions between proteins because they seem to lack DNA-binding domains. In the current working model of the molecular pathogenesis of synovial sarcoma, t(X;18) subverts normal transcriptional regulation by directing SYT-mediated transcriptional activation to targets presumably recognized by the carboxy end of SSX and normally inhibited by the latter's amino-terminal transcriptional repressor domain. The genes normally repressed by SSX1 and SSX2, and presumably aberrantly activated by the SYT-SSX gene product, are unknown.

Whether the precise location of the X chromosome breakpoint correlates with the morphology of synovial sarcoma has been debated for some time. Results of fluorescence in situ hybridization (FISH) from three independent groups suggested a relation between the two histologic subtypes of synovial sarcoma and breakpoints in the OATL1 or OATL2 region, now known respectively to contain the SSX1 and SSX2 genes.¹²⁻¹⁴ However, another group failed to confirm these findings.^{5,15,16} Moreover, no studies have compared the effects of SSX1 and SSX2 in t(X;18) on clinical outcome. To address these issues, we compared the type of SYT-SSX fusion, as determined by the reverse-transcriptase polymerase chain reaction (RT-PCR), with relevant clinicopathological data in 45 patients with synovial sarcoma.

METHODS

Patients and Tumors

The study included 45 patients with histologically verified synovial sarcoma, treated at Memorial Sloan-Kettering Cancer Center between 1982 and 1997, who were enrolled exclusively on the basis of the availability of frozen tumor for molecular analysis. Partial clinical and molecular data on 34 patients were included in a previous report.¹⁷ There were 25 male and 20 female patients. The age at diagnosis ranged from 13 to 70 years (mean, 33). Thirty-four primary tumors were located in the extremities (33 in the lower extremity including the buttocks and groin, and 1 in the upper extremity) and 11 in the central axis (Table 1). The size of the tumor, represented by the largest tumor dimension in the resected specimen, ranged from 2 to 21 cm (mean, 9.5). At the time of diagnosis, 39 patients had localized disease and 6 patients had distant metastases.

The tumor samples studied were obtained from primary tumor in 29 cases, metastatic deposits in 12, and locally recurrent tumor in 4. The histopathological findings were reviewed by two physicians who were unaware of the results of molecular genetic analyses. All tumors had morphologic and immunohistochemical features consistent with synovial sarcoma. Tumors were classified as biphasic on the basis of architectural evidence of epithelial differentiation, such as glandular structures. All tumors were considered high grade, and all were deep-seated. All primary tumors were surgically treated with curative intent. Negative surgical margins were achieved in 44 patients, and 1 patient had a microscopically positive margin. Radiation therapy was given to 19 patients (external radiation in 10 patients and brachytherapy in 9 patients).

Chemotherapy including doxorubicin, ifosfamide, or both¹⁸ was administered to 21 patients. The postoperative follow-up period ranged from 2 to 180 months (mean, 40; median, 26). Ex-

tended follow-up data (>60 months) were available on seven patients. These included four patients with SYT-SSX1, two of whom were alive with disease at 144 and 180 months (Patients 31 and 9, respectively) and two of whom had no apparent disease at 61 and 78 months (Patients 10 and 25), and three patients with SYT-SSX2, of whom two were alive with disease at 172 and 180 months (Patients 7 and 2) and one had died of the disease at 62 months (Patient 8).

RT-PCR Analysis

Total RNA was isolated from snap-frozen tumor samples according to the acid-guanidinium-phenol-chloroform method, then 1 µg was reverse-transcribed with Superscript II reverse transcriptase (GIBCO BRL, Gaithersburg, Md.) and random hexamers. The resulting complementary DNA was subjected to PCR amplification with the forward primer 5'CAACAGCAAGATGCATACCA3' for SYT³ and one of the following reverse primers: 5'CACTTGCTATGCACCTGATG3' (consensus) for SSX,³ 5'GGTGCAGTTGTTTCCCATCG3' for SSX1,⁴ 5'GGCACAGC-TCTTCCCATCA3' for SSX2,⁴ or 5'CCCCITTTGGGTC-AGATATCA3' for SSX3. The amplification conditions consisted of denaturation at 95°C for 1 minute, annealing at 60°C for 1 minute, and extension at 72°C for 1 minute for 35 cycles, with a final period of extension at 72°C for 10 minutes. The products were separated by electrophoresis in agarose gels and visualized with ethidium bromide.

