American Journal of Pathology, Vol. 148, No. 2, February 1996

Chromosomal Translocation t(X;18) in Human Synovial Sarcomas Analyzed by Fluorescence *in Situ* Hybridization using Paraffin-Embedded Tissue

Katsuhito Nagao,* Hisao Ito,* and Haruhiko Yoshida†

From the Department of Pathology, Faculty of Medicine, and College of Medical Care Technology,† Tottori University, Yonago, Tottori, Japan*

Synovial sarcoma is characterized cytogenetically by translocation t(X;18)(p11.2;q11.2). In this study, 28 cases that had been diagnosed initially as synovial sarcoma, including 2 fibrosarcomas, and 1 leiomyosarcoma were collected and examined for translocation t(X;18) on paraffin-embedded tissues by fluorescence* in situ *hybridization (FISH). Of the synovial sarcomas, 25 showed findings consistent with translocation t(X;18) with an additional copy signal for the total probe of X and 18 chromosomes. The other three cases, as well as the two fibrosarcomas and the leiomyosarcoma, did not show this translocation. One (case 26) of three negative cases was diagnosed finally as leiomyosarcoma and another (case 27) as malignant peripheral nerve sheath tumor from histological and immunohistochemical analysis. Thus, in all, 25 (96%) of 26 synovial sarcomas showed findings consistent with translocation t(X;18). In summary, translocation t(X;18) is a chromosomal aberration specific for synovial sarcoma. The fluorescence* in situ *hybridization technique can be used even on cells from paraffin-embedded tissues, and is a useful diagnostic aid for synovial sarcoma. (Am J Pathol 1996, 148:601–609)

Synovial sarcoma (SS) is a highly malignant tumor that occurs mainly in adolescents and young adults, usually in the extremities in the vicinity of joints, most commonly the knee and lower thigh region. There are four distinct histological types of synovial sarcoma: 1) the biphasic type with distinct epithelial and spindle cell components in varying proportions, 2) the monophasic fibrous type, 3) the rare monophasic epithelial type, and 4) the poorly differentiated type.[1]

Diagnosis of SS may pose a considerable challenge to pathologists owing to the lack of an epithelial component. Additional diagnostic aids such as immunohistochemical staining or electron microscopy have been used in cases with predominant monophasic features to discriminate them from fibrosarcoma, leiomyosarcoma, and neurogenic tumors. Positive immunohistochemical staining for cytokeratin is observed in nearly all biphasic SS and in most tumor cells of the monophasic SS. A small number of fibrosarcomas, leiomyosarcomas, and neurogenic tumors also reveal unusual immunoreactivity for cytokeratin, and thus immunohistochemical staining for cytokeratin is not a useful diagnostic marker.[2]

The chromosomal translocation t(X;18) was first reported in an SS by Limon et al,[3] and subsequently reported in several cases of SS as a specific chromosomal abnormality by karyotypic analysis.[4–26] However, it remains unclear whether this translocation is present in all cases and would thus be useful for diagnosis.

Pinkel et al[27] provided the means to analyze the numerical and structural aberrations of tumor cells, even in interphase. In 1993, Lee et al[28] reported the identification of translocation t(X;18) in SS by fluorescence *in situ* hybridization (FISH).[28] In the present study, we examined translocation t(X;18) in the nuclei isolated from paraffin-embedded tissues of tu-

Supported by a grant (no. 04151019) from the Ministry of Education, Tokyo, Japan.

Accepted for publication October 19, 1995.

Address reprint requests to Dr. Katsuhito Nagao, M. D., Department of Pathology, Faculty of Medicine, Tottori University, 86 Nishimachi, Yonago, Tottori 683, Japan.

