Clinical Outcome of Patients With Ewing Sarcoma Family of Tumors of Bone in Japan

The Japanese Musculoskeletal Oncology Group Cooperative Study

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BACKGROUND. Ewing sarcoma family of tumors (ESFT) of bone is extremely rare in Japan. The objectives of the current study were to assess the clinical outcome and prognostic factors of patients with ESFT of bone in Japan and to compare them between Euro-American and Japanese populations.

METHODS. The authors conducted a retrospective analysis of 243 patients who were treated for ESFT of bone in Japan between 1981 and 2003. Local therapy was surgery in 35% of patients, surgery combined with radiotherapy in 40% of patients, radiotherapy alone in 22% of patients, and no local treatment in 3% of patients. All but 3 patients received various regimens of multidrug chemotherapy.

RESULTS. The median patient age was 16 years. The primary disease sites were the trunk in 53% of patients and the extremities in 47% of patients. Forty-one patients had metastases at presentation. The median follow-up was 66 months. A univariate survival analysis demonstrated that patients who had metastases at presentation, primary site in the trunk, age $\geq$16 years, tumor size $\geq$10 cm, tumor that responded poorly to induction chemotherapy, and local treatment with radiotherapy alone had a significantly worse event-free survival (EFS). A multivariate analysis further verified that the former 3 factors were significant adverse prognostic factors. Of 201 patients with localized disease, 45 patients who received current chemotherapy regimens that included ifosfamide and etoposide had a significantly better 5-year EFS rate (67.6%) compared with other patients.

CONCLUSIONS. The clinical outcome of patients with localized ESFT of bone in Japan has improved markedly with the use of current chemotherapy regimens that include ifosfamide and etoposide and has become comparable to the outcomes observed in other major series of Euro-American patients. The prognostic factors are also almost identical. *Cancer* 2007;109:767–75. © *2007 American Cancer Society.*

KEYWORDS: sarcoma, Ewing, Asian Continental Ancestry Group, treatment outcome.

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Ewing sarcoma family tumors (ESFT) are highly malignant, small, round cell tumors of neuroectodermal origin arising from bone and soft tissue. ESFT of bone is the second most common primary malignant bone tumor after osteosarcoma in children and adolescents. It is well known that there is an interracial variation in the incidence of ESFT. The incidence is remarkably lower in black populations and in East and Southeast Asians compared with the incidence in Euro-American populations.[1–3] Therefore, in the past, there was no clinical study targeting large populations of patients with ESFT in Japan and other Asian countries. These facts inspired the Japanese Musculoskeletal Oncology Group (JMOG) to conduct a multi-institutional, retrospective study. The objectives of the current study were 1) to assess the clinical outcome of patients with ESFT of bone in Japan, 2) to identify prognostic factors from which to develop a therapeutic strategy for future studies, and 3) to determine whether there are any differences in patients' characteristics, clinical outcome, or prognostic factors between Euro-American and Japanese populations.

MATERIALS AND METHODS

Patients

This study was designed as a multi-institutional, retrospective analysis by the JMOG, which consists of representative Japanese tertiary referral hospitals and cancer centers for musculoskeletal tumors. For this study, the JMOG conducted a survey of clinical outcomes of patients who had primary bone tumors that were diagnosed histologically as ESFT of bone and were treated at the institutions of the JMOG between January 1981 and May 2003. Patients who had received previous anticancer therapy for Ewing sarcoma were excluded from the study. Two hundred forty-three patients from 29 institutions finally were enrolled into the study. Clinical staging was determined based on diagnostic imaging examinations according to the Musculoskeletal Tumor Society surgical staging system.[4] Primary tumor size was based on the greatest tumor dimension on radiographic images, including computed tomography scans and magnetic resonance imaging.

Treatment

Although treatment strategy varied to some extent according to the time of referral and the policy of the institution, fundamentally, it was a combination of systemic chemotherapy and localized, definitive surgery and/or radiotherapy. Patients and/or their guardians were informed and consented to their treatment. Of the 243 patients in this study, 183 patients underwent definitive surgery as primary local treatment. The primary lesions were excised with a wide margin in 145 patients, with a marginal margin in 17 patients, with an intralesional margin in 19 patients, and with unspecified surgical margins in 2 patients. Of those 183 patients, 96 patients (52.5%) also received radiotherapy. The modes of combined radiotherapy were preoperative for 70 patients, postoperative for 21 patients, both postoperative and preoperative for 3 patients, intraoperative and postoperative for 1 patient, and not specified for 1 patient. Fifty-three patients received local radiotherapy alone. Carbon ion beam radiotherapy was applied to 4 patients. The total dose of radiotherapy ranged from 14 grays (Gy) to 95.5 Gy (mean, 47.9 Gy, not including the dose of carbon ion beam radiotherapy). Seven patients received only systemic chemotherapy without local treatment.

All but 3 patients received chemotherapy, and those 3 patients were excluded from the survival analysis. Although the majority of chemotherapy was administered according to regimens that have been reported previously in major prospective studies, some regimens were unique to an institution. The regimens that were applied are listed in Table 1.[5–19] High-dose chemotherapy (HDC) with hematopoietic stem cell rescue was received by 51 patients, including 48 patients who received autologous peripheral blood stem cell transfusion (PBSCT) and 3 patients who underwent autologous bone marrow transplantation.

Evaluation of Response to Chemotherapy

Resected tumor specimens were examined specifically to evaluate the surgical margins and the rate of necrosis by induction chemotherapy. For 126 tumors, the extent of viable tumor cells was evaluated histologically, and the response to chemotherapy was graded according to the modified criteria of Rosen et al.[20,21] as follows: grade 3, 100% tumor necrosis; grade 2, <10% area of viable tumor; grade 1, from 10% to 50% area of viable tumor; grade 0, from 50% to 100% area of viable tumor.

The mass-reductive effect of chemotherapy with or without radiotherapy was evaluated radiographically for 190 primary lesions. The effectiveness of chemotherapy was defined according to the criteria of the Japanese Orthopedic Association (JOA) Committee of Tumors[21] as follows: A complete response was defined as the disappearance of extraosseous mass that continued for ≥3 weeks, a partial response was defined as a reduction ≥30% of extraosseous mass that continued for ≥3 weeks, no change was defined as from 10% expansion to 30% reduction of extraoss-

TABLE 1
Chemotherapy Regimens

Regimen	Agents	No. of patients
VACD+IE-based regimens		41
NCI protocol (Wexler et al., 1996[5]; Grier et al., 2003[6])	VCR,DOX,CYC+IFO,ETO	10
CCG 7942/POG 9354 Regimen A (Granowetter et al., 2001[7])	VCR,DOX (ACT),CYC+IFO,ETO	7
P6 (Kolb et al., 2003[8])	VCR,DOX,CYC+IFO,ETO	3
SE 91-CNR (Rosito et al., 1999[9])	VCR,DOX,CYC+VCR,ACT,IFO+IFO,ETO	2
CCCH (for poor responders) (Kimura et al., 2002[10])*	VCR,DOX,CYC+IFO,ETO	2
Protocol of the PBSCT Study Group†	VCR,THP,CYC (+ACT,CYC)+IFO,ETO	9
Other VACD+IE-based regimens		8
T-16‡	IFO,ETO,DOX+CYC+IFO,ETO,DOX	11
EVAIA (Paulussen et al., 1998[11])	ETO,VCR,DOX,IFO,ACT	3
KS-1§	ETO,CDDP,THP,IFO	7
T-6 (Rosen et al., 1981[12])	ACT,CYC,BLM,VCR+MTX,CYC,DOX+CYC,BCNU	4
T-11 (Rosen, 1982[13]) and modified T-11	CYC,DOX,MTX,VCR+BLM, CYC,ACT+CYC,DOX,MTX	47
VAC (Nesbit et al., 1981[14]) and modified VAC	VCR,ACT,CYC	6
VACA (Burgert et al., 1990[15]; and Jurgens et al., 1988[16])		22
and modified VACA	VCR,ACT,CYC,DOX	
VAIA (Paulussen et al., 2001[17])	VCR,ACT,IFO,DOX	22
CCCH (for good responders) (Kimura et al., 2002[10])*	VCR,DOX,CYC+IFO	8
K2 (Tsuchiya et al., 1998[18])	DOX,CDDP,caffeine	9
VCD-based regimens	VCR,CYC,DOX, etc	10
VCD+I-based regimens	VCR,CYC,DOX,IFO, etc	12
CDDP- or CBDCA-based regimens	CDDP or CBDCA, etc	10
CYVADIC (Bramwell et al., 1994[19])	CYC,VCR,DOX,DTIC	2
DOX+CDDP-based regimens	DOX,CDDP, etc	12
Others		12
Not specified		2
No chemotherapy		3
Total		243

VACD indicates vincristine (VCR), actinomycin D (ACT), cyclophosphamide (CYC), and doxorubicin (DOX); IE, ifosfamide (IFO) and etoposide (ETO); NCI, National Cancer Institute; CCG, Children's Cancer Group; POG, Pediatric Oncology Group; CCCH; PBSCT, peripheral blood stem cell transfusion; THP, therarubicin; CDDP, cisplatin; BLM, bleomycin; MTX, methotrexate; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosurea; CBDCA; carboplatin; DTIC; dacarbazine.

* VCR (1.5 mg/m^2), DOX (60 mg/m^2/48 h), CYC (900 mg/m^2 × 2 d), and IFO (16 g/m^2/8 d) repeated twice and local treatment followed by VCR, DOX, CYC, and IFO (16 g/m^2/8 d) repeated 3 times (for good responders) or VCR, DOX, CYC, and IFO (9 g/m^2/5 d) and ETO (500 mg/m^2) repeated 4 times (for poor responders).

† VCR (2 mg/m^2), THP (80 mg/m^2/48 h), CYC (2.2 g/m^2), and IFO (14 g/m^2/5 d), ETO (600 mg/m^2/5 d) repeated 5 times with or without ACT (600 μg/m^2 × 2) and CYC (600 mg/m^2 × 2).