We identified the amplified fragments on the basis of their size on agarose gels and confirmed the results if necessary by blotting the PCR products onto nylon membranes and hybridizing them with internal and junction-specific probes, or by sequencing with the dideoxy chain-termination method modified for fluorescent-based DNA sequencing with a DNA sequencer (model 373, Applied Biosystems, Foster City, Calif.). As an internal control for PCR and for quality assessment of the tumor RNAs, a 247-bp portion of the ubiquitously expressed phosphoglycerate kinase transcript was amplified with primers 5'CAGTTTGGAGCTCC-GGAAG3' and 5'TGCAAATCCAGGGTGCAGTIG3' under identical PCR conditions. Negative controls included reactions lacking RNA and reactions lacking reverse transcriptase.

Statistical Analysis

Survival curves were estimated according to the method of Kaplan and Meier from the date of primary-tumor surgery to the time of metastatic recurrence or death.¹⁹ The differences in survival curves were examined with the log-rank test. Multivariate analysis was performed by Cox proportional-hazards analysis. To arrive at a parsimonious multivariate model, covariates were selected with a stepwise regression model using backward elimination. Associations between variables were studied with Fisher's exact test. All P values are two-sided.

RESULTS

Of the 45 synovial sarcomas, 29 (64 percent) had a SYT-SSX1 fusion transcript and 16 (36 percent) contained a SYT-SSX2 fusion transcript (Fig. 1). Representative histologic sections from two patients are shown in Figure 2. Because the SSX1 primer we used might misprime from SSX3, all tumors positive for SYT-SSX1 were also analyzed with SYT and an SSX3-specific reverse primer. None of these samples contained an SYT-SSX3 fusion transcript.

The results of all analyses of fusion transcripts and the clinicopathological features of the 45 patients are summarized in Table 1. There were 12 biphasic and 33 monophasic tumors. All 12 biphasic synovial sarcomas had a SYT-SSX1 fusion transcript, where-

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TABLE 1. CLINICAL, HISTOLOGIC, AND MOLECULAR CHARACTERISTICS OF THE STUDY PATIENTS.*

PATIENT No.	AGE (YR)/SEX	PRIMARY SITE	HISTOLOGIC SUBTYPE	SAMPLE SOURCE	SYT-SSX FUSION TYPE	ADJUVANT THERAPY	TIME TO RECURRENCE (MO)	METASTASIS		FOLLOW-UP (MO)	STATUS
								SITE	TIME AFTER DIAGNOSIS (MO)		
1	56/M	Foot	Monophasic	Met	1	C	8	Lung	24	36	DOD
2	29/M	Popliteal	Monophasic	Met	2	C	29	Lung	168	180	AWD
3	19/M	Foot	Monophasic	P	1	C	—	Lung	At diagnosis	14	DOD
4	46/M	Lower leg	Monophasic	P	1	C	—	Lung	At diagnosis	23	DOD
5	28/F	Popliteal	Monophasic	Met	1	C	—	Lung	11	20	DOD
7	24/F	Knee	Monophasic	Met	2	C	—	Soft tissue	64	172	AWD
8	21/F	Groin	Monophasic	P	2	C, R	—	Lung	36	62	DOD
9	15/F	Abdomen	Monophasic	Met	1	C, R	120	Lung	60	180	AWD
10	14/F	Thigh	Biphasic	P	1	R	—	—	—	61	NED
11	40/F	Thigh	Biphasic	P	1†	C	—	Lung	At diagnosis	14	DOD
12	36/F	Thigh	Monophasic	P	2	R	—	Lung	45	51	AWD
13	34/F	Thigh	Monophasic	P	2†	—	—	—	—	24	NED
14	17/M	Neck	Monophasic	P	1	R	—	Lung	7	38	DOD
15	24/M	Thigh	Monophasic	Met	1	R	18	Lung	14	26	AWD
16	13/F	Chest wall	Monophasic	P	2	C, R	—	Lung	At diagnosis	10	DOD
17	35/M	Knee	Biphasic	P	1	C	6	Lung	6	26	DOD
18	18/M	Buttock	Monophasic	P	1	—	—	—	—	11	NED
20	31/M	Abdomen	Monophasic	P	1	R	15	Bone	16	36	DOD
25	34/M	Thigh	Biphasic	P	1	C, R	—	—	—	78	NED
26	28/F	Buttock	Monophasic	P	1	C	—	Lung	At diagnosis	15	DOD
29	48/F	Foot	Monophasic	P	1	C, R	11	Lung	17	36	DOD
30	34/F	Groin	Monophasic	P	2	—	—	—	—	36	NED
31	50/F	Foot	Monophasic	Met	1	—	—	Lung	80	144	AWD
36	70/F	Popliteal	Monophasic	P	1	—	—	—	—	47	NED
37	52/M	Groin	Biphasic	Met	1†	—	6	—	—	12	DOD
38	26/F	Chest wall	Monophasic	Rec	2 var	C	28	Liver	28	60	AWD
39	36/M	Thigh	Monophasic	Met	2	—	39	Soft tissue	31	43	AWD
41	32/F	Thigh	Monophasic	P	1	C, R	—	Lung	7	15	DOD
42	20/M	Groin	Monophasic	P	2	—	—	—	—	22	NED
43	39/M	Thigh	Biphasic	P	1	R	16	Lung	16	33	AWD
44	35/M	Popliteal	Monophasic	P	2†	—	—	—	—	30	NED
47	26/F	Buttock	Monophasic	Met	1†	C, R	—	Lung	12	15	AWD
49	23/M	Breast	Monophasic	Met	1	R	—	Lung	23	37	DOD
50	29/F	Thigh	Monophasic	P	2	R	—	—	—	18	NED
51	52/F	Foot	Biphasic	P	1	R	—	—	—	17	NED
53	51/M	Elbow	Biphasic	P	1	C, R	—	—	—	13	NED
55	43/M	Foot	Biphasic	Met	1	C	—	Lung	13	22	AWD
56	36/F	Mediastinum	Monophasic	P	2	—	—	—	—	2	NED
57	26/M	Foot	Biphasic	Rec	1	—	—	—	—	13	NED
58	26/M	Abdomen	Monophasic	P	2	—	4	—	—	7	AWD
60	39/M	Chest wall	Monophasic	P	1	—	—	—	—	5	NED
62	57/M	Foot	Biphasic	P	1	C	—	—	—	3	NED
63	45/M	Thigh	Biphasic	P	1	C	—	Lung	At diagnosis	2	AWD
64	21/M	Chest wall	Monophasic	Rec	2	R	24	—	—	30	NED
65	28/M	Chest wall	Monophasic	Rec	2	—	4	—	—	55	AWD