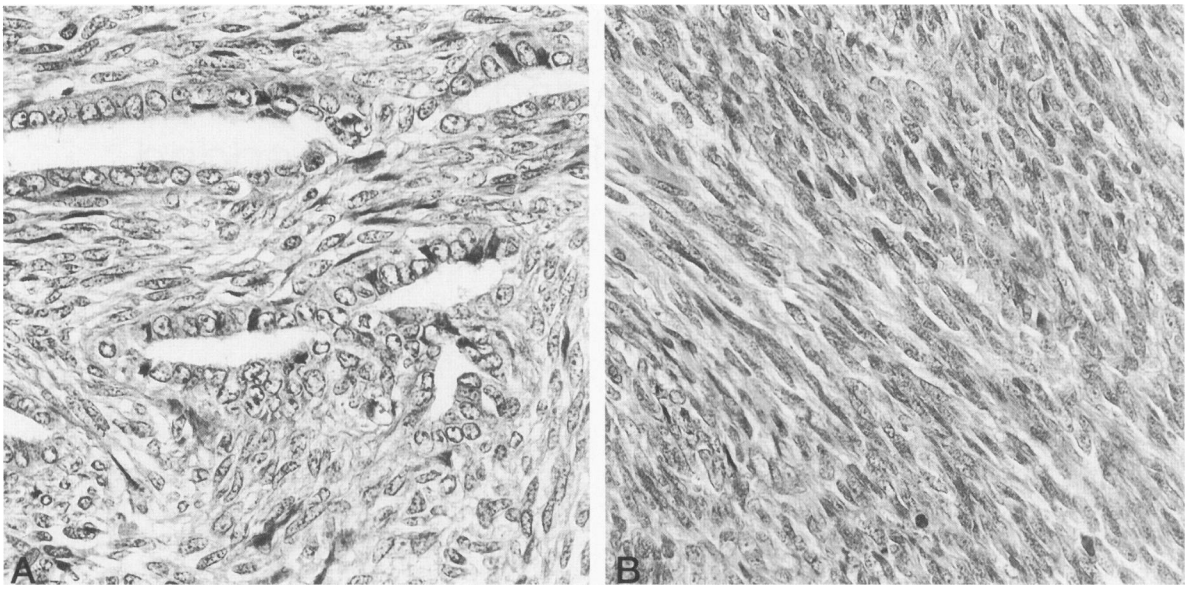

Figure 1. (**A**) *Typical SS with distinctive biphasic pattern: epithelial cells surrounded by fibrosarcoma-like spindle cell elements.* (**B**) *Monophasic fibrous SS: tumor consisted of a packed cell proliferation of round to plump spindle-shaped cells in a somewhat fasciculated arrangement (original magnification ×100).*

mors from 28 SS cases by FISH with chromosome-specific DNA probes.

Materials and Methods

Tissues

Twenty-eight cases of SS were collected from surgical specimens registered at the Departments of Pathology, Tottori University and Hiroshima University, Japan, from 1965 to 1994. The clinical data of the twenty-eight SS cases are shown in Table 1. Paraffin-embedded blocks were stored from 2 months to 29 years. For light microscopy, paraffin sections were stained with hematoxylin and eosin.

Immunohistochemistry

Immunohistochemical staining was carried out on paraffin-embedded sections of tissue from all cases, employing the avidin-biotin peroxidase complex method after reaction with anti-cytokeratin (polyclonal, 1:50, Nichirei, Tokyo, Japan), and anti-epithelial membrane antigen (EMA) (monoclonal, 1:50, Nichirei) antibodies. Briefly, 5-μm sections were deparaffinized, rehydrated, and incubated with primary antibodies for 2 hours at 37°C. Immunoreactivity was visualized using 3,3′-diaminobenzidine and hydrogen peroxide. In cases 26 to 28, antibodies against the following antigens were used: carcinoembryonic antigen (monoclonal, 1:50, Nichirei), vimentin (monoclonal, 1:100, Dakopatts, Copenhagen, Denmark), S-100 protein (polyclonal, 1:100, Nichirei), neuron-specific enolase (polyclonal, 1:100, Nichirei), α-smooth muscle actin (monoclonal, 1:50, Nichirei), and desmin (monoclonal, 1:50, Nichirei).

FISH

FISH was performed on formalin-fixed, paraffin-embedded tissue. The tissues were cut to a thickness of 50 μm from paraffin blocks and disaggregated according to the method reported by Schutte et al.[29] Briefly, the sections were dewaxed with xylene three times and rehydrated. The suspensions were treated with 0.3% trypsin citrate buffer (pH 7.4, 37°C, overnight) and centrifuged. The pellets were washed in distilled water, and this was followed by fixation in 70% ethanol. Drops of the cell preparations were then placed on glass slides and allowed to dry overnight. The FISH procedure was performed essentially as described by Pinkel et al.[27] The slides were treated in proteinase K (0.01 to 0.1 mg/ml, Wako, Tokyo, Japan) for 5 to 15 minutes at 37°C, then fixed in 4% paraformaldehyde for 25 minutes at 4°C. Biotinylated α-satellite centromeric probes for chromosomes X and 18 (DXZ1, D18Z1), and whole chromosome painting probes (Coatasome X, Coatasome 18) were purchased from Oncor. DNA of the tumor cell nuclei on the slides was denatured for 3 minutes at 75°C in 70% formamide (Boehringer Mannheim, Mannheim, Germany), 2X standard sodium citrate, and dehydrated. The probes were denatured for 10 minutes at 75°C, and aliquots of 10 μl were placed