‡ IFO (10 g/m^2/5 d), ETO (360 mg/m^2/3 d), DOX (60 mg/m^2 48 h), CYC (2500 mg/m^2 × 2 d), and IFO, ETO, DOX repeated 4 times.

§ ETO (500 mg/m^2/5 d), CDDP (125 mg/m^2/5 d), THP (40 mg/m^2), IFO (4.2 g/m^2/3 d).

eous mass that continued for ≥3 weeks, and progressive disease was defined as expansion >10% of extraosseous mass or other newly emerged lesions.

Statistical Analysis

Event-free survival (EFS) and overall survival (OAS) rates were estimated by using the Kaplan-Meier method.[22] Both EFS and OAS were calculated from the date of initial treatment. An event against EFS was defined as disease recurrence or progression; onset of a secondary, therapy-related neoplasm; or death from any other causes. A terminal point of OAS was defined as the time of death from disease or from chemotherapy-related toxicity. Deaths from concurrent causes were estimated as censored deaths in the OAS analysis. Local control rates also were calculated by using Kaplan-Meier estimation based on the period from the initiation of treatment to the date of local recurrence. Log-rank tests and generalized Wilcoxon tests were used to evaluate the significance of differences between groups of patients. A Cox proportional hazards model was used to identify independent factors that were predictive of survival for multivariate analysis. Patient age also was evaluated as a continuous variable in the Cox regression model. These statistical analyses were performed using the JMP version 5.01 statistical analysis software package for personal computers (SAS Institute Inc., Cary, NC).

RESULTS

In this study, 243 patients (136 men and 107 women) from 29 institutions were enrolled. The patients'

TABLE 2
Patient Characteristics

Characteristic	Patients (N = 243) No.	%
Sex		
Men	136	56.0
Women	107	44.0
Age at diagnosis, y		
Median	16	
Range	0–49	
Primary tumor site		
Extremity	115	47.3
Humerus	23	9.5
Radius	3	1.2
Ulna	3	1.2
Hand	4	1.6
Femur	43	17.7
Tibia	19	7.8
Fibula	13	5.3
Foot	7	2.9
Trunk	128	52.7
Skull	5	2.1
Clavicle	9	3.7
Scapula	15	6.2
Rib cage	24	9.9
Thoracic spine	9	3.7
Lumbar spine	4	1.6
Pelvis	62	25.5
Disease extension at diagnosis		
Localized	202	83.1
Stage IIA (intracompartmental)	15	6.2
Stage IIB (extracompartmental)	176	72.4
Not specified	11	4.5
Metastatic	41	16.9

characteristics are summarized in Table 2. The median patient age at diagnosis was 16 years (range, 0–49 years). At the time of this analysis, the median follow-up for the survivors was 66 months (range, 4–248 months). There were 41 patients (16.9%) who had metastases at presentation. The primary site was the extremities in 115 patients (47.3%) and the trunk in 128 patients (52.7%).

Local Control

Local control was evaluated in 229 patients; the other 14 patients were excluded either because they received no chemotherapy (3 patients) or because they had incomplete records (11 patients). Of the 229 evaluable patients, 180 patients (78.6%) underwent definitive surgery with or without combined radiotherapy as local treatment, and 25 patients (13.9%) developed local recurrences during follow-up. Conversely, of 49 patients who received treatment with radiotherapy alone, 14 patients (28.6%) developed local recurrences; this difference in the local recurrence rate was statistically significant ($P = .015$; chi-square test). There was no difference in the rate between patients who underwent surgery alone and patients who underwent surgery combined with radiotherapy. However, of the 49 patients who received radiotherapy alone as local treatment, 36 patients (73.5%) had primary tumor sites in the trunk. The ratio of axial sites in the radiotherapy group was significantly higher compared with the ratio in axial sites among patients who underwent surgery (81 of 180 patients; 45.0%; $P < .001$; chi-square test). With regard to surgical margins, the rate of local recurrence was 38.9% (7 of 18 patients) for those who had intralesional margins, 35.3% (6 of 17 patients) for those who had marginal margins, and 8.4% (12 of 143 patients) for those who had wide or radical margins (including amputations). The rate was significantly lower for patients who had tumors excised with wide or radical margins compared with patients who had tumors excised with intralesional or marginal margins ($P < .001$; chi-square test).

The local recurrence-free rate also was calculated by using the method of Kaplan and Meier for 224 assessable patients (another 5 patients were excluded because of unspecified periods to local recurrence). The cumulative local recurrence-free rate for patients who underwent surgery with or without combined radiotherapy was significantly higher for patients who received radiotherapy alone (5-year local recurrence-free survival rate, 87.4% vs 68.2%, respectively; $P = .0016$ [log-rank test] and $P = .0006$ [Wilcoxon test]).

Survival Rate

Of 243 patients who were enrolled in the current study, 3 patients were excluded from the survival analysis because they did not receive chemotherapy. Of the remaining 240 patients, 97 patients (40.4%) remained continuously free of disease during follow-up. Four patients (1.7%) died of chemotherapy-related toxicity (3 patients died of sepsis, and 1 patient died of rhabdomyolysis). Of 202 patients who did not have any metastatic disease at presentation, 27 patients (13.4%) developed local recurrences, and 97 patients (48.0%) developed distant recurrences. In 1 patient, secondary chronic myeloid leukemia developed at an interval of 50 months after HDC with autologous PBSCT rescue. This patient was treated with further chemotherapy and had no evidence of disease at the time of final follow-up.

The 5-year OAS and EFS rates, which were estimated by using the Kaplan-Meier method, were 48.7% and 40.7%, respectively (Fig. 1a). In patients without metastasis at presentation, the 5-year OAS and EFS rates were 54.9% and 46.6%, respectively

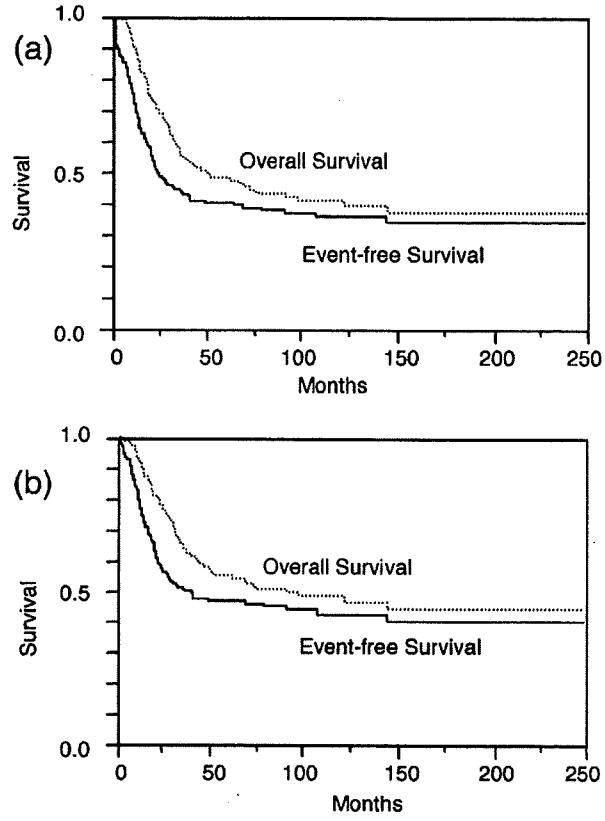

FIGURE 1. Kaplan-Meier estimated overall survival (dotted line) and event-free survival (solid line) are illustrated for all patients (n = 240 patients) (a) and for patients without metastatic disease at presentation (n = 201 patients) (b).

(Fig. 1b). Conversely, the 5-year OAS and EFS rates for patients who had metastatic disease at presentation were 13.2% and 6.8%, respectively; both rates were significantly less favorable than the rates for patients without metastasis ($P < .0001$; log-rank test and Wilcoxon test).

Univariate Analysis for Survival

The clinical variables and their prognostic impact on EFS are listed in Table 3. With regard to pretreatment factors, extent of tumor (metastatic), primary tumor site (trunk or pelvis), age (≥16 years), and tumor size (≥10 cm) were significantly predictive of poor survival. For the age variable, a cut-off age of 16 years was chosen. When it was evaluated as a continuous variable, older age was predictive of poor EFS in the Cox regression model with a relative risk of 1.029 (95% confidence interval, 1.008–1.048; $P = .006$). For the tumor size variable, different cut-off sizes (5 cm, 10 cm, and 15 cm) were tested, but differences were observed only for tumor sizes of 5 cm and 10 cm.

TABLE 3
Univariate Analysis for Event-free Survival

Variable	No. of patients (N = 240)	5-Year EFS, %	P	
			Log-rank	Wilcoxon
Disease extension				
Localized	201	46.6	<.0001	<.0001
Metastatic	39	6.8		
Primary tumor site				
Trunk	126	30.3	.0001	<.0001
Extremities	114	51.7		
Primary tumor site				
Pelvic	62	22.3	<.0001	<.0001
Extrapelvic	178	47.1		
Tumor size, cm				
<10	151	43.9	.019	.0057
≥10	68	31.8		
Age at diagnosis, y				
<16	119	50.1	.0024	.0081
≥16	121	31.1		
Sex				
Men	135	39.4	.53	.27
Women	105	41.8		
Histologic response to chemotherapy				
Grade 3 (100% necrosis)	59	58.0	.013	.0083
Grade 2–0 (necrosis <100%)	67	37.7		
Histologic response to chemotherapy				
Grade 3–2 (necrosis >90%))	90	50.1	.093	.022
Grade 1–0 (necrosis ≤90%)	36	39.2		
Radiographic response to chemotherapy				
Complete response	48	52.8		
Partial response	106	40.4	<.0001	<.0001
No change	22	30.3		
Progressive disease	14	0.0		
Local treatment				
Surgery with or without radiotherapy	181	44.8	.0025	.0002
Radiotherapy alone	52	27.7		

EFS indicates event-free survival.

