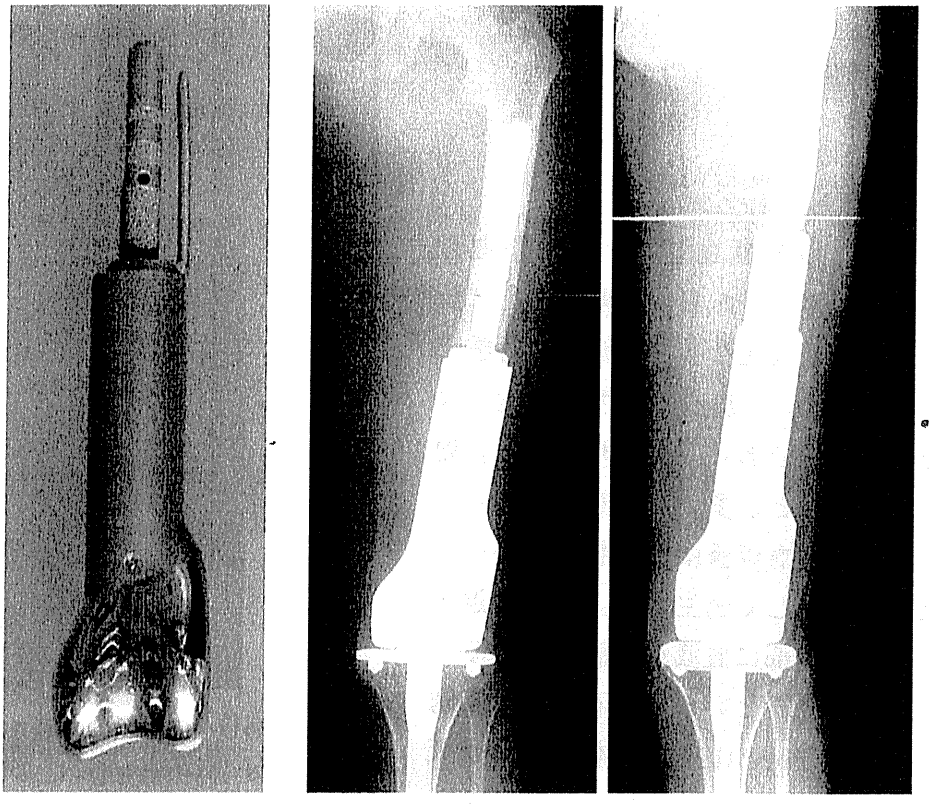

（A）　　（B）　　（C）

図15　延長型腫瘍用人工関節
成長により脚長差が予想される小児の四肢悪性骨腫瘍の再建に対して用いられる．
（A）実物の写真，（B）挿入直後，（C）延長後

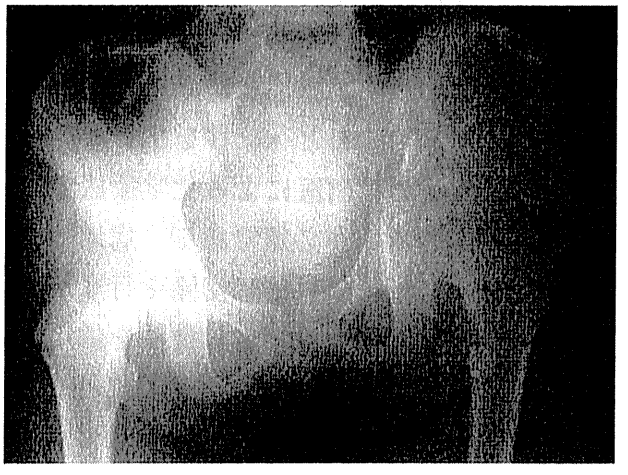

図16　骨盤発生ユーイング肉腫
腫瘍は左腸骨全体に及び病的骨折により骨盤は変形している．

一般的である。治療成績は，5年生存率で60%近くまで改善されたが，初診時から肺転移がある，腫瘍が大きい，脊椎や骨盤などの体幹発生などの例では，依然として予後不良である。