Table 1. *Clinical Data and Histological Diagnosis*

Case	Age/Sex	Location	Histological diagnosis*		Immunophenotype	
			Original	Revised	Keratin	EMA
1	32/F	R foot†	B(D)	B(D)	+	+
2	45/F	L foot	B(D)	B(D)	+	+
3	27/F	R upper arm	B(D)	B(D)	+	+
4	25/F	R axilla	B(D)	B(D)	+	+
5	47/F	R ankle	MF(D)	MF(D)	+	+
6	9/F	R foot	MF(D)	MF(D)	+	−
7	51/F	L lower leg	MF(D)	MF(D)	+	−
8	57/F	R foot	MF(S)	B(D)	−	−
9	24/F	L lower leg	M(S)	M(D)	−	−
10	45/F	R poples	M(S)	M(D)	−	−
11	45/M	R thigh	B(D)	B(D)	+	+
12	49/M	R foot	B(D)	B(D)	+	+
13	13/M	R thigh	B(D)	B(D)	+	+
14	81/M	R chest wall	MF(D)	MF(D)	+	+
15	48/M	L thigh	MF(D)	MF(D)	+	+
16	13/M	R forearm	MF(D)	MF(D)	+	+
17	18/M	L lower leg	MF(D)	MF(D)	+	+
18	38/M	R mentum	MF(D)	MF(D)	+	+
19	35/M	R elbow	MF(D)	MF(D)	+	−
20	29/M	R lower leg	MF(S)	MF(D)	−	−
21	56/M	L forearm	MF(S)	MF(D)	−	−
22	28/M	R thigh	MF(S)	MF(D)	−	−
23	43/M	L buttock	MF(S)	MF(D)	−	−
24	62/M	L thigh	M(S)	M(D)	−	−
25	39/M	R shoulder	M(S)	M(D)	−	−
26	76/M	R knee	M(S)	LMS(S)	−	−
27	67/M	R first toe	M(S)	MPNST(S)	−	−
28	53/M	L ankle	M(Q)	M(Q)	−	−

*B = biphasic, MF = monophasic fibrous, M = monophasic, subtype not provided; D = definite, S = suspected, Q = questionable
LMS: leiomyosarcoma, MPNST: malignant peripheral nerve sheath tumor.
†R = right, L = left.

on the slide glasses and covered with a 22 × 22 mm glass coverslip. These preparations were incubated for 17 hours at 37°C.

For visualization of the hybridized chromosomes, the nuclei were treated with fluorescein isothiocyanate (FITC)-avidin for 30 minutes at 37°C. The biotinylated anti-avidin-conjugated FITC avidin system was used to amplify the fluorescent signals. The nuclei were counterstained with propidium iodide (0.1 μg/ml, Sigma Chemical Co., St. Louis, MO). Hybridization signals were observed under a fluorescent microscope (Nikon, Tokyo, Japan). The number of signals was counted in 100 to 200 nuclei for evaluation of the possible translocation. Paraffin blocks from two fibrosarcomas and one leiomyosarcoma were used as controls.

Translocation t(X;18) of Epithelial Cells and Spindle Cells

In the biphasic type of SS, the signals were counted in about 100 nuclei of each of epithelial cells and spindle cells. When the tissues were cut to a thickness of 50 μm from paraffin blocks, the epithelial component forming glandular structure in paraffin block was trimmed. A round nucleus with a disaggregated shape was regarded as the nucleus of epithelial cells. The spindle-shaped to long-oval nuclei in disaggregated shape were regarded as those of spindle cells. When testing the effects of differentiation on the comparison between epithelial cells and spindle cells, the paired *t*-test was used; $P < 0.05$ was taken to indicate significance.

Results

Histological Findings

The SS were classified as either monophasic or biphasic type based on the presence or absence of epithelial components. Histological examination revealed 7 biphasic and 21 monophasic (14 cases were of the distinct fibrous type). The microscopic biphasic pattern of SS was distinctive, revealing two morphologically different types of cells: epithelial cells, which often made glandular structures; and spindle cells, which showed fibrosarcoma-like proliferation (Figure 1A). In monophasic fibrous types of SS, the tumors consisted mainly of packed cell proliferation of round to plump spindle-shaped cells in a

Table 2. *Immunophenotype Data of Cases 26 to 28*

Case	Immunophenotype							
	Keratin	EMA	CEA	Vimentin	S-100	NSE	SMA	Desmin
26	−	−	−	+	−	−	+	−
27	−	−	−	+	+	+	−	−
28	−	−	−	+	−	−	−	−

CEA = carcinoembryonic antigen NSE = neuron-specific enolase, SMA = smooth muscle actin

somewhat fasciculated arrangement. There was no epithelial component forming glandular structures (Figure 1B).

Immunohistochemical Findings

Immunohistochemical examination showed positive immunoreactivity for cytokeratin in 16 (57%), and for EMA in 13 (36%) of 28 SS cases. All seven biphasic SS were immunoreactive for cytokeratin and EMA. Of the 21 monophasic SS, tumor cells showed immunoreactivity for cytokeratin in 9 cases (43%) and for EMA in 6 (29%) (Table 1).

Most tumor cells of three cases (cases 26 to 28) stained positively for vimentin. Immunohistochemical staining of case 26 revealed immunoreactivity for α-smooth muscle actin in most tumor cells. No immunoreactivity was seen for cytokeratin, EMA, carcinoembryonic antigen, S-100 protein, neuron-specific enolase, or desmin. In case 27, immunoreactivity was seen for S-100 protein and neuron-specific enolase in most tumor cells, but the other antibodies showed no immunolabeling. Also, in case 28, immunoreactivity was seen only for vimentin, but not with the other antibodies (Table 2).