The number of patients who had tumors ≥15 cm, however, was very small (14 patients) in the current analysis.

With regard to treatment-related variables, both histologic and radiographic responses to induction chemotherapy and local treatment were significant factors. The histologic response was assessable in 126 patients. Patients who had tumors with a grade 3 response (good responders; n = 59) had a significantly higher EFS rate compared with other patients (poor responders; n = 67) (Table 3). Patients who had tumors with grade 3 and 2 responses were defined as good responders (n = 90 patients) and had a significantly higher EFS rate compared with poor responders (n = 36 patients) according to the Wilcoxon test ($P = .022$) but not according to the log-rank test

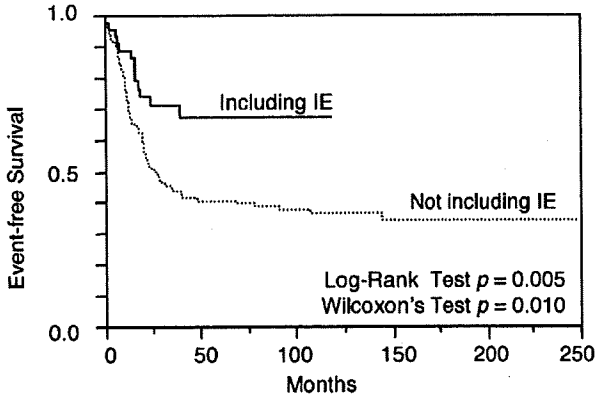

FIGURE 2. Kaplan-Meier estimated event-free survival is illustrated for patients without metastatic disease at presentation by chemotherapy regimens. Comparison between chemotherapy regimens that included ifosfamide and etoposide (IE) (n = 45 patients; solid line) and regimens that did not include IE (n = 156 patients; dotted line) (see Tables 1 and 5).

($P = .093$). The radiographic response was assessable in 190 patients. It was apparent statistically that poorer tumor responses, from the best response (complete response) to the worst response (progressive disease), resulted in worse EFS rates ($P < .0001$; both log-rank test and Wilcoxon test) (Table 3).

Patients who received local treatment with radiotherapy alone (n = 52 patients) had a significantly lower EFS rate compared with patients who underwent surgery with or without radiotherapy (n = 181 patients) (Table 3), but the distribution of patients in each group was biased with regard to the primary tumor site. When the analysis was conducted exclusively among patients who had nonmetastatic tumors that were located in the extremities (n = 102 patients), there was no significant difference in the rate of EFS between the 2 groups.

Chemotherapy regimens varied according to the treatment period and the policy of each institution. Recently, it was reported that the addition of ifosfamide and etoposide (IE) to previous standard chemotherapy regimens contributed significantly to improvements in clinical outcome for patients with nonmetastatic ESFT of bone.[6] Of 202 patients without metastasis at presentation, 201 patients received chemotherapy. Of these, 45 patients who received chemotherapy regimens that included (IE) (vincristine, actinomycin D, cyclophosphamide, and doxorubicin [VACD] plus IE-based regimens; T-16, EVAIA, KS-1) (see Table 1) had significantly better EFS (5-year EFS rate, 67.6%) compared with the other patients (n = 156 patients; 5-year EFS rate, 41.2%; $P = .0054$ [log-rank test] and $P = .010$ [Wilcoxon test]) (Fig. 2).

TABLE 4
Multivariate Analysis for Event-free Survival

Variable	RR	95% CI	P
Disease extension			
Localized	1		<.0001
Metastatic	1.81	1.46–2.24	
Primary tumor site			
Extremities	1		.0357
Trunk	1.22	1.01–1.47	
Tumor size, cm			
<10	1		.0617
≥10	1.20	0.99–1.44	
Age at diagnosis, y			
<16	1		.0393
≥16	1.21	1.01–1.46	

RR indicates relative risk; 95% CI, 95% confidence interval.

Moreover, of the various chemotherapy regimens, we compared outcomes of nonmetastatic patients who received 2 typical regimens, the T-11 protocol (see Table 1) and VACD plus IE-based regimens. The results indicated that patients who received VACD plus IE-based regimens had significantly better EFS (n = 32 patients; 5-year EFS rate, 67.3%) than patients who received the T-11 protocol (n = 43 patients; 5-year EFS rate, 41.6%; $P = .032$ [log-rank test] and $P = .041$ [Wilcoxon test]).

Multivariate Analysis for Survival

Based on the results from the univariate analyses, we performed multivariate analyses using a Cox proportional hazards model. Among the parameters that were identified in the univariate analyses, response to chemotherapy was excluded, because the number of assessable patients was small. The type of local treatment also was excluded, because the distribution of patients in each group was biased. The model (n = 219 patients) indicated that the risk of an event increased when a patient had the following characteristics: metastatic disease at presentation, primary tumor located in the trunk, and age ≥16 years. Tumor size lost its statistical significance in this set of multivariate analyses (Table 4).

A second multivariate analysis was performed that included only the 181 assessable patients who did not have metastases at presentation. In that analysis, the parameters chemotherapy regimen, primary tumor site, age, and tumor size were estimated. The results indicated that not only primary tumor site in the trunk and age ≥16 years but also chemotherapy regimens that did not include IE (Table 1) had an adverse prognostic impact on EFS (Table 5).

TABLE 5
Multivariate Analysis for Event-free Survival in Patients Without Metastatic Disease

Variable	RR	95% CI	P
Primary tumor site			
Extremities	1		.0109
Trunk	1.31	1.06–1.62	
Tumor size, cm			
<10	1		.0656
≥10	1.23	0.99–1.53	
Age at diagnosis, y			
<16	1		.0085
≥16	1.32	1.07–1.64	
Chemotherapy regimen			
Including IE*	1		.0033
Not including IE†	1.50	1.14–2.06	

RR indicates relative risk; 95% CI, 95% confidence interval; IE, ifosfamide and etoposide.
* Vincristine, actinomycin D, cyclophosphamide (VAC), and doxorubicin (VACD)- and IE-based regimens; T-16, EVAIA, and KS-1 (see Table 1).
† T-6, T-11, and modified T-11; VAC and modified VAC, VACA and modified VACA; VAIA, CCCH (for good responders), K2; VDC-based regimens; VDC+I-based regimens; CDDP- or CBDCA-based regimens; CYVADIC; DOX+CDDP-based regimens, and others (see Table 1).

HDC with Hematopoietic Stem Cell Rescue for High-risk Patients

In the multivariate survival analysis, when high-risk patients were defined as patients with metastatic disease at presentation, primary tumors located in the trunk, or age ≥16 years, 178 patients were classified into this group. Of those, 39 patients received HDC followed by hematopoietic stem cell rescue. However, those patients did not have a significantly better EFS than the other patients in the high-risk group ($P = .86$ [log-rank test] and $P = .85$ [Wilcoxon test]).

DISCUSSION

It has been noted that ESFT is very rare in black populations and in East and Southeast Asian[1] or Chinese and Japanese populations.[2] According to the Japanese annual registry of primary malignant bone tumors by the JOA,[21] only 156 patients with Ewing sarcoma of bone were registered for the 6 years between 1989 and 1994. Previously, the extreme rarity of the disease may have prevented a large mass clinical study, which may have led to improvements in clinical outcome among patients with ESFT in Japan. The current study, however, revealed that the clinical outcome of patients with localized ESFT who received current chemotherapy regimens that included IE attained 67.6% of the 5-year EFS rate. This result was comparable to the reports from other major series in ESFT of bone among Euro-American populations.[6,8,9,23–27]

Compared with the international variation in incidence, there was no considerable difference in the ratio of men to women, age distribution, or site distribution between Japanese patients and Euro-American patients (Table 2). Parkin et al. also noted that there was no suggestion of any geographic or ethnic difference in the site distribution.[1]

In the current study, the factors that were associated with decreased EFS were similar to those identified in previous retrospective studies.[27–29] It has been well confirmed that ESFT patients with larger tumors have a poorer outcome.[6,17,27,29,30] However, recent studies have demonstrated that this classic prognostic factor may become less critical when it is accompanied by the application of more aggressive treatment, such as EW-92,[25] SE 91-CNR,[9] and P6[8] (see Table 1).

Although the histologic response to initial chemotherapy was among the most reliable predictive factors,[16,17,28,31,32] even with the limitation that tumors had to be resected to determine response, we also used simple radiographic evaluations based on changes in extraosseous tumor size. These evaluations also demonstrated sufficient predictive value.

The most advantageous local treatment for Ewing sarcoma of bone remains controversial. Although some studies demonstrated no significant differences in the rates of local recurrence and/or survival between patients who received radiotherapy alone and patients who underwent surgery,[9,26,33] other studies demonstrated that surgery improved clinical outcomes significantly.[34,35] Results from 1058 patients who were treated on Cooperative Ewing Sarcoma Study 81 (CESS 81), CESS 86, and European Intergroup Cooperative Ewing Sarcoma Study 92 demonstrated that local control and EFS among patients who received definitive radiotherapy was significantly lower than EFS among patients who underwent surgery with or without receiving additional radiotherapy, although the former subgroup of patients represented a negatively selected population with unfavorable tumor sites.[35] Similar results were obtained in the current study. To eliminate such a selection bias, Bacci et al. retrospectively evaluated patients with tumors located exclusively in the extremities. Their study demonstrated the significant superiority of local surgical treatment.[34] These results indicate that surgery should be considered primarily in the local treatment of ESFT if the tumor is resectable with an adequate surgical margin.