*Met denotes metastasis, C chemotherapy, DOD died of disease, AWD alive with disease, P primary tumor, R radiation, NED no evidence of disease, Rec local recurrence, and var variant fusion product.¹⁷

†This tumor was previously reported as negative for the fusion transcript.¹⁷

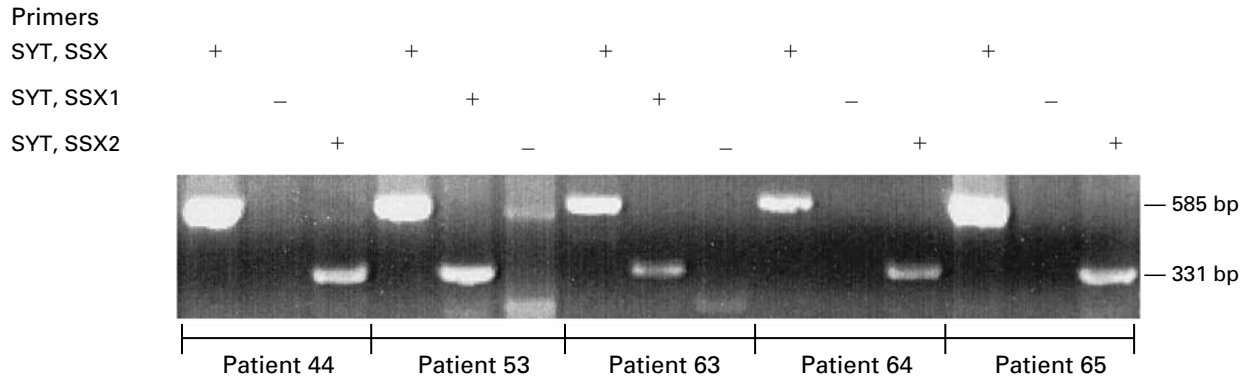


Figure 1. Analysis of *SYT-SSX* Fusion Transcripts in Synovial Sarcoma.

We performed RT-PCR on total RNA from five patients with synovial sarcoma, using the *SYT*, *SSX* (consensus), *SSX1*, and *SSX2* primers. The PCR products were then separated by electrophoresis on an agarose gel. The size of the specific *SYT-SSX* product is 585 bp, and the *SYT-SSX1* and *SYT-SSX2* products are both 331 bp. Tumors from Patients 44, 64, and 65 contain the *SYT-SSX1* fusion transcript. Tumors from Patients 53 and 63 contain the *SYT-SSX2* fusion transcript. Fainter nonspecific RT-PCR products are seen with the *SYT* and *SSX* primers in Patients 53 and 63.