Fluorescence in Situ Hybridization

We analyzed paraffin-embedded tissue sections of SS tumors from 28 patients between 9 and 81 years of age. The cumulative results of FISH analysis are shown in Tables 3 to 5.

In tumor cells from two fibrosarcomas and one leiomyosarcoma, the nuclei showed two signals (one signal was seen in tissue from male patients) at a rate of 88 to 96% with the centromeric probes for chromosome X and 18 (Figure 2A). Similarly, two fluorescent painting signals (although only one signal was seen in the male tissue) were defined at 81 to 90% with the painting probes for chromosomes X and 18 (Figure 2B).

In females (cases 1 to 10) the nuclei showed two signals at a rate of 75 to 94% with the centromeric probe for the X chromosome (Figure 2C) and at 83 to 93% with that for chromosome 18 (Figure 2E). Three fluorescent signals were defined at 55 to 68% with the painting probe for the X chromosome (Figure 2D) and at 54 to 70% with that for chromosome 18 (Figure 2F). In males (cases 11 to 25) the nuclei showed one signal at a rate of 87 to 95% with the centromeric probe for the X chromosome and two signals at 81 to

Table 3. *FISH Analysis of Biphasic SS**

Case		Copy number									
		Chromosome X					Chromosome 18				
		0	1	2	3	4≦	0	1	2	3	4≦
Female											
1	C	4	10	80	4	2	1	7	88	3	1
	T	0	3	30	64	3	0	8	17	70	5
2	C	3	19	75	2	1	1	6	88	3	2
	T	0	3	29	67	1	2	3	24	70	1
3	C	1	12	80	7	0	1	8	89	2	0
	T	0	4	31	62	3	1	8	29	60	2
4	C	0	5	92	2	1	1	3	93	2	1
	T	1	10	32	55	2	3	11	32	54	0
Male											
11	C	0	90	8	1	1	2	3	87	7	1
	T	3	29	60	6	2	0	3	28	65	4
12	C	0	87	11	2	0	1	6	90	3	0
	T	1	24	67	6	2	0	6	29	61	4
13	C	0	91	8	1	0	1	5	92	2	0
	T	0	31	63	5	1	1	9	29	60	1

* Numbers in each column indicate percentages.
C = centromeric probe; T = total probe.

Table 4. *FISH Analysis of Monophasic SS**

Case		Copy number: Chromosome X 0	1	2	3	4≦	Chromosome 18 0	1	2	3	4≦
Female											
5	C	0	7	90	2	1	1	6	89	4	0
	T	1	7	34	56	2	2	9	29	59	1
6	C	0	5	91	3	1	2	6	90	2	0
	T	1	8	30	58	3	0	6	33	59	2
7	C	1	3	94	1	1	1	3	91	4	1
	T	1	11	27	60	1	0	9	28	60	3
8	C	2	12	85	1	0	4	7	83	5	1
	T	2	10	19	68	1	2	10	17	70	1
9	C	0	5	89	6	0	1	9	88	2	0
	T	1	2	29	67	1	0	2	34	63	1
10	C	3	11	85	1	0	2	7	88	3	0
	T	7	12	19	61	1	4	8	31	57	0
Male											
14	C	3	92	4	1	0	0	15	81	3	1
	T	0	23	65	10	2	0	15	21	61	3
15	C	4	92	3	1	0	1	5	88	6	0
	T	1	25	61	12	1	1	8	22	64	5
16	C	2	87	10	1	0	0	5	90	5	0
	T	2	32	64	2	0	1	2	27	68	2
17	C	1	95	3	1	0	1	5	88	6	0
	T	1	20	65	1	3	1	8	24	64	3
18	C	1	91	8	0	0	0	11	86	3	0
	T	4	37	55	4	0	2	10	33	54	1
19	C	5	90	3	2	0	2	4	88	6	0
	T	2	35	60	3	0	1	9	29	58	3
20	C	2	89	8	1	0	1	7	90	2	0
	T	0	30	57	12	1	2	24	16	53	5
21	C	5	92	2	1	0	0	4	93	2	1
	T	2	23	69	6	0	1	5	20	72	2
22	C	1	89	8	2	0	2	4	86	7	1
	T	2	30	60	8	0	1	3	31	62	3
23	C	4	90	5	1	0	0	6	91	3	0
	T	2	31	62	5	0	1	7	29	61	2
24	C	2	89	7	2	0	1	5	90	3	1
	T	0	24	67	7	2	0	9	22	69	0
25	C	2	95	2	1	0	1	4	90	5	0
	T	0	28	69	3	0	0	7	29	62	2

* Numbers in each column indicate percentages.
C = centromeric probe; T = total probe.

93% with that for chromosome 18. Two fluorescent signals with an additional small signal were seen at 55 to 69% with the painting probe for the X chromosome, and three fluorescent signals were seen at 53 to 72% with that for chromosome 18.