In the current study, we observed that Rosen's T-11 protocol was used widely used in 1980s, and the 5-year EFS rate of patients without metastasis at presentation who received the T-11 protocol was only 41.6%. The St. Jude Children's Hospital study, EW-87,[36] showed that the combined administration of IE was very active in untreated patients with ESFT. Similarly, the Pediatric

Branch of the National Cancer Institute's pilot study demonstrated the efficacy of adding IE to the core regimen of vincristine, cyclophosphamide, and doxorubicin.[5] Furthermore, the first Pediatric Oncology Group (POG)-Children's Cancer Group (CCG) randomized study (POG 8850/CCG 7881)[6] demonstrated that the addition of IE to a standard regimen improved outcomes significantly among patients with nonmetastatic ESFT of bone. In the current study, the majority of JMOG institutions introduced chemotherapy regimens that included IE in and after 1990. Although progress in surgical and radiation expertise also contributed in part to the recent improvement in clinical outcome of patients with nonmetastatic ESFT of bone in Japan, it is believed that this improvement resulted mostly from the application of these newer chemotherapy regimens.

For patients with high-risk ESFT, more aggressive treatment with HDC followed by hematopoietic stem cell rescue have been used to achieve better survival. Many studies have been conducted to assess the benefit of this megachemotherapy. Some studies suggested an improvement in clinical outcomes,[37–39] whereas others did not.[40,41] In the current study, HDC with hematopoietic stem cell rescue failed to improve EFS significantly among high-risk patients. Precise assessment of the utility of HDC for patients with high-risk ESFT will require massive prospective, controlled studies.

In conclusion, this is the first report to our knowledge of a large series of patients with ESFT of bone in Japan as a representative cohort of East Asians. The current results demonstrated that 1) the incidence of ESFT is remarkably lower in Japan than in Western countries, 2) the recent clinical outcome of patients with localized ESFT of bone in Japan was virtually comparable to outcomes reported in other major series of Euro-American patients with ESFT, 3) the identified prognostic factors also are almost the same, and 4) the recent improvement in clinical outcome resulted mostly from the application of current chemotherapy regimens that included IE. In the future, for the establishment of highly effective therapy against ESFT, a large-scale, prospective study also will be required in Japan, even if it is conducted in collaboration with the major Euro-American group studies.

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Construct validity of the Enneking score for measuring function in patients with malignant or aggressive benign tumours of the upper limb

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We evaluated the construct validity of the Musculoskeletal Tumour Society rating scale (Enneking score) as a functional measure for patients with sarcoma involving the upper limb. We compared the Enneking score by examining the correlation between two patient-derived outcome measures, the Disability of the Arm, Shoulder, and Hand (DASH) questionnaire and the Medical Outcomes Study Short Form-36 (SF-36) as indicators of functional status in 40 patients with malignant or aggressive benign bone and soft-tissue tumours of the upper limb who had undergone surgical treatment.

The frequency distributions were similar among the three scoring systems. As for the validity, Spearman's rank correlation coefficient of the Enneking score to the DASH questionnaire was -0.79 and that of the Enneking to the SF-36 subscales ranged from 0.38 to 0.60. Despite being a measure from the surgeon's perspective, the Enneking score was shown to be a valid indicator of physical disability in patients with malignant or aggressive benign tumours of the upper limb and reflected their opinion.

The function of the arm in a patient with musculoskeletal sarcoma has been assessed widely using the Musculoskeletal Tumour Society rating scale (Enneking score) which was originally described by Enneking in 1987[1] and also by Enneking et al[2] in 1993. This score is based on an analysis of factors pertinent to the patient as a whole and of those specific to the affected upper limb. Each of these is assigned a value of 0 to 5 points (maximum overall score, 30 points) on the basis of established criteria. To date there has been no study which has shown the Enneking score to be valid as an outcome measure for patients with sarcoma affecting the arm.

Recently, attention has been focused on the impact of a disorder on the ability to function in daily life with a move towards measures of health-related quality of life, both generic and disease- or domain-specific. These often use self-administered questionnaires. Patients with sarcoma involving a limb have had little attention directed towards evaluating the functional outcome from their perspective.[3] The Enneking score is a disease-specific but physician-based measure. Potential existing patient-based measures for patients with sarcoma of the arm include region-specific functional questionnaires, the Disabilities of the Arm, Shoulder, and Hand (DASH) questionnaire[4,5] and general health-status questionnaires such as the Medical Outcomes Study Short Form-36 (SF-36).[6,7] Previous reports have validated the DASH score for patients with disorders of the upper limb[5,8,9] and the SF-36 for those with musculoskeletal complaints.[10,11]

Our aim was to determine the construct validity of the Enneking score used to measure disability and symptoms of patients with sarcoma of the arm. Construct validity indicates the degree to which a scoring system is associated with other measures that are hypothesised to have a specific relationship with the system. Testing of construct validity builds confidence in a scoring system. We examined the correlation of the Enneking score with the DASH and SF-36 scores to describe a series of patients who had surgery for sarcoma of the arm. The underlying hypothesis was that the three different measures would perform in a similar manner (i.e. be correlated with each other) since they were conceptually similar in their goals and design.

Patients and Methods

A series of 40 patients with malignant or aggressive benign bone and soft-tissue tumours of the upper limb, who had undergone surgery and were scheduled to be seen in three co-operative musculoskeletal oncology centres (Sapporo Medical University Hospital, Sapporo National Cancer Centre Hospital, Tokyo

Table I. A modified Enneking[12] functional scoring system

Score	Pain	Function	Emotional acceptance	Hand position	Manual dexterity	Lifting ability
5	No pain	Not restricted	Enthused	Unlimited	No limitations	Normal load
4	Intermediate	Intermediate	Intermediate	Intermediate	Intermediate	Intermediate
3	Modest/non-disabling	Recreational restriction	Satisfied	Not above shoulder/ no prosupination	Loss of fine movements	Limited
2	Intermediate	Intermediate	Intermediate	Intermediate	Intermediate	Intermediate
1	Moderate/intermittently disabling	Partial occupational restriction	Accepts	Not above waist	Cannot pinch	Helping only
0	Severe/continuously disabling	Total occupational restriction	Dislikes	None	Cannot grasp	Cannot help

Table II. Details of outcome measures used to assess function in patients with sarcoma of the upper limb

Outcome measure*	Who evaluates?	Dimension/number of items	Scaling of responses	Ease of scoring	Usable questionnaires (n = 40)
Enneking	Physician	Function, pain, emotional acceptance, hand position, dexterity, lifting ability	5-point scale of each arm	Easy	40 (*100*)
DASH (JSSH version)	Patients	30-item disability/symptom scale	5-response choices of each item	Moderate	38 (*95*)
SF-36	Patients	Physical function, social function, emotional role, physical role function, mental health, energy, pain, general, health perceptions	Variety of scales, used as a generic measure of health status	Moderate	40 (*100*)

* DASH (JSSH version), Disability of the Arm, Shoulder and Hand, The Japanese Society of Surgery of the Hand version; SF-36, Short-Form-36

and Kannmon Medical Centre, Yamaguchi) agreed to participate in the study which was performed between December 2004 and December 2005. The inclusion criteria were the ability to read and write in Japanese in order to complete the questionnaires and a willingness to participate in the study. The protocol was approved by the ethics board of each hospital and informed consent was obtained from all the patients.

There were 23 men and 17 women with a mean age of 47 years (18 to 81). The diagnoses included 14 bone tumours (35%) (five giant cell tumours of bone, four osteosarcomas, two Ewing's sarcomas, and three others) and 26 soft-tissue tumours (65%) (12 malignant fibrous histocytomas, four synovial sarcomas, two liposarcomas, two desmoid tumours and six others). The tumours were located at the shoulder girdle in seven patients, the upper arm in eight, the elbow in seven, the forearm in ten, and the wrist and hand in eight. Of the 40 patients, 21 (52.5%) were continuously free from disease, 16 (40%) had no evidence of disease and three (7.5%) had active disease. The mean period from surgery to assessment was 41 months (4 to 360). The operative procedures included reconstruction after resection of the tumour in 21 (52.5%), resection without reconstruction in 12 (30%) and amputation in seven (17.5%) patients.

The Enneking scoring system[2] and its Japanese version[12] were used to assess function. Categories within this system included pain, function, emotional acceptance, hand positioning, strength and manual dexterity with each having a maximum of five points representing normal or full function (Table I). The DASH questionnaire is designed to measure total functional disability of the upper limb with a score of 0 representing the least and 100 the most disability. The SF-36 is based on the definition of health of the World Health Organisation and has a broad content recommended for health-status measures from the patient's perspective. We used The Japanese Society of Surgery of the Hand version of the DASH score[5] and the official Japanese version of the SF-36, version 2[6,7] (Table II).

The Enneking score for each patient was completed by the surgeon as a part of the routine clinical examination. After the examination, the purpose of the study was explained to the patient and their participation was requested. If they were willing, they completed the questionnaires.

Statistical analysis. The scores were calculated for all three systems and analyses were performed using SPSS software version 13.0J (SPSS Inc., Chicago, Illinois).

The data obtained with the Enneking score and the DASH and SF-36 questionnaires were described in terms of the frequency distribution, the central tendency (the mean), spread (the SD and the range of responses) and the presence of a ceiling or floor effect. We assumed that more

Table III. Spearman rank correlation coefficients of the Enneking score with the Disability of the Arm, Shoulder and Hand (DASH) and short-form-36 (SF-36) subscales with 95% confidence interval in parentheses

Instruments	Patients							
Outcome measure	All (n = 40)		p-value	With tumours located proximal to the elbow (n = 18)		p-value	With tumours located distal to the forearm (n = 22)	p-value
DASH	-0.79*	(-0.62 to -0.88)	< 0.001	-0.84*	(-0.65 to -0.93)	< 0.001	-0.64* (-0.23 to -0.86)	0.007
SF-36								
Physical functioning	0.46*	(0.17 to 0.68)	0.003	0.40	(0.00 to 0.70)	0.069	0.58* (0.14 to 0.83)	0.012
Role physical	0.60*	(0.35 to 0.77)	0.001	0.50*	(0.17 to 0.79)	0.008	0.64* (0.23 to 0.86)	0.005
Body pain	-0.06	(-0.37 to +0.26)	0.706	-0.08	(-0.49 to +0.35)	0.698	0.02 (-0.47 to +0.50)	0.937
General health	-0.02	(-0.34 to +0.29)	0.876	-0.21	(-0.58 to +0.23)	0.353	0.03 (-0.17 to +0.70)	0.185
Vitality	0.06	(-0.26 to +0.37)	0.720	-0.02	(-0.44 to +0.41)	0.936	0.24 (-0.28 to +0.64)	0.362
Social functioning	0.43*	(0.14 to 0.66)	0.006	0.53*	(0.15 to 0.78)	0.010	0.31 (-0.21 to +0.68)	0.232
Role emotional	0.38†	(0.07 to 0.62)	0.017	0.34	(-0.09 to +0.67)	0.117	0.46 (-0.02 to +0.77)	0.061
Mental health	0.10	(-0.22 to +0.40)	0.551	-0.05	(-0.46 to +0.38)	0.838	0.19 (-0.33 to +0.61)	0.470

* $p < 0.01$
† $p < 0.05$

severely disabled individuals (those with a high score on the DASH questionnaire and a low score on the SF-36) would have a lower Enneking score. Spearman's rank correlation coefficients were used to determine the association between the Enneking score and the responses on the patient-completed functional questionnaires. Statistical significance was set at a p-value ≤ 0.05.