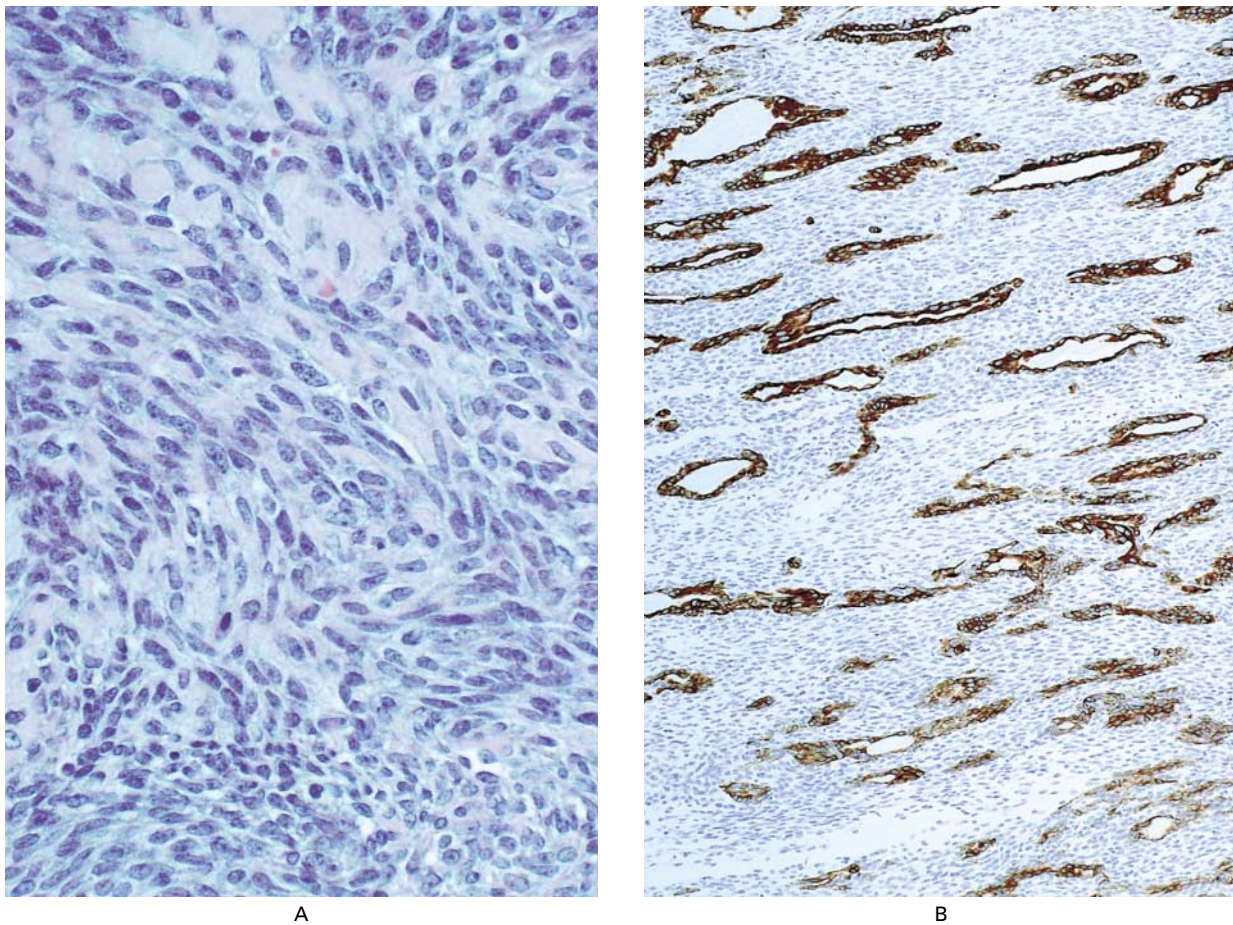


Figure 2. Monophasic and Biphasic Synovial Sarcoma.

Panel A shows monophasic synovial sarcoma from Patient 47 (hematoxylin and eosin, $\times 100$). Panel B shows biphasic synovial sarcoma from Patient 11 immunostained with cytokeratin antibody to highlight the epithelial cells lining the gland-like spaces ($\times 40$). Both tumors expressed *SYT-SSX1*.

as 17 monophasic synovial sarcomas contained an *SYT-SSX1* fusion transcript and 16 had an *SYT-SSX2* fusion transcript. There was a statistically significant ($P=0.003$) relation between the histologic subtype and the presence of *SSX1* or *SSX2* in the fusion transcript. There were no significant correlations between the type of transcript and age, sex, tumor location or size, or whether there were metastases at diagnosis (Table 2).