In cases 26 to 28, the nuclei showed one signal at rates of 75 to 90% with the centromeric and painting probes for the X chromosome. The nuclei showed two signals at rates of 79 to 89% with the centromeric and painting probes for chromosome 18.

Table 5. *FISH Analysis of Cases 26 to 28 (All Male)**

Case		Copy number: Chromosome X 0	1	2	3	4≦	Chromosome 18 0	1	2	3	4≦
26	C	2	81	9	7	1	3	5	89	2	1
	T	0	82	8	9	1	1	7	85	7	0
27	C	2	81	9	7	1	1	8	83	6	2
	T	1	75	14	8	2	0	12	79	7	2
28	C	1	90	7	2	0	2	6	89	2	1
	T	1	85	9	4	1	2	10	83	5	0

*Numbers in each column indicate percentages.
C = centromeric probe; T = total probe.

Translocation t(X;18) in Epithelial and Spindle Cells

It was possible to distinguish epithelial cells from spindle cells by their large size and round shape on the slides. In epithelial cells of biphasic SS (cases 1 to 4), the average proportions of nuclei showing two signals with the centromeric probes for chromo-

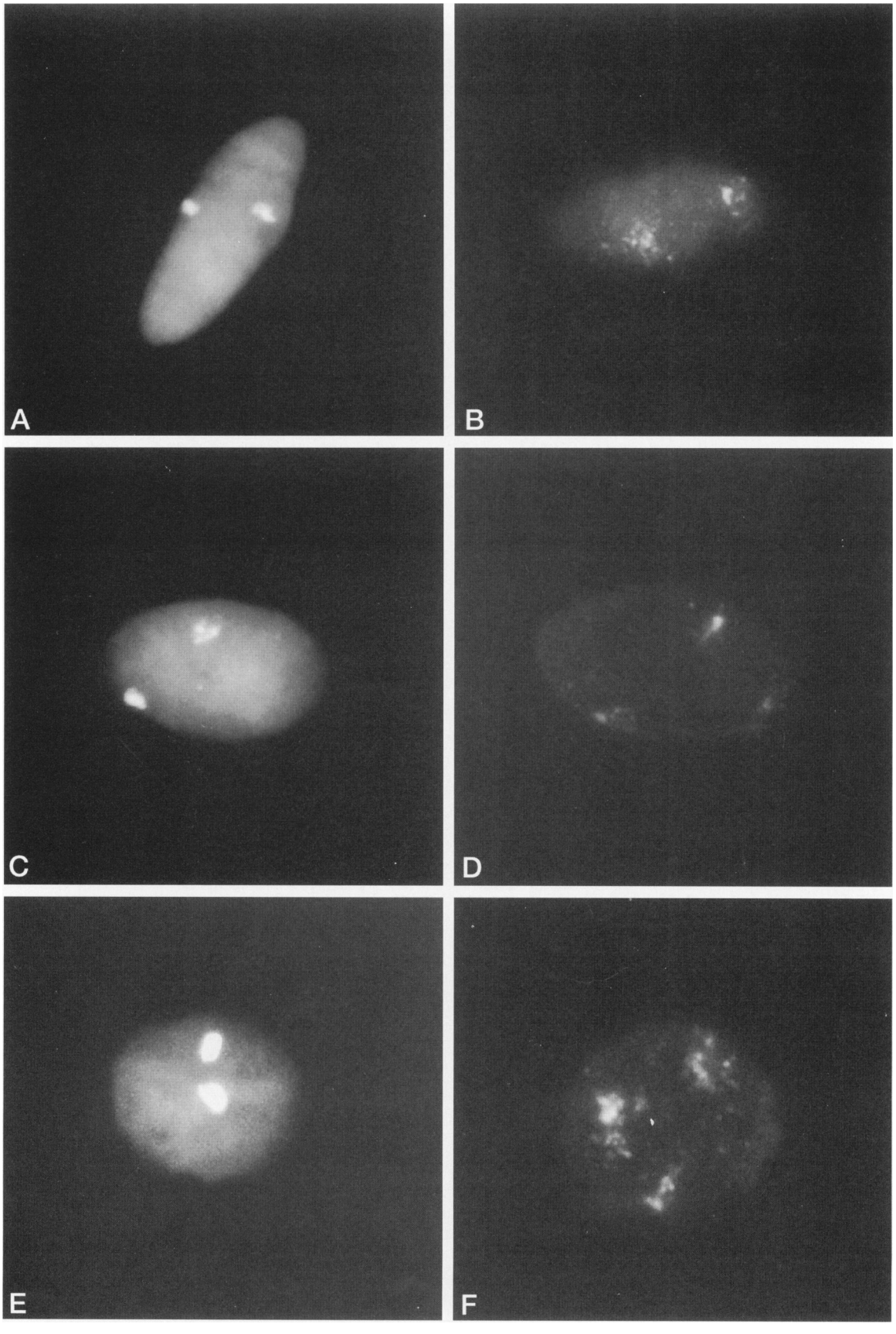
A
B
C
D
E
F

Table 6. *Proportion of Translocation t*(X;18) in Epithelial Cells and Spindle Cells