Results

Completeness of item responses. Of the 40 patients, ten (25%) did not answer one or more items of the DASH questionnaire, (all omitting item 21 regarding sexual activity). Among them, two patients age 72 and 81 years, respectively did not respond to sufficient numbers of items to allow complete scoring of the DASH questionnaire. These patients were excluded from the validity study. Regarding the SF-36, three patients did not answer one of the 36 items but the SF-36 subscale could be calculated with an estimated value. There was no missing item in any of the Enneking scores since they were completed by medical staff (Table II).

Distribution of responses. The mean Enneking score was 72 (23 to 100), the mean DASH score was 27 (10 to 100), the mean SF-36 physical functioning subscale was 79 (10 to 100) and the mean SF-36 role physical subscale was 70 (0 to 100; Fig. 1). A ceiling effect but no floor effect was noted in association with the Enneking score and the SF-36 physical functioning subscale. A floor effect but no ceiling effect was noted in association with the DASH. Both ceiling and floor effects were shown in association with the SF-36 role physical subscale.

Validation. The Spearman rank correlation coefficient of the Enneking score to the DASH and the SF-36 subscales are shown in Table III. The Enneking score was shown to have a high negative correlation ($r = -0.79$, $p < 0.001$) with DASH and to have moderate positive correlation with the SF-36 physical functioning subscale ($r = 0.46$, $p = 0.003$), the role physical subscale ($r = 0.60$, $p < 0.001$), social function ($r = 0.43$, $p = 0.006$) and the role emotional subscale ($r = 0.38$, $p = 0.017$). The correlations were shown for all patients irrespective of the location of their tumour.

Discussion

Our comparison of the Enneking score with the DASH and the SF-36 subscales as measures of post-operative outcome for 40 patients with sarcoma of the arm showed high and moderate correlations of the Enneking score with the DASH and SF-36 scores regardless of the location of the tumour. The correlation of the Enneking score with the well-established DASH and SF-36 scores supports its validity.

Since it was published in 1987[1] and revised in 1993,[2] the Enneking scoring system has been widely accepted as a functional measure of the upper and lower limb in patients undergoing surgery for a tumour. It is unclear whether the measure was intended to describe and evaluate change in status and if the intent was to use the measure with groups and individuals. The only validation of the Enneking score relates to a study on sarcoma of the lower limb performed by Davis et al.[8] They compared the Enneking score, the Toronto Extremity Salvage Score (TESS)[3] and the SF-36 as measures of functional status. The TESS is a validated measure which was developed to evaluate physical disability in patients with sarcoma of the lower limb from the perspective of the patient. The authors indicated that the Enneking score was shown to have a moderate positive correlation with the TESS. The latter was also shown to have a high positive correlation with the physical function subscale of the SF-36 and to have moderate positive correlations with the social function and mental health subscales.

In the absence of a disease-specific and patient-based questionnaire to measure function of patients with a sarcoma of the upper limb, we chose the DASH and SF-36 scores to evaluate the construct validity of the Enneking score. The DASH questionnaire is a standardised instru-

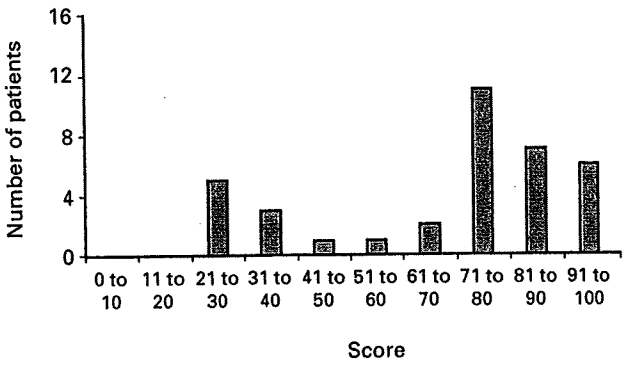

Fig. 1a

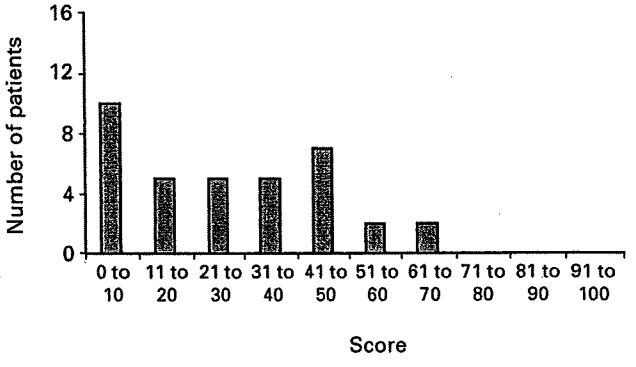

Fig. 1b

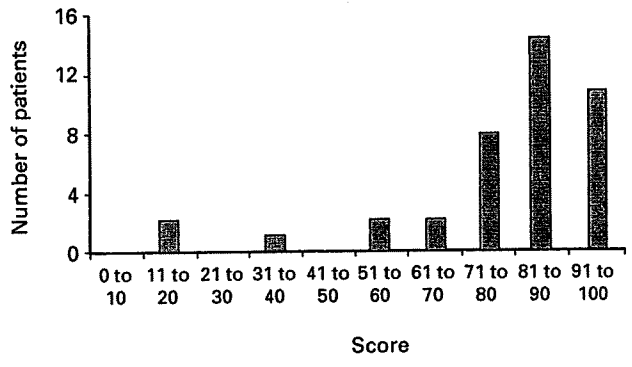

Fig. 1c

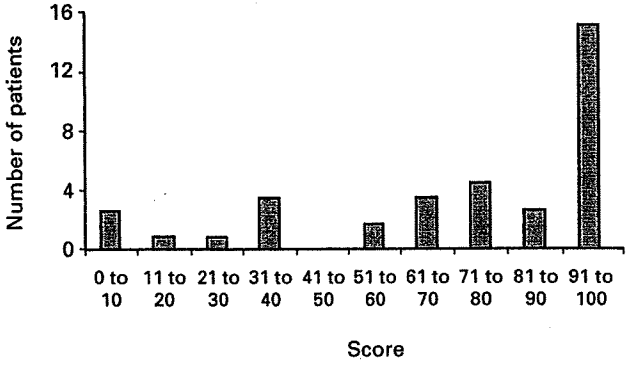

Fig. 1d

Barcharts showing a) the Enneking score, b) the Disability of the Arm, Shoulder and Hand, c) the short-form 36 (SF-36) physical function subscale anc d) the SF-36 role physical subscale for the 40 patients.

ment which measures the patient's own perspective of his/her disability of the upper limb. It has been developed with careful attention to the psychometric principles of instrument design. The outcome measure can be reliable, can discriminate between severities of functional impact, can be sensitive to change over time, and can be statistically valid.[5,8,13] The SF-36 score has been validated in patients with musculoskeletal complaints and is used widely for measuring health outcomes. However, it is a generic questionnaire and has the potential disadvantage of being less sensitive to clinical change in patients with complaints specific to an anatomical region or disease process.[11,14,15]

The correlation between disease- or region-specific outcome measures with the DASH and SF-36 scores has been evaluated. The correlation coefficient of -0.79 of the Enneking score with the DASH score was consistent with that seen in validation studies of outcome measures for the elbow and shoulder. Turchin, Beaton and Richards[16] observed correlations of five observer-based elbow scoring systems and the DASH score ranging from 0.55 to 0.74. Kirkley et al[17] showed that the correlation coefficient between a disease-specific quality-of-life measurement tool for shoulder instability and the DASH score was 0.77. By contrast, that of the Enneking score with the SF-36 was consistent with that seen in validation studies of outcome measures for the shoulder and knee. Beaton and Richards[13] observed correlations of five shoulder questionnaires with the SF-36 physical functioning and bodily pain subscales ranging from 0.58 to 0.72.

The correlation between the Enneking and the DASH scores (r = -0.79) was stronger than that between the Enneking and the SF-36 physical functioning (r = 0.46) and role physical (r = 0.60) scores. This may have been due to the fact that the Enneking score and the DASH questionnaire enquire only about function and symptoms of the upper limb. Only three of ten items on the SF-36 physical functioning are devoted to the function of the arm.

In patient self-reported measures of outcomes, such as the DASH and SF-36, patients may not respond in sufficient numbers to certain items to allow complete scoring of the questionnaire. Indeed, two of the ten patients did not respond to sufficient numbers of items to allow complete scoring of their DASH questionnaires. The DASH and the SF-36 were also not developed for use with patients under the age of 16 or 17 years in whom osteosarcoma and Ewing's

sarcoma occur frequently. The Enneking score is not limited by any age constraint.

The major limitation of our study is that the reliability of the Enneking score was not assessed because we performed a cross-sectional comparative study. Inter- and intra-observer reliability of the Enneking score also warrants investigation as was reported in a scoring system of foot and ankle disorders.[18] Another potential limitation was our inclusion of more than one type of tumour and anatomical location in the arm. We did so to allow generalisation of the results to a wide variety of tumours and treatments of the upper limb.