2．転移性骨腫瘍

神経芽細胞腫の骨転移（bone metastasis of neuroblastoma）

小児の代表的悪性腫瘍で，交感神経節に関連して発生する。骨転移を高率に伴い，単純X線では溶骨像を示す。2歳以下の発症がほとんどであり，発熱や体重減少などの全身症状があり，手術と化学療法，放射線治療を併用する治療が行われる。

3．その他

白血病の骨病変（leukemia）

白血病は造血器の悪性腫瘍であり，その約半数は単純X線で骨に何らかの変化を認める。このような変化を認める例は，幼年期の症例が多い。長管骨の骨幹端から骨端部など，関節近傍に見られ，関節痛や腫脹を伴う。

Ⅳ．軟部腫瘍

1．良性軟部腫瘍

1）血管腫（hemangioma）

毛細血管腫，海綿状血管腫，静脈型血管腫，動静脈型血管腫がある。小児期で主に認められるのは，毛細血管腫と海綿状血管腫である。毛細血管腫は，皮膚の軽度の隆起を伴う赤い母斑状病変であり，出生時または乳児期に発見されることが多い。自然消退することもある。海綿状血管腫は，四肢の皮膚や皮下から深部に連続して発生する。自然消退することはなく，内部に血液を充満している。単純X線で，静脈石といわれる石灰化像を認め診断に有用である（図17）。本症に血小板減少性紫斑病を伴うものはKasabach-Merritt症候群といわれている。保存療法に抵抗する疼痛や関節拘縮などの機能障害があれば手術を考慮する。

2）リンパ管腫（lymphangioma）

出生時あるいは幼児期に発見されることが多いことから，先天的に生じているリンパ管の形成異常と考えられている。好発部位は頸部，腋窩であるが，四肢に発見されることもある。腫瘍の内部は，リンパ液で満たされ，触診でやわらかい腫瘤として触知する。

3）脂肪芽腫（lipoblastoma）

3歳以下に発生する稀な腫瘍であり，男性にやや多い。四肢の皮下に限局して発生するものと，びまん性のものがある。完全に切除を行えれば，再発することは少ない。

4）乳幼児指趾線維腫症（infantile digital fibromatosis）

乳児の指趾に発生する線維性腫瘍。関節の背側や側面に生じることが多く，多発例もある。切除後の再発率が高く，関節拘縮などの問題がなければ経過観察を行う。

2．悪性軟部腫瘍

1）横紋筋肉腫（rhabdomyosarcoma）

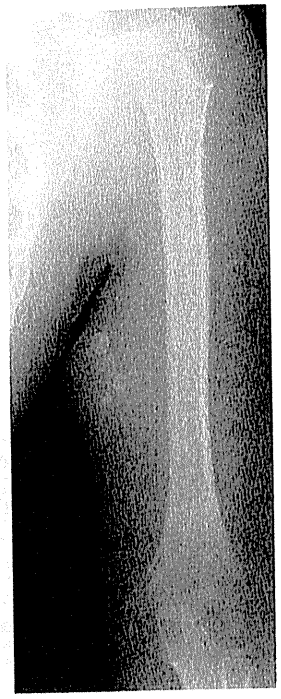

図17　血管腫の単純X線像
上腕部内側軟部組織内に多数の石灰化陰影を認める．

通常，胎児型，胞巣型，多形型の3型に分類される。胎児型と胞巣型が小児期に発生する。好発部位は頭頸部，泌尿生殖器，四肢・体幹の軟部組織であり，四肢の筋肉内発生は胞巣型が多い。悪性度が高く，早期から骨・リンパ節・肺などへ転移を生じるため，小児悪性腫瘍の専門医による化学療法を中心とした集学的治療が必須である。病巣の切除が可能であれば，外科的に切除を行うが，完全切除が困難な場合は放射線療法を併用する。5年生存率は50％以上に改善しているが，初診時遠隔転移例，腫瘍の大きいもの，局所再発例などは予後不良である。