Case	Epithelial cells		Spindle cells	
	C	T	C	T
1	85.5	68.0	82.5	66.0
2	79.5	66.5	83.5	70.5
3	84.5	62.0	84.5	60.0
4	94.0	55.5	91.0	53.5
11	87.0	62.0	90.0	63.0
12	87.5	65.5	89.5	62.5
13	92.5	62.5	90.5	60.5
Mean ± SD	87.2 ± 4.90*	63.1 ± 4.12**	87.4 ± 3.68*	62.3 ± 5.28**

Numbers in each column indicate percentages.
C = centromeric probe; T = total probe.
*, **: Not significant ($P < 0.05$).

somes X and 18 were 79.5 to 94.0%. Three signals with the painting probes for chromosomes X and 18 were detected at rates of 55.5 to 68.0%. In spindle cells of biphasic SS (cases 1 to 4), the average proportions of nuclei showing two signals with the centromeric probe for chromosomes X and 18 were 82.5 to 91.0%. Three signals were detected with the painting probes for chromosomes X and 18 at rates of 53.5 to 70.5%. Also, in epithelial cells of cases 11 to 13, the average proportions of nuclei showing one signal with the centromeric probe for the X chromosome and two signals with that for chromosome 18 were 87.0 to 92.5%. Two signals with the painting probe for the X chromosome and three signals with that for chromosome 18 were detected at rates of 62.0 to 65.5%. In spindle cells of cases 11 to 13, the average proportions of nuclei showing one signal with the centromeric probe for the X chromosome and two signals with that for chromosome 18 were 89.5 to 90.5%. Two signals were detected with the painting probe for X chromosome and three signals with that for chromosome 18 at rates of 60.5 to 63.0%. There were no significant differences in proportion of cells showing translocation t(X;18) between epithelial and spindle cells as determined using the paired *t*-test (Table 6).

Discussion

SS accounts for 5 to 10% of all soft-tissue sarcomas and is characterized histologically as a biphasic tumor with distinct epithelial and spindle-shaped cells. As a result, SS is difficult to distinguish from other spindle-cell sarcomas such as fibrosarcoma, leiomyosarcoma, and neurogenic tumors, even with the help of electron microscopy and immunohistochemistry, when composed of a monophasic spindle cell proliferation with the lack of an epithelial component. The chromosomal rearrangement translocation t(X;18) was shown to be present in more than 40 cases of SS by cytogenetic analysis.[3–26] Sreekantaiah et al[24] identified a specific translocation t(X;18)(p11.2;q11.2) presented in 38 tumors (90%) of 42 specimens with karyotypes described.

In this study, 25 of 28 cases of SS showed findings consistent with translocation t(X;18) by FISH. Of 21 monophasic SS, 12 (57%) were negative for cytokeratin and EMA. Nine cases showed this translocation, while the other three cases did not show this translocation, among which two cases (cases 26 and 27) were finally diagnosed as leiomyosarcoma and malignant peripheral nerve sheath tumor, respectively.

The other case (case 28) could not be assigned a final diagnosis even after immunohistochemical staining, but was still suggested to be monophasic SS despite an absence of translocation t(X;18). Knight et al[22] reported two cases of monophasic SS without translocation t(X;18) as shown by cytogenetic and molecular analysis. According to these results, 25 (96%) of 26 definite SS showed findings consistent with translocation t(X;18). However, a few other sarcomas, including fibrosarcoma[30] and malignant fibrous histiocytoma,[6] have been reported to have the same translocation, but these two cases might represent histological varieties of SS.[21] Although it remains unclear whether this translocation is present in all cases, it was found here in more than 90% of SS.

Figure 2. *Labeling with the centromeric and total probes for the X chromosome in control fibrosarcoma, illustrating the presence of only two centromeric signals* (**A**) *and painting signals* (**B**). *Labeling with the centromeric and total probes for the X chromosome in spindle cells of case 2, demonstrating the presence of two centromeric signals* (**C**) *and three painting signals* (**D**), *and labeling with the centromeric and total probes for chromosome 18 in epithelial cells of case 2, showing two centromeric signals* (**E**) *and three painting signals* (**F**) (*original magnification* ×*1000*).

FISH, introduced by Pinkel et al,[27] has provided the means to analyze the numerical and structural aberrations of tumor cells, even in interphase. FISH has been applied to various solid tumors including gastric,[31] bladder,[32, 33], breast,[34] and testicular carcinomas,[35] and can even be used to stain tumor cells from paraffin-embedded tissues. FISH has also been used in bone[36] and soft tissue tumors,[37] in which numerical aberrations of chromosomes, gene deletion, and translocation have been successfully demonstrated. Lee et al[28] reported the occurrence of translocation t(X;18) in SS by FISH with chromosome-specific DNA probes. They found that the storage time of the paraffin blocks, even for as long as 10 years, did not affect the quality of FISH results. We detected chromosomal aberrations in paraffin-embedded tissues stored for as long as 29 years. Also, this technique can detect chromosomal aberrations, allowing screening for specific chromosomal rearrangements in SS as well as other histological types of sarcomas, using a wide panel of DNA probes.