Despite being a measure from the surgeon's perspective, the Enneking score has been shown to be a validated measure for evaluating physical disability of patients with sarcomas involving the upper limb which reflects their opinion. We propose that a patient-derived questionnaire to measure the functional status of patients with sarcoma of the upper limb could be developed from the Enneking score.

Supplementary Material


A further opinion by Mr S. R. Cannon is available with the electronic version of this article on our website at www.jbjs.org.uk

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Gene expression analysis of soft tissue sarcomas: characterization and reclassification of malignant fibrous histiocytoma

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In soft tissue sarcomas, the diagnosis of malignant fibrous histiocytoma (MFH) has been a very controversial issue, and MFH is now considered to be reclassified into pleomorphic subtypes of other sarcomas. To characterize MFH genetically, we used an oligonucleotide microarray to analyze gene expression in 105 samples from 10 types of soft tissue tumors. Spindle cell and pleomorphic sarcomas, such as dedifferentiated liposarcoma, myxofibrosarcoma, leiomyosarcoma, malignant peripheral nerve sheath tumor (MPNST), fibrosarcoma and MFH, showed similar gene expression patterns compared to other tumors. Samples from those five sarcoma types could be classified into respective clusters based on gene expression by excluding MFH samples. We calculated distances between MFH samples and other five sarcoma types (dedifferentiated liposarcoma, myxofibrosarcoma, leiomyosarcoma, MPNST and fibrosarcoma) based on differentially expressed genes and evaluated similarities. Three of the 21 MFH samples showed marked similarities to one of the five sarcoma types, which were supported by histological findings. Although most of the remaining 18 MFH samples showed little or no histological resemblance to one of the five sarcoma types, 12 of them showed moderate similarities in terms of gene expression. These results explain the heterogeneity of MFH and show that the majority of MFHs could be reclassified into pleomorphic subtypes of other sarcomas. Taken together, gene expression profiling could be a useful tool to unveil the difference in the underlying molecular backgrounds, which leads to a rational taxonomy and diagnosis of a diverse group of soft tissue sarcomas.
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Keywords: gene expression; malignant fibrous histiocytoma; myxofibrosarcoma; soft tissue sarcoma; reclassification; undifferentiated pleomorphic sarcoma

Malignant soft tissue tumors are a diverse group of tumors of mesenchymal origin, which have generally been classified according to their histological resemblance to normal tissue. Understanding of molecular pathology gained in recent decades shows that some soft tissue tumors exhibit single recurrent genetic aberrations, such as chromosomal translocations resulting in gene fusions (*SYT-SSX* in synovial sarcoma, *TLS-CHOP* in myxoid/round cell liposarcoma) or somatic mutations (*KIT* in gastrointestinal stromal tumors), and they are now classified by these molecular markers specific to each tumor.[1] In contrast, other malignant soft tissue tumors, such as malignant fibrous histiocytoma (MFH), fibrosarcoma and leiomyosarcoma, are characterized by numerous, nonrecurrent complex chromosomal aberrations, and frequently show overlapping histological appearance and immunohistochemical phenotypes that are often difficult to

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interpret by pathologists.[2] Among them, diagnosis of MFH has been the most controversial issue.[3–5] MFH has been considered the most common soft tissue sarcoma of adults; it is manifested by a broad range of histological appearances and consists of four subtypes: storiform and pleomorphic type, myxoid type, giant cell type and inflammatory type. Recent clinicopathological, ultrastructural and immunohistochemical studies revealed that MFH shows no evidence of true histiocytic differentiation and that it is not a single entity but rather a heterogeneous collection of pleomorphic subtypes of other sarcomas. Since each type of sarcoma other than MFH shows distinct biological behavior, particularly in local recurrence or metastasis rate, MFH showing a variety of clinicopathological characteristics should be further reclassified to correctly evaluate the malignant potential of each case. In the latest edition of the WHO classification, myxoid type MFH was classified as myxofibrosarcoma in the fibroblastic category, and other subtypes of MFH without any evidence of differentiation were classified as undifferentiated high grade pleomorphic sarcoma.[6] WHO classification also suggested that the term 'MFH' might disappear when criteria for the diagnosis of pleomorphic sarcomas showing a distinct differentiation state can be reproducibly defined.[6] In this work, we used the term 'MFH' to identify tumors diagnosed as storiform and pleomorphic type MFH, and the term 'myxofibrosarcoma' for so-called MFH with predominant (>50%) myxoid features conventionally diagnosed as myxoid type MFH.

Recently, several studies report gene expression profiling of soft tissue tumors using microarray technologies to provide new insights into the tumor characterization. They described distinct patterns of gene expression in respective tumors with single, recurrent genetic aberrations, such as synovial sarcoma, myxoid/round cell liposarcoma, clear cell sarcoma or gastrointestinal stromal tumors, and heterogeneous patterns in spindle cell and pleomorphic sarcomas which are generally characterized by complex chromosomal aberrations.[7–12] No further detailed analysis of gene expression in spindle cell and pleomorphic sarcomas have been reported so far.

In this study, we analyzed gene expression profile of total 105 cases representing 10 types of soft tissue tumors to identify their molecular characteristics. We observed similarity in gene expression among spindle cell and pleomorphic sarcomas, forming a relatively loose cluster, which is separated from the distinct clusters of synovial sarcoma, myxoid/round cell liposarcoma and lipoma + well-differentiated liposarcoma. Next, we primarily analyzed 64 samples of spindle cell and pleomorphic sarcomas and showed heterogeneity of MFH in terms of gene expression. We selected genes that could clearly distinguish between dedifferentiated liposarcoma, myxofibrosarcoma, leiomyosarcoma, malignant peripheral nerve sheath tumor (MPNST) and fibrosarcoma and quantified similarities as distances between MFH samples and the five sarcoma types.

Materials and methods

Patients and Tumor Samples

Characteristics of 105 soft tissue tumors used in this study are shown in Supplementary data 1. Among them, 35 samples were previously analyzed in a different method.[13] All patients received histological diagnosis of primary soft tissue tumor at National Cancer Center Hospital, Tokyo, from 1996 to 2002. In this paper, we use the term 'MFH' to describe samples diagnosed as storiform and pleomorphic type MFH showing predominant pleomorphic features without immunohistochemical phenotypes characteristic of specific differentiation, and the term 'myxofibrosarcoma' to describe MFH with predominant (>50%) myxoid features conventionally diagnosed as myxoid type MFH. Before the gene expression analysis pathologists confirmed the diagnosis of MFH was appropriate at the time of primary diagnosis. Tumor samples were collected from the part with macroscopically high tumor content by pathologists immediately after surgical excision and cryopreserved in liquid nitrogen until use. This study was approved by the ethics committee of National Cancer Center and conducted according to tenets of the Declaration of Helsinki.

Gene Expression Profiling

Total RNA was isolated using TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instruction. Samples were analyzed with a GeneChip Human Genome U133A array (Affymetrix, Santa Clara, CA, USA) containing 22 283 probe sets. Target cRNA preparation from total RNA, hybridization to the microarray, washing and staining with an antibody amplification procedure and scanning were all carried out according to the manufacturer's instructions. The expression value (Signal) of each probe set was calculated using GeneChip Operating Software (GCOS) ver. 1.3 (Affymetrix), so that the mean of expression values in each experiment was set at 100 to adjust for minor differences between experiments.

Statistical Analysis

Gene expression data were subsequently imported into GeneSpring GX7.2 software (Agilent Technologies, Santa Clara, CA, USA) and normalized to the median of all samples enrolled in the analysis and log-transformed for each gene. Hierarchical clustering analysis was performed using Pearson's correlation. To select appropriate probe sets defining five

types of spindle cell and pleomorphic sarcomas (dedifferentiated liposarcoma, myxofibrosarcoma, leiomyosarcoma, MPNST and fibrosarcoma), we performed Student's *t*-tests between one and the other four sarcoma types. The top 50 probe sets with low *P*-values in each *t*-test were summed. The centroid of each sarcoma type was determined by calculating the average of the selected probe sets. The distance (D) from a centroid to a sample was defined as $D=1-r$, using Pearson's correlation coefficient (r, $-1 \leq r \leq 1$). Inter-centroid distances were also calculated using Pearson's correlation coefficient.

Histological Analysis

Histological sections of the tumors were stained with hematoxylin and eosin and reviewed for all samples, and representative sections were examined immunohistochemically using the labeled streptavidin–biotin method. Sections were dewaxed, rehydrated and moistened with phosphate-buffered saline (pH 7.4), autoclaved at 121°C for 10 min in 10 mM citrate buffer (pH 6.0) and incubated with antibodies to the following molecules on an automated immunostaining system i6000 (BioGenex, San Ramon, CA, USA) for 30 min, as described previously:[14] vimentin, desmin, α-smooth muscle actin (αSMA), muscle-specific actin, h-caldesmon, CD34, S-100 protein, epithelial membrane antigen, cytokeratin and neurofilament. Heat-induced epitope retrieval was not undertaken when sections were stained with antibodies to S-100 protein and epithelial membrane antigen.

Quantitative RT-PCR

Real-time quantitative reverse transcription (RT)-PCR was carried out using the 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) with FastStart TaqMan Probe Master (Rox) and Universal ProbeLibrary (Roche Applied Science, Mannheim, Germany). One microgram of total RNA from 17 tumor samples (myxofibrosarcoma ($n=5$), MFH ($n=7$), leiomyosarcoma ($n=2$) and MPNST ($n=3$)) was reverse-transcribed to synthesize single-stranded cDNAs using SuperScript III (Invitrogen), and 1/100 of the cDNA was used for each PCR. Probes and primers were designed using Probe Finder software (Roche Applied Science) (Supplementary data 2). Transcript levels were normalized to that of the *ACTB* transcript.