2）乳（幼）児型線維肉腫（infantile fibrosarcoma）

5歳以下に発生する悪性腫瘍であり，男児に多い。四肢に発生する無痛性腫瘤で急速に増大する（図18）。治療は広範切除が一般的だが，近年，化学療法の有効性も報告されている。成人例より予後良好で，5年生存率は約80％と改善している。

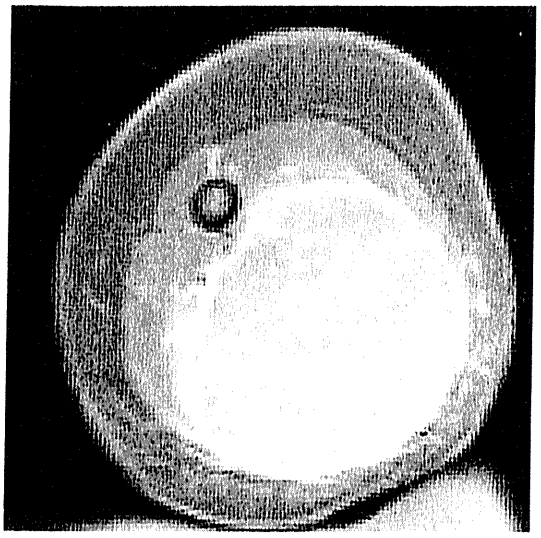

図18　乳児型線維肉腫
MRI　T1強調 Gd 造影画像：上腕内側に辺縁優位に造影される巨大な腫瘤を認める．

V．まとめ

小児期の運動器に発生する腫瘍性疾患について述べた。良性腫瘍の場合，成長に伴い自然治癒傾向を有する疾患も存在する。しかし悪性腫瘍の多くは，病巣が急速に広がり予後不良な疾患が多く，治療は骨・軟部腫瘍専門医と小児悪性腫瘍専門医の連携のもとに進められる必要がある。これらの専門医への道筋をつけるのは，常に一般臨床医であり，この分野においては，整形外科医のみならず，一般小児科医の果たす役割が少なくないと考える。これらの点から，本稿が一般小児科診療に少しでも有益な情報をもたらし，患者の予後改善につながることを期待している。

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ORIGINAL ARTICLE

Revision of tumor prosthesis of the knee joint

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Abstract
Background Among 40 patients with primary malignant tumors of the knee joint who underwent reconstruction of the affected limb with tumor prosthesis, revision was required in 7 due to stem breakage or loosening.
Subjects and methods In the 7 cases undergoing revision, conditions and background factors at the time of breakage, the breakage site, time of revision, models of previous and new prostheses, stem diameters before and after revision, details of the revision (blood loss, operative time), and the presence or absence of adjuvant therapy were determined.
Results The replacement site was the distal femur in 5 and proximal tibia in 2. Revision was performed 6 years and 2 months after the previous prosthesis placement on average. The broken prosthesis model was KMFTR in 4 and HMRS and the physio-hinge type in one each. Revision due to loosening was performed in a case requiring replacement with Growing Kotz prosthesis. The model was switched to HMRS in 3, and the stem diameter was changed to 12 mm in 3 KMFTR breakage cases. The mean stem diameters were 11.2 and 10.2 mm in the non-revision and revision groups. The respective resection rates were 36 and 45%. The mean functional evaluation was 70.1% before and 76.2% after revision.
Conclusion To reduce the risk of tumor prosthesis breakage, the amount of bone resection should be limited to 30% or less in the affected bone, the stem diameter should be at least 12 mm, and the stem shape should be fitted to the anatomical shape of the femur.