There is the interesting question as to whether the pathways leading to epithelial- or mesenchymal-like differentiation are determined by epigenetic or environmental factors, or are related to distinct events at the chromosomal level. In this study, there were no significant differences in proportion of cells positive for translocation t(X;18) between epithelial and spindle cells in the biphasic type of SS, suggesting the same progenitor cells of epithelial cells as spindle cells. The results of Janz et al,[38] although representing only a limited number of cases, support the hypothesis that the biphasic type of SS have a breakpoint within the ornithine aminotransferase (OAT)L1 cluster, whereas the majority of the monophasic type of SS show a breakpoint mapping to the OATL2 cluster. Clark et al[39] detected a genomic rearrangement of the *SYT* gene in OATL2 cluster on chromosome 18 in SS and indicated that *SYT* undergoes fusion with a gene named *SSX* on chromosome X, resulting in the formation of an *SYT-SSX* fusion protein. This protein might be responsible for the pathogenesis of SS.

In summary, we detected translocation t(X;18) in SS by FISH with chromosome-specific DNA probes in paraffin-embedded tissue sections. It is our conclusion that translocation t(X;18) is a chromosomal aberration specific for SS, and that the FISH technique is suitable for analysis of tumor cells from paraffin-embedded tissues. This method is a useful aid for correct diagnosis of SS, particularly the monophasic type.

Acknowledgments

The authors would like to thank Dr. K. Taniyama of the Clinical Pathology, Kure Mutual Aid Hospital, Kure, Hiroshima, Japan for providing tumor materials.

References

1. Enzinger FM, Weiss SW: Soft Tissue Tumors, ed 3. St. Louis, CV Mosby, 1995, pp 757–786
2. Salisbury JR, Isaacson PG: Synovial sarcoma: an immunohistochemical study. J Pathol 1985, 147:49–57
3. 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
4. Griffin CA, Emanuel BS: Translocation t(X;18) in a synovial sarcoma. Cancer Genet Cytogenet 1987, 26: 181–183
5. Wang-Wuu S, Soukup SW, Lange BJ: Another synovial sarcoma with t(X;18). Cancer Genet Cytogenet 1987, 29:179–181
6. 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
7. Noguera R, Lopez-Gines C, Gil R, Carda C, Pellin A, Llombart-Bosch A: Translocation t(X;18) in a synovial sarcoma: a new case. Cancer Genet Cytogenet 1988, 33:311–312
8. Bridge JA, Bridge RS, Borek DA, Shaffer B, Norris CW: Translocation t(X;18) in orofacial synovial sarcoma. Cancer 1988, 62:935–937
9. Llombart-Bosch A, Carda C, Boix J, Pellin A, Peydro-Olaya A: Value of nude mice xenografts in the expression of cell heterogeneity of human sarcomas of bone and soft tissue. Pathol Res Pract 1988, 183:683–692
10. Ueda T, Aozasa K, Tsujimoto M, Uchida A, Taniwaki M, Abe T: Translocation X;18 and insertion 15;11 in a case of synovial sarcoma. Cancer Genet Cytogenet 1988, 30:183–185
11. 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
12. Molenaar WM, DeJong B, Buist J, Idenburg VJS, Seruca R, Vos AM, Hoekstra HJ: Chromosomal analysis and the classification of soft tissue sarcomas. Lab Invest 1989, 60:266–274
13. Calabrese G, Palka G, Di Virgilio C, Cianchetti E, Caracino A, Stuppia L, Angelucci D: Translocation t(X;18) in synovial sarcoma: AluI endonuclease digestion for breakpoint detection in two cases. Cancer Genet Cytogenet 1990, 50:277–279