The overall survival rate at five years for all 45 patients was 55 percent. In the study group as a whole, the presence of metastases at diagnosis was the only significant factor related to overall survival ($P=0.001$ by multivariate analysis). Further analysis was performed on the 39 patients who had localized tumor at diagnosis. In this subgroup, the 15 patients with tumors containing the *SYT-SSX2* fusion transcript had a significantly longer metastasis-free survival than the 24 patients with tumors containing an *SYT-SSX1* fusion transcript ($P=0.03$ by multivariate analysis; relative risk, 3.0; 95 percent confidence interval, 1.1 to 8.0) (Fig. 3). In the multivariate analysis, the type of fusion transcript emerged as the only variable associated with metastasis-free survival (Table 3). There was also a trend toward better overall survival in patients with localized tumors containing the *SYT-SSX2* fusion transcript ($P=0.11$ by multivariate analysis) (data not shown). The histologic subtype did not affect survival in any subgroup of patients.

Because some patients were treated before the widespread use of ifosfamide, one of the few effective agents against synovial sarcoma,^{20,21} we examined the distribution of ifosfamide-treated patients in the *SYT-SSX1* and *SYT-SSX2* groups. Among the 29 patients in the *SYT-SSX1* group, 10 had received ifosfamide, as compared with 2 of 14 patients in the *SYT-SSX2* group (Table 2). This difference was not statistically significant ($P=0.16$ by Fisher's exact test), suggesting that the survival advantage for the *SYT-SSX2* group was not related to treatment with this agent. Not surprisingly, adjuvant chemotherapy, with or without ifosfamide, had no significant effect on outcomes in this historical cohort of patients who did not undergo randomization for adjuvant chemotherapy.

DISCUSSION

Specific chromosomal translocations have come to define many types of sarcomas.^{8,9} The translocation $t(X;18)(p11;q11)$ occurs in over 90 percent of synovial sarcomas.^{2,22} Cloning of the translocation breakpoints has shown that two novel genes are thereby rearranged, *SYT* (at 18q11) and a duplicated gene, *SSX* (at Xp11).³ Identification of the *SYT-SSX* chimeric transcript provides a sensitive diagnostic test for synovial sarcoma.^{5,16,17} Previously, we and Crew et al.^{5,17} found a specific *SYT-SSX* RT-PCR product in

TABLE 2. COMPARISON OF FUSION TYPES AND CLINICOPATHOLOGICAL FEATURES.

CHARACTERISTIC	SYT-SSX1 (N=29)	SYT-SSX2 (N=16)	P VALUE
Age			0.13
<30 yr	11	10	
≥30 yr	18	6	
Sex			0.35
Male	18	7	
Female	11	9	
Location of tumor			0.16
Extremity	24	10	
Central axis	5	6	
Histologic subtype			0.003
Biphasic	12	0	
Monophasic	17	16	
Greatest dimension of tumor			0.48
<5 cm	6	5	
≥5 cm	23	11	
Metastasis at diagnosis			0.40
No	24	15	
Yes	5	1	
Ifosfamide-based adjuvant chemotherapy			0.16
Yes	10	2	
No	19	14	

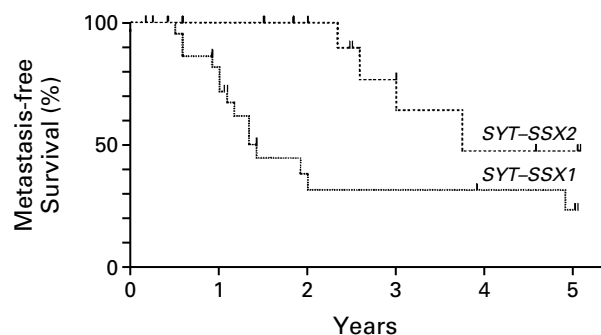


Figure 3. Metastasis-free Survival in Patients with Localized Tumors.

Metastasis-free survival was significantly longer among those with the *SYT-SSX2* fusion transcript than among those with *SYT-SSX1* ($P=0.03$ by multivariate analysis).

58 of 64 (91 percent) synovial sarcomas tested. Analysis of additional material subsequently obtained from several of our patients with initially negative results yielded positive results, indicating that the prevalence of this fusion transcript in synovial sarcoma approaches 100 percent when there is material adequate for molecular analysis. This finding is clinically important, because the differential diagnosis of synovial sarcoma is broad and often problematic.¹

The aim of our study was to determine whether

TABLE 3. ANALYSIS OF FACTORS PREDICTING METASTASIS-FREE SURVIVAL IN PATIENTS WITH LOCALIZED SYNOVIAL SARCOMA.