Results

Overview of Gene Expression in Soft Tissue Sarcomas

Gene expression data of 105 soft tissue tumor samples consisting of synovial sarcoma ($n=16$), myxoid/round cell liposarcoma ($n=19$), lipoma ($n=3$), well-differentiated liposarcoma ($n=3$), dedifferentiated liposarcoma ($n=15$), myxofibrosarcoma ($n=15$), leiomyosarcoma ($n=6$), MPNST ($n=3$), fibrosarcoma ($n=4$) and MFH ($n=21$) were obtained using an oligonucleotide microarray containing 22 283 probe sets. Among them, 12 599 probe sets whose expression values were not less than 100 in at least 3 of 105 samples were analyzed. To overview the transcriptome of sarcomas in our data set, we first performed principal component analysis with 12 599 probe sets (Figure 1a), which is a decomposition technique to reduce multidimensional data into several specialized dimensions. The x and y axes in Figure 1a indicate the first and second principal components, respectively, representing the top and second largest fractions of the overall variability. In this analysis, 105 samples were roughly classified into four groups based on their position relative to the first and second principal components. Both synovial sarcoma and myxoid/round cell liposarcoma samples were located on the negative side of the first principal component, while well-differentiated liposarcoma, dedifferentiated liposarcoma and other spindle cell and pleomorphic sarcoma samples were on the positive side. On the negative side of the second principal component were myxoid/round cell liposarcoma, well-differentiated liposarcoma and lipoma samples, all of which are adipocytic tumors. Interestingly, some dedifferentiated liposarcoma samples were distributed close to well-differentiated liposarcoma samples, while others were midway between well-differentiated liposarcoma and other spindle cell and pleomorphic sarcoma samples. These results suggest that the first principal component was associated with the difference between synovial sarcoma + myxoid/round cell liposarcoma and spindle cell and pleomorphic sarcomas, and that the second principal component was associated with adipocytic differentiation. Probe sets contributing significantly to the first and second principal components are listed in Supplementary data 3.

To identify genes whose expression differed in a statistically significant manner among all sarcoma types, we performed an analysis of variance (ANOVA) among 10 tumor types and selected 2590 probe sets with *P*-values of less than 1.0×10^{-5}. Two-dimensional hierarchical clustering analysis using those 2590 probe sets showed that synovial sarcoma and myxoid/round cell liposarcoma samples displayed distinct gene expression profiles and formed robust clusters (Figure 1b). On the other hand, myxofibrosarcoma, leiomyosarcoma, MPNST, fibrosarcoma and MFH samples did not show distinct gene expression profiles, but rather formed a single loose cluster and shared a similar expression profile. We also found that lipoma and well-differentiated liposarcoma samples and some of the dedifferentiated liposarcoma samples displayed similar gene expression profiles and formed a cluster, whereas

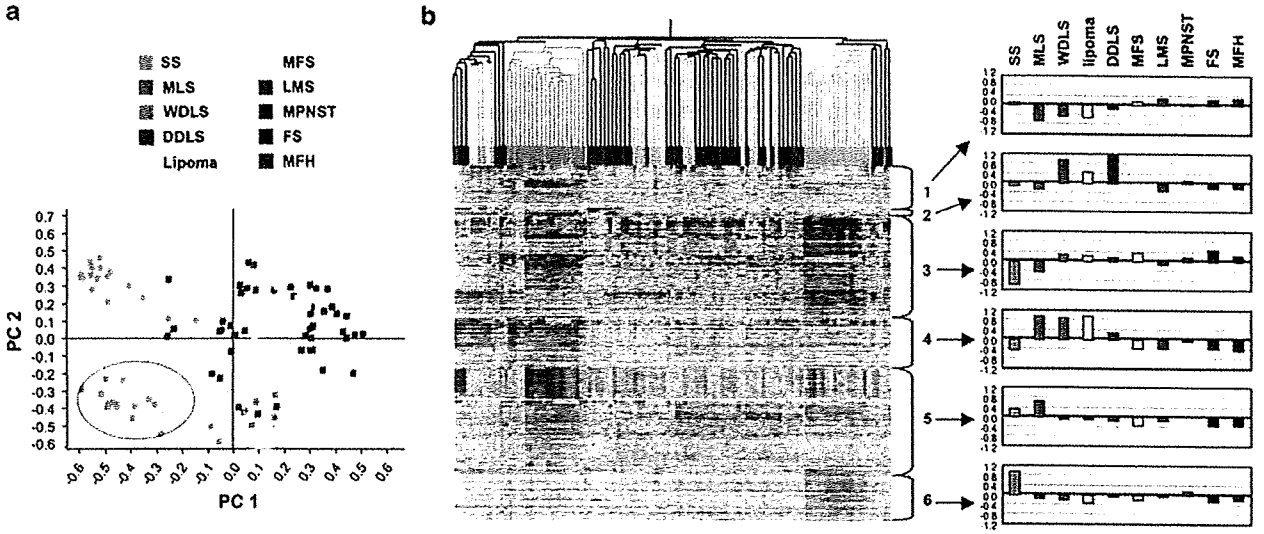

Figure 1 Gene expression overview of 105 soft tissue tumors. (a) Principal component analysis. A total of 12 599 probe sets with expression values not less than 100 in at least three samples were used in this analysis. *x* and *y* axes represent the first and second principal components (PC1 and PC2), respectively. Each dot represents a sample colored by its histological type. (**b**) Two-dimensional hierarchical clustering analysis. A total of 2590 probe sets differentially expressed among histological types ($P<1.0\times10^{-5}$ by ANOVA) were used. Columns represent samples and rows represent probe sets. Red and green indicate high and low expression, respectively. The 2590 probe sets were roughly divided into six clusters (clusters 1–6). The six graphs on the right show averages of normalized expression values of those clusters for each histological type. Note that spindle cell and pleomorphic sarcomas, such as dedifferentiated liposarcoma, myxofibrosarcoma, leiomyosarcoma, MPNST, fibrosarcoma and MFH, form a loose cluster and share a similar expression profile compared with synovial sarcoma, myxoid/round cell liposarcoma, well-differentiated liposarcoma and lipoma. SS, synovial sarcoma; MLS, myxoid/round cell liposarcoma; WDLS, well-differentiated liposarcoma; DDLS, dedifferentiated liposarcoma; MFS, myxofibrosarcoma; LMS, leiomyosarcoma and FS, fibrosarcoma.

the other dedifferentiated liposarcoma samples did not share that profile but instead formed a loose cluster with fibrosarcoma, myxofibrosarcoma and MFH samples.

The 2590 probe sets were classified into six clusters according to their expression patterns (Figure 1b and Supplementary data 4). Interestingly, we found two major clusters (clusters 3 and 5) whose expression patterns were similar between synovial sarcoma and myxoid/round cell liposarcoma samples. Cluster 3, whose expression was low in synovial sarcoma and myxoid/round cell liposarcoma, contained many HLA genes, and cluster 5, whose expression was high in both synovial sarcoma and myxoid/round cell liposarcoma, contained many genes encoding ribosomal proteins and cancer testis antigens, such as *CTAG1B*, *CTAG2* and *PRAME*. Of note, these genes contributed largely to the first principal component (see Supplementary data 3). On the other hand, cluster 1, whose expression was low in myxoid/round cell liposarcoma, well-differentiated liposarcoma and lipoma samples, included cell cycle associated genes such as *CCNB1*, *CDKN3*, and *CDC20*, while cluster 4, whose expression was high in myxoid/round cell liposarcoma, well-differentiated liposarcoma and lipoma samples, included adipocytic differentiation-associated genes such as *LPL*, *ACACB* and *PLIN*. These genes contributed largely to the second principal component (see Supplementary data 3). Cluster 6, whose expression was high in synovial sarcoma, included *COL2A1*, *COL9A3*, *SSX1* and *SSX2*. The small but robust cluster, cluster 2, consisted of *MDM2*, *CDK4* and other genes located in 12q13-15, which are known to be amplified in both well-differentiated liposarcoma and dedifferentiated liposarcoma.

Heterogeneity of MFH in Gene Expression and Classification of Spindle Cell and Pleomorphic Sarcomas

Spindle cell and pleomorphic sarcomas frequently display overlapping histological appearance and immunohistochemical phenotypes. Samples from these types of sarcoma did not separate into distinct histological types in the analysis using whole samples (Figure 1). To determine whether they could be grouped by gene expression, we analyzed 64 samples of spindle cell and pleomorphic sarcomas (dedifferentiated liposarcoma, myxofibrosarcoma, leiomyosarcoma, MPNST, fibrosarcoma and MFH). We performed principal component analysis with 11 300 probe sets whose expression values were not less than 100 in at least three of 64 samples, and two-dimensional hierarchical clustering analysis using 1671 probe sets selected by ANOVA among six sarcoma types ($P<0.01$) (Supplementary data 5). In the clustering analysis,