14. Nojima T, Wang YS, Abe S, Matsuno T, Yamawaki S, Nagashima K: Morphological and cytogenetic studies of a human synovial sarcoma xenotransplanted into nude mice. Acta Pathol Jpn 1990, 40:486–493
15. Gilgenkrantz S, Chery M, Teboul M, Mujica P, Leotard B, Gregoire MJ, Boman F, Duprez A, Hanauer A: Sublocalisation of the X breakpoint in the translocation t(X;18)(p11.2;q11.2) primary change in synovial sarcomas. Oncogene 1990, 5:1063–1066
16. le Marc'Hadour F, Pasquier B, Leroux D, Jacrot M: Mediastinal synovial sarcoma with t(X;18). Cancer Genet Cytogenet 1991, 55:265–267
17. Verhest A, Limon J, Mrozek K, Nedoszytko B: Translocations t(X;7) and t(7;14) in a synovial sarcoma. Genes Chromosomes & Cancer 1991, 3:71–73
18. Limon J, Mrozek K, Mandahl N, Nedoszytko B, Verhest A, Rys J, Niezabitowski A, Babinska M, Nosek H, Ochalek T, Kopacz A, Willen H, Rydholm A, Heim S, Mitelman F: Cytogenetics of synovial sarcoma: presentation of ten new cases and review of the literature. Genes Chromosomes & Cancer 1991, 3:338–345
19. Fletcher JA, Kozakewich HP, Hoffer FA, Lage JM, Weidner N, Tepper R, Pinkus GS, Morton CC, Corson JM: Diagnostic relevance of clonal cytogenetic aberrations in malignant soft-tissue tumors. N Engl J Med 1991, 324:436–442
20. Miozzo M, Sozzi G, Cariani CT, Di Palma S, Luksch R, Azzarelli A, Pierotti MA, Della Porta G: A synovial sarcoma with t(X;18)(p11;q11) in a patient with Turner's syndrome. Genes Chromosomes & Cancer 1992, 4:192–193
21. Dal Cin P, Rao U, Jani-Sait S, Karakousis C, Sandberg AA: Chromosomes in the diagnosis of soft tissue tumors. I. Synovial sarcoma. Mod Pathol 1992, 5:357–362
22. Knight JC, Reeves BR, Smith S, Clark J, Fisher C, Fletcher CDM, Gusterson BA, Cooper CS: Cytogenetic and molecular analysis of synovial sarcoma. Int J Oncol 1992, 1:747–752
23. Karn CM, Socinski MA, Fletcher JA, Corson JM, Craighead JE: Cardiac synovial sarcoma with translocation t(X;18) associated with asbestos exposure. Cancer 1994, 73:74–78
24. 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
25. Nagao K, Gomyo Y, Ito H, Yamamoto K, Yoshida H: A case report of synovial sarcoma with translocation t(X;18). Application of fluorescence *in situ* hybridization to paraffin embedded tissue. Virchows Arch 1995, 426:519–522
26. Sandberg AA, Bridge JA: The Cytogenetics of Bone and Soft Tissue Tumors. Austin, TX, R. G. Landes Co., 1994, pp 430–441
27. Pinkel D, Straume T, Gray JW: Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Proc Natl Acad Sci USA 1986, 83:2934–2938
28. 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
29. Schutte B, Reynders MMJ, Bosman FT, Blijham GH: Flow cytometric determination of DNA ploidy level in nuclei isolated from paraffin-embedded tissue. Cytometry 1985, 6:26–30
30. 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
31. Gomyo K, Andachi H, Nagao K, Ikeguchi M, Ito H: Interphase cytogenetics of gastric carcinoma: fluorescence *in situ* hybridization (FISH) applied to cells obtained from formalin-fixed paraffin-embedded tissues. Pathol Int 1995, 45:227–232
32. Hopman AHN, Moesker O, Smeets AWGB, Pauwels RPE, Vooijs GP, Ramaekers FCS: Numerical chromosome 1,7,9, and 11 aberrations in bladder cancer detected by *in situ* hybridization. Cancer Res 1991, 51: 644–651
33. Sauter G, Deng G, Moch H, Kerschmann R, Matsumura K, De Vries S, George T, Fuentes J, Carroll P, Mihatsch MJ, Waldman FM: Physical deletion of the *p53* gene in bladder cancer: detection by fluorescence *in situ* hybridization. Am J Pathol 1994, 144:756–766
34. Matsumura K, Kallioniemi A, Kallioniemi O, Chen L, Smith HS, Pinkel D, Gray J, Waldman FM: Deletion of chromosome 17p loci in breast cancer cells detected by fluorescence *in situ* hybridization. Cancer Res 1992, 52:3474–3477
35. Emmerich P, Jauch A, Hofmann MC, Cremer T, Walt H: Interphase cytogenetics in paraffin embedded sections from human testicular germ cell tumor xenografts and in corresponding cultured cells. Lab Invest 1989, 61:235–242
36. Bridge JA, DeBoer J, Travis J, Johansson SL, Elmberger G, Noel SM, Neff JR: Simultaneous interphase cytogenetic analysis and fluorescence immunophenotyping of dedifferentiated chondrosarcoma: implications for histopathogenesis. Am J Pathol 1994, 144: 215–220
37. Taylor C, Patel K, Jones T, Kiely F, De Stavola BL, Sheer D: Diagnosis of Ewing's sarcoma and peripheral neuroectodermal tumour based on the detection of t(11;22) using fluorescence *in situ* hybridisation. Br J Cancer 1993, 67:128–133
38. Janz M, Leeuw B de, Olde Weghuis D, Werner M, Nolte M, Geurts van Kessel AD, Nordheim A, Hipskind RA: Interphase cytogenetic analysis of distinct X-chromosomal translocation breakpoints in synovial sarcoma. J Pathol 1995, 175:391–396
39. 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 the t(X;18)(p11.2; q11.2) translocation found in human synovial sarcoma. Nature Genet 1994, 7:502–508