FACTOR	NO. OF PATIENTS (N=39)	5-YR SURVIVAL RATE (%)	UNIVARIATE P VALUE	MULTIVARIATE P VALUE*	RELATIVE RISK (95% CI)†
Age			0.48		
<30 yr	18	49			
≥30 yr	21	28			
Sex			0.51		
Male	22	28			
Female	17	49			
Location of tumor			0.65		
Extremity	29	38			
Central axis	10	0			
Histologic subtype			0.98		
Biphasic	10	50			
Monophasic	29	28			
Greatest dimension of tumor			0.04	0.11	2.6 (0.8–8.2)
<5 cm	11	75			
≥5 cm	28	28			
Fusion transcript			0.04	0.03	3.0 (1.1–8.0)
<i>SYT-SSX2</i>	15	48			
<i>SYT-SSX1</i>	24	24			
Adjuvant chemotherapy (any type)			0.51		
No	24	38			
Yes	15	38			
Ifosfamide-based adjuvant chemotherapy‡			0.18		
No	31	42			
Yes	8	21			

*Factors with a P value less than 0.25 are included in the multivariate analysis.

†CI denotes confidence interval.

‡This factor was not included in the multivariate analysis.

the two alternative forms of the *SYT-SSX* fusion transcript (*SYT-SSX1* and *SYT-SSX2*) are related to the histologic and clinical characteristics of synovial sarcoma. Among 45 tumors analyzed, 29 (64 percent) contained an *SYT-SSX1* fusion transcript and 16 (36 percent) had an *SYT-SSX2* fusion transcript, a ratio concordant with that obtained elsewhere.⁵ *SSX3*, a third member of the *SSX* gene cluster at Xp11, was not involved in any case or in a previous series of 15 synovial sarcomas.⁶

We found a significant correlation between the *SSX* gene involved in the fusion transcript and the histologic subtype of tumors. All 16 synovial sarcomas with *SSX2* involvement were monophasic, whereas all 12 biphasic synovial sarcomas showed *SSX1* involvement. This observation confirms earlier FISH results from three independent studies including a total of 23 tumors, which found a correlation between the location of the X chromosome breakpoint and the histologic subtype: in aggregate, all eight biphasic tumors had a breakpoint in the OATL1 region (*SSX1* gene).¹²⁻¹⁴ Another group has reported the *SYT-SSX2* fusion in two biphasic synovial sarcomas,^{5,16} of which only one could be histologically confirmed to

contain focal glandular structures (Fisher C: personal communication). Thus, the present analysis and previous studies suggest a significant relation between the type of fusion and the histologic findings, but it is not a simple one, since over half of tumors containing *SYT-SSX1* are monophasic. Hypothetically, the *SYT-SSX1* fusion protein, although not sufficient by itself to induce architectural epithelial differentiation (gland formation), may be more permissive than *SYT-SSX2* with respect to this process.

Prognosis in synovial sarcoma has been correlated with age, site, tumor size, mitotic rate, necrosis, and histologic subtype.²³⁻²⁸ Our study confirms that the presence of metastases at diagnosis is the most important prognostic factor for synovial sarcomas. The prognostic value of the histologic subtype has been controversial. Some early studies reported a more favorable outcome in patients with biphasic tumors,^{23,29} whereas other groups found no differences in survival between patients with monophasic tumors and those with biphasic tumors.²⁵⁻²⁸ Our results confirm the lack of prognostic importance of the histologic subtype. In most sarcomas arising in the extremities, grade and depth are usually also of prognostic im-

portance,³⁰ but they are of little value in defining prognostic subgroups in synovial sarcomas, because these tumors are uniformly high grade and almost all are deep-seated.¹ It is therefore important to look for other prognostic variables.

Our data suggest that the type of *SYT-SSX* fusion transcript may be a major prognostic factor in synovial sarcoma. This result does not appear to be biased by unequal distribution of the patients, because possible prognostic factors for patients with localized tumors containing an *SYT-SSX2* transcript (mean age, 29 years; tumor size, 8.9 cm; adjuvant chemotherapy, 4 of 15 patients; median follow-up period, 36 months) were similar to those for patients with tumors containing an *SYT-SSX1* transcript (mean age, 37 years; tumor size, 8.4 cm; adjuvant chemotherapy, 11 of 24 patients; median follow-up period, 26 months). Moreover, in multivariate analysis, the type of *SYT-SSX* fusion transcript was the sole independent prognostic factor for metastasis-free survival in localized tumors ($P=0.03$). We examined metastasis-free survival because local recurrence of sarcomas is primarily related to the quality of local surgical control.³⁰ Since the type of *SYT-SSX* fusion transcript was significantly correlated with both the clinical course and the histologic subtype, but the latter alone had no prognostic importance, the biologic mechanisms underlying these two different effects of fusion type may be independent.