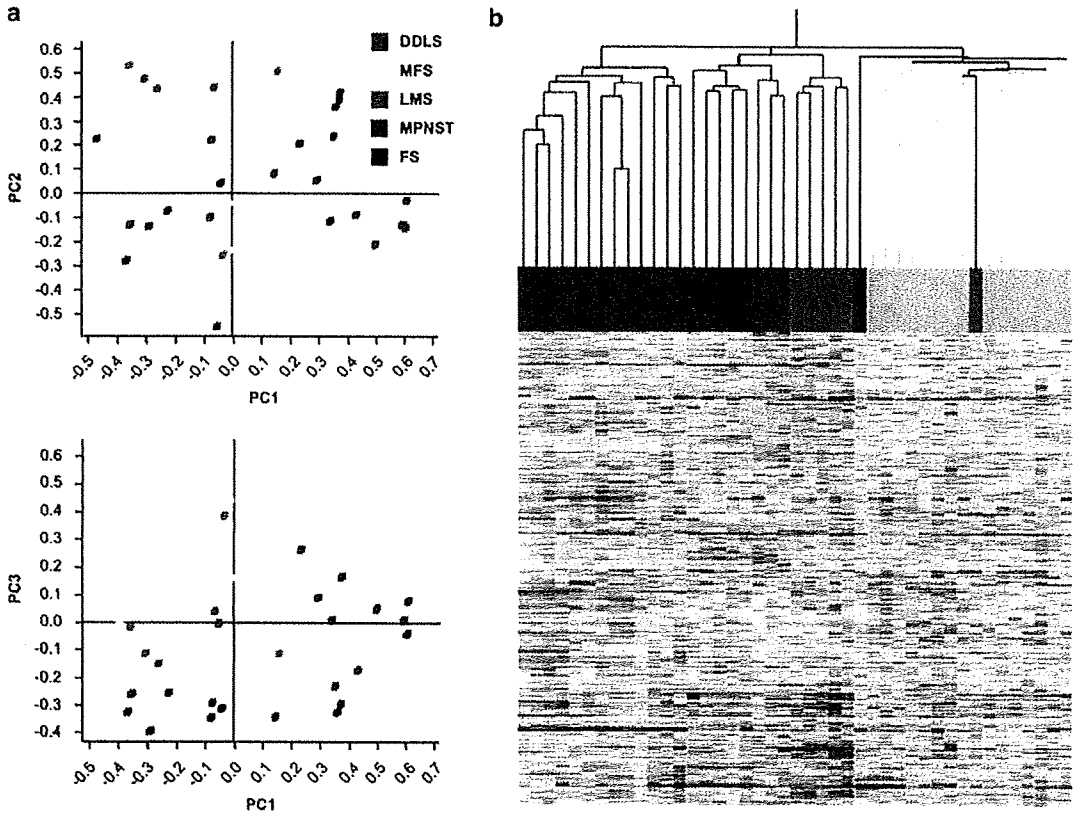

Figure 2 Classification of spindle cell and pleomorphic sarcomas without MFH. (a) Principal component analysis. A total of 11 300 probe sets with expression values not less than 100 in at least three of 64 spindle cell and pleomorphic sarcoma samples including MFH were used in this analysis. *x* and *y* axes in the upper panel represent the first and second principal components (PC1 and PC2), and *x* and *y* axes in the lower panel represent the first and third principal components (PC1 and PC3), respectively. (b) Two-dimensional hierarchical clustering analysis. A total of 1457 probe sets differentially expressed among five types of spindle cell and pleomorphic sarcomas ($P<0.01$ by ANOVA) were used. Columns represent samples and rows represent probe sets. Red and green indicate high and low expression, respectively. Note that most samples formed clusters corresponding to their histology. DDLS, dedifferentiated liposarcoma; MFS, myxofibrosarcoma; LMS, leiomyosarcoma and FS, fibrosarcoma.

dedifferentiated liposarcoma, myxofibrosarcoma, leiomyosarcoma, MPNST and fibrosarcoma samples appeared to form their own clusters, whereas those of MFH partitioned into several groups, some close to clusters of other sarcomas. These results suggest that MFH is heterogeneous in terms of gene expression as observed histologically.

Next, we analyzed 43 samples of five spindle cell and pleomorphic sarcomas (dedifferentiated liposarcoma, myxofibrosarcoma, leiomyosarcoma, MPNST and fibrosarcoma) and excluded MFH samples. In principal component analysis with 11 300 probe sets, samples of the same tumor type appeared to cluster (Figure 2a). We then performed two-dimensional hierarchical clustering analysis with 1457 probe sets selected from the 11 300 probe sets by ANOVA among five sarcoma types ($P<0.01$) (Figure 2b). Although we found three exceptions (one leiomyosarcoma and two dedifferentiated liposarcoma samples), almost all dedifferentiated liposarcoma, myxofibrosarcoma, leiomyosarcoma, MPNST and fibrosarcoma samples formed their own respective clusters suggesting that each type of spindle cell and pleomorphic sarcoma formed a homogeneous group in terms of gene expression by excluding MFH samples.

Distances of MFH Samples from Other Spindle Cell and Pleomorphic Sarcomas

Since MFH samples did not form a clearly distinctive cluster, we next addressed a question whether MFH could be reclassified into other types of spindle cell and pleomorphic sarcomas by gene expression and quantified similarities between MFH samples and those sarcoma types using differentially expressed genes. To select appropriate probe sets defining spindle cell and pleomorphic sarcomas, we performed the Student's *t*-test between one and the other four of the five sarcoma types, namely, dedifferentiated liposarcoma, myxofibrosarcoma, leiomyosarcoma, MPNST and fibrosarcoma. In this analysis, we excluded three exceptional samples

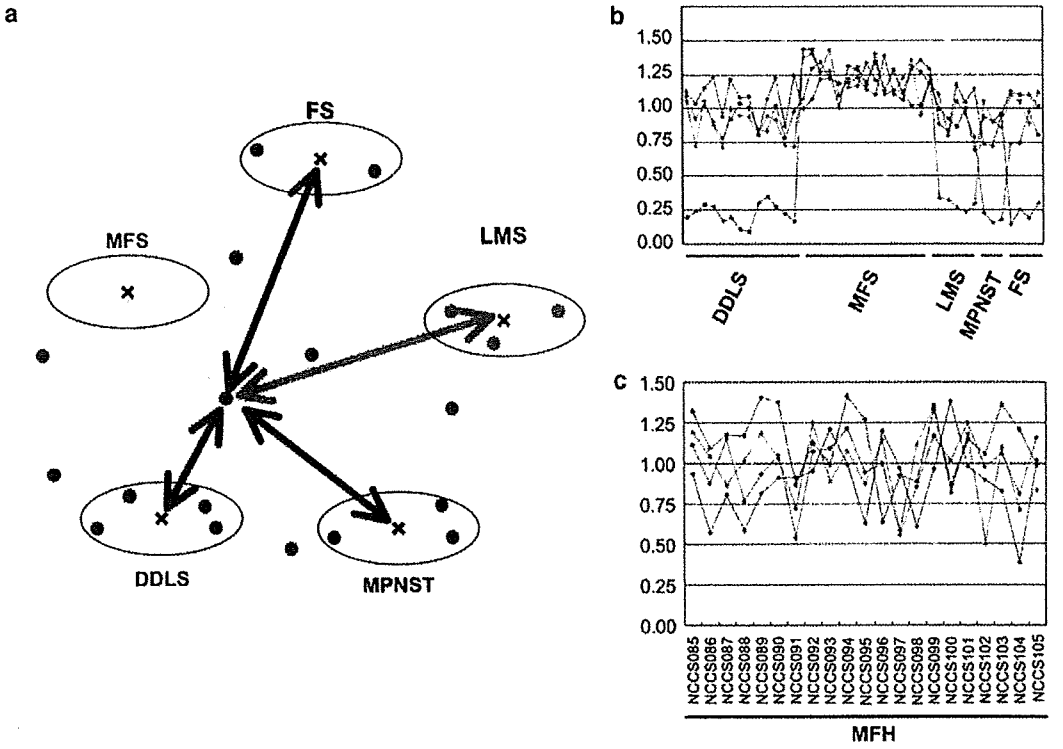

Figure 3 Distance evaluation of spindle cell and pleomorphic sarcoma samples from five sarcoma types. (**a**) Scheme of distance calculation. Each dot represents a sample colored according to its histology. Each ×-mark represents the centroid of each histological type of sarcoma. Each arrow indicates the distance from a sample to a centroid colored by the histology. (**b**) Distances of 40 control samples from the five centroids. Note that the closest centroids matched their histology. (**c**) Distances of 21 MFH samples from the five centroids. DDLS, dedifferentiated liposarcoma; MFS, myxofibrosarcoma; LMS, leiomyosarcoma and FS, fibrosarcoma.

that did not fall into the appropriate cluster (Figure 2b). The top 50 probe sets with low *P*-values in each *t*-test were summed to obtain 248 probe sets (Supplementary data 6). On the basis of the expression of these 248 probe sets, the centroids of those five sarcoma types were calculated in advance, and inter-centroid distances and distances from five centroids to each control sample ($n=40$) were evaluated (Supplementary data 7 and Figure 3a and b). All inter-centroid distances were greater than 0.77 and the closest centroids for 40 control samples matched their histological types (Figure 3b), indicating that the evaluated distances were good indicators of sarcoma classification. We then evaluated the distances of each MFH sample from the five centroids (Figure 3c) and focused on determining the minimum (D_{min}) of the five distances. Small D_{min} values indicate high similarity to one of the five histological types in terms of gene expression. We used two cutoff values of 0.5 and 0.75 to evaluate similarity, because the majority of D_{min} values in control samples were less than 0.5 and most of the remaining four distances in each control sample were greater than 0.75. Among 21 samples, 3 showed marked similarity ($D_{min} \leq 0.5$), 12 showed moderate similarity ($0.5 < D_{min} \leq 0.75$) and the remaining 6 showed little similarity ($D_{min} > 0.75$). Among 15 MFH samples showing high or moderate similarity ($D_{min} \leq 0.75$), 6 were similar to myxofibrosarcoma, 5 to fibrosarcoma, 2 to MPNST and 1 each to dedifferentiated liposarcoma and leiomyosarcoma.

Histological Reviews

We re-examined the histology of 21 MFH samples with the knowledge of similarity to other types of spindle cell and pleomorphic sarcomas based on gene expression. Three MFH samples that showed high gene expression similarity ($D_{min} \leq 0.5$) displayed marked pleomorphism, indicating that a diagnosis of MFH was appropriate at the time of diagnosis. However, these samples also showed histological signatures of relevant subtypes. The NCCS099 sample, which was significantly close to the myxofibrosarcoma centroid ($D_{min} = 0.46$), showed prominent myxoid features very similar to myxofibrosarcoma in one third of the tumor (Figure 4a). The NCCS102 sample which was very close to the leiomyosarcoma centroid ($D_{min} = 0.50$) was positive for desmin and αSMA (Figure 4b–d). The NCCS104 sample, which was very close to the fibrosarcoma centroid ($D_{min} = 0.39$), showed focal