Remarkably, there were no deaths due to cancer in patients with tumors containing the *SYT-SSX2* fusion transcript in the first five years after surgery (data not shown). Analysis of metastasis-free survival showed that after the first two years, the survival curve of patients with tumors containing *SYT-SSX2* began to drop and became almost parallel to that of patients with tumors containing *SYT-SSX1* (Fig. 3). These findings indicate that patients with tumors positive for *SYT-SSX2* had a low risk of early relapse, but the cumulative risk of distant metastasis may be similar in both groups. A relatively high incidence of late metastases is characteristic of synovial sarcoma.¹ It is possible that tumors with *SYT-SSX2* could account for this clinical observation in synovial sarcoma. Studies with a larger number of patients and longer follow-up are needed to confirm these observations. We have developed a method for detecting *SYT-SSX* transcripts in archival formalin-fixed, paraffin-embedded material³¹ that should facilitate retrospective studies of historical cohorts of patients with extended follow-up.

The biologic basis of our results is unclear. Comparative functional studies of the two types of *SYT-SSX* fusion proteins have not yet been performed. Hypothetically, the differences in 13 amino acids among the 78 amino acids of the carboxy terminal of the SSX proteins included in *SYT-SSX*^{4,5} may influence specific protein-protein interactions and,

hence, alter the target gene specificity of *SYT-SSX* chimeric proteins or the degree of transactivation of target-gene subgroups, thereby influencing the biologic behavior of synovial sarcoma.

Different fusion products generated by cytogenetically identical chromosomal translocation can have major clinical correlates. This was first demonstrated for the *BCR-ABL* rearrangement (the Philadelphia chromosome), in which the position of the breakpoint within the *BCR* gene determines which *BCR* exons are included in the encoded chimeric tyrosine kinase, thereby leading to either chronic myelogenous leukemia or acute lymphoblastic leukemia.³² More recently, the type of *PAX-FKHR* chimeric transcription factor (i.e., *PAX3-FKHR* or *PAX7-FKHR*) has been shown to influence the clinical presentation and course of alveolar rhabdomyosarcoma.³³ We and others have also found that the precise exon composition of the *EWS-FLI1* fusion transcript is a prognostic factor in the Ewing's sarcoma tumor group.^{34,35} The structure of the fusion transcript appears to be a novel marker of clinical behavior in sarcomas and leukemias with specific chromosomal translocations that show molecular heterogeneity.

In conclusion, the *SYT-SSX* gene fusion resulting from the translocation $t(X;18)$, already presumed to be a primary pathogenetic event in synovial sarcoma, also appears to influence its morphology and subsequent clinical behavior. Analysis of *SYT-SSX* fusion transcripts provides both a useful diagnostic marker for synovial sarcoma and important independent prognostic information, because *SYT-SSX1* and *SYT-SSX2* may define subtly different types of synovial sarcoma.

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REFERENCES

1. Enzinger FM, Weiss SW. Synovial sarcoma. In: Enzinger FM, Weiss SW, eds. Soft tissue tumors. 3rd ed. St. Louis: Mosby, 1995:757-86.
2. 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;144:1121-34.
3. Clark J, Rocques PJ, Crew AJ, et al. Identification of novel genes, SYT and SSX, involved in the $t(X;18)(p11.2;q11.2)$ translocation found in human synovial sarcoma. *Nat Genet* 1994;7:502-8.
4. 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;4:1097-9.
5. Crew AJ, Clark J, Fisher C, et al. 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;14:2333-40.
6. de Leeuw B, Balemans M, Geurts van Kessel A. A novel Kruppel-associated box containing the SSX gene (SSX3) on the human X chromosome is not implicated in $t(X;18)$ -positive synovial sarcomas. *Cytogenet Cell Genet* 1996;73:179-83.
7. Chand A, Clark J, Cooper CS, Craig IW. Long-range organization of reiterated sequences, including the SSX1 cDNA at the OATL1 cluster in Xp11.23. *Genomics* 1995;30:545-52.
8. Ladanyi M. The emerging molecular genetics of sarcoma translocations. *Diagn Mol Pathol* 1995;4:162-73.
9. Sorensen PH, Triche TJ. Gene fusions encoding chimaeric transcription factors in solid tumours. *Semin Cancer Biol* 1996;7:3-14.

10. Brett D, Whitehouse S, Antonson P, Shipley J, Cooper C, Goodwin G. The SYT protein involved in the t(X;18) synovial sarcoma translocation is a transcriptional activator localised in nuclear bodies. *Hum Mol Genet* 1997;6:1559-64.
11. dos Santos NR, de Bruijn DRH, Baemans M, et al. Nuclear localization of SYT, SSX and the synovial sarcoma-associated SYT-SSX fusion proteins. *Hum Mol Genet* 1997;6:1549-58.
12. de Leeuw B, Suijkerbuijk RF, Olde Weghuis D, et al. 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.
13. Janz M, De Leeuw B, Weghuis DO, et al. Interphase cytogenetic analysis of distinct X-chromosomal translocation breakpoints in synovial sarcoma. *J Pathol* 1995;175:391-6.
14. Renwick PJ, Reeves BR, Dal Cin P, et al. Two categories of synovial sarcoma defined by divergent chromosome translocation breakpoints in Xp11.2, with implications for the histologic sub-classification of synovial sarcoma. *Cytogenet Cell Genet* 1995;70:58-63.
15. Shipley JM, Clark J, Crew AJ, et al. The t(X;18)(p11.2;q11.2) translocation found in human synovial sarcomas involves two distinct loci on the X chromosome. *Oncogene* 1994;9:1447-53.
16. Shipley J, Crew J, Birdsall S, et al. Interphase fluorescence in situ hybridization and reverse transcription polymerase chain reaction as a diagnostic aid for synovial sarcoma. *Am J Pathol* 1996;148:559-67.
17. Fligman I, Lonardo F, Jhanwar SC, Gerald WL, Woodruff J, Ladanyi M. Molecular diagnosis of synovial sarcoma and characterization of a variant SYT-SSX2 fusion transcript. *Am J Pathol* 1995;147:1592-9.
18. Kampe CE, Rosen G, Eilber F, et al. Synovial sarcoma: a study of intensive chemotherapy in 14 patients with localized disease. *Cancer* 1993;72:2161-9.
19. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958;53:457-81.
20. Rosen G, Forscher C, Lowenbraun S, et al. Synovial sarcoma: uniform response of metastases to high dose ifosfamide. *Cancer* 1994;73:2506-11.
21. Connelly EF, Budd GT. Ifosfamide in the treatment of soft tissue sarcomas. *Semin Oncol* 1996;23:Suppl 6:16-21.
22. Limon J, Mrozek K, Mandahl N, et al. Cytogenetics of synovial sarcoma: presentation of ten new cases and review of the literature. *Genes Chromosomes Cancer* 1991;3:338-45.
23. Cagle LA, Mirra JM, Storm FK, Roe DJ, Eilber FR. Histologic features relating to prognosis in synovial sarcoma. *Cancer* 1987;59:1810-4.
24. Rooser B, Willen H, Hugoson A, Rydholm A. Prognostic factors in synovial sarcoma. *Cancer* 1989;63:2182-5.
25. Brodsky JT, Burt ME, Hajdu SI, Casper ES, Brennan MF. Tendosynovial sarcoma: clinicopathologic features, treatment, and prognosis. *Cancer* 1992;70:484-9.
26. 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.
27. Pappo AS, Fontanesi J, Luo X, et al. Synovial sarcoma in children and adolescents: the St Jude Children's Research Hospital experience. *J Clin Oncol* 1994;12:2360-6.
28. Singer S, Baldini EH, Demetri GD, Fletcher JA, Corson JM. Synovial sarcoma: prognostic significance of tumor size, margin of resection, and mitotic activity for survival. *J Clin Oncol* 1996;14:1201-8.
29. Krall RA, Kostianovsky M, Patchefsky AS. Synovial sarcoma: a clinical, pathological, and ultrastructural study of 26 cases supporting the recognition of a monophasic variant. *Am J Surg Pathol* 1981;5:137-51.
30. Pisters PW, Leung DH, Woodruff J, Shi W, Brennan MF. Analysis of prognostic factors in 1,041 patients with localized soft tissue sarcomas of the extremities. *J Clin Oncol* 1996;14:1679-89.
31. Argani P, Zakowski ME, Klimstra DS, Rosai J, Ladanyi M. Detection of the SYT-SSX chimeric RNA of synovial sarcoma in paraffin-embedded tissue and its application in problematic cases. *Mod Pathol* (in press).
32. Melo JV. The diversity of BCR-ABL fusion proteins and their relationship to leukemia phenotype. *Blood* 1996;88:2375-84.
33. Kelly KM, Womer RB, Sorensen PHB, Xiong QB, Barr FG. Common and variant gene fusions predict distinct clinical phenotypes in rhabdomyosarcoma. *J Clin Oncol* 1997;15:1831-6.
34. Zoubek A, Dockhorn-Dworniczak B, Delattre O, et al. Does expression of different EWS chimeric transcripts define clinically distinct risk groups of Ewing tumor patients? *J Clin Oncol* 1996;14:1245-51.
35. de Alava E, Kawai A, Healey JH, et al. EWS-FLI1 fusion transcript structure is an independent determinant of prognosis in Ewing's sarcoma. *J Clin Oncol* (in press).

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