Keywords Limb salvage · Revision · Tumor prostheses · Malignant bone tumor

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Background

Various methods, such as allografting [1, 2], pasteurized autografting [3], and bone elongation [4], have recently been employed for the reconstruction of limbs affected by malignant bone tumors. However, reconstruction with tumor prosthesis remains the primary treatment. Tumor prosthesis use is advantageous in that it facilitates stable function of the affected limb and an early return to social activities. The survival rate of patients treated with tumor prosthesis was satisfactory in a recent report [5]. However, there are potentially serious complications, including infection, breakage, and loosening. We recently experienced a case in which tumor prosthesis of the knee joint (Howmedica Modular Reconstruction System (HRMS) broke 13 years after surgery. Reportedly, tumor prostheses breakage is caused

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by increased patient activity and loosening of the stem. When wide resection is performed for a malignant bone tumor around the knee joint, the surrounding soft tissue is also resected. Thus, a hinge-type prosthesis is inevitably needed to stabilize the knee joint. Breakage of the stem is thus reportedly due to the design of the prosthesis [5]. The causes of breakage are thought to include the stem diameter, the length of resected bone, and prosthesis design. Forty patients with primary malignant tumors around the knee joint underwent limb salvage by reconstruction with prosthesis use. To investigate the causes underlying breakage of prostheses, we divided these patients into those with (7) and without (33) revision of a broken or loose stem. The prosthesis model, stem diameter, length of resected bone, and International Symposium on Limb Salvage (ISOLS) X-ray evaluation were determined in each case. In the 7 revision cases, elements assumed to be causative were analyzed in detail to identify problems and possible countermeasures.

Materials and methods

The 40 subjects had primary malignant tumors of the knee joint and underwent limb salvage by reconstruction with a prosthesis in our department between 1979 and 2008.

The subjects comprised 20 women and 20 men, ranging in age from 7 to 82 years (mean age, 27.5 years). The pathological diagnosis of the primary lesion was osteosarcoma in 28, chondrosarcoma in 5, bone malignant fibrous histiocytoma (MFH) in 3, and a giant cell tumor GCT (grade 3), synovial sarcoma, Ewing's sarcoma, and a primitive neuroectodermal tumor (PNET)in one each. The duration of follow-up ranged from 1 year and 5 months to 19 years (mean: 11 years and 2 months). The 7 patients who underwent replacement were 6 women and 1 man, ranging in age from 7 to 44 years (mean age, 26.5 years).The primary lesion was osteosarcoma in 4, and PNET, GCT (grade 3), and synovial sarcoma in one each. The duration of follow-up after the initial examination ranged from 12 to 19 years (mean: 16 years). The prosthesis model, replacement site, stem diameter, length of resected bone, resection rate, and ISOLS X-ray evaluation were investigated in all 40 cases. In the 7 cases undergoing revision, conditions and background factors at the time of breakage, breakage site, time of revision, models of previous and new prostheses, stem diameters before and after revision, details of the revision (blood loss, operative time, surgical procedure), and the presence or absence of adjuvant therapy were also investigated. In addition, ISOLS X-ray and functional evaluations were performed before revision and at the final follow-up.

Results

Replacement site and time of revision

The prosthesis replacement site was the distal femur in 28 and the proximal tibia in 12 cases. Among the 7 cases requiring revision due to stem breakage or loosening, the replacement site was the distal femur in 5 and the proximal tibia in 2. The shortest and longest times until revision for breakage and loosening, after the initial wide resection followed by reconstruction of the tumor affected limb or an elongation-type prosthesis placement, were 10 months and 11 years, respectively, with a mean of 6 years and 2 months.

Prosthesis models used in the initial replacement and the revision

At the initial replacement in our 40 cases, the Howmedica Modular Reconstruction System (HMRS) was used in 22, and the rotating hinge type in two. The Kotz Modular Femur and Tibia Reconstruction System (KMFTR) was used in 6, the Growing Kotz in 10, the Kyocera custom-made prosthesis in one, and the Kyocera PH1 (physio-hinge type 1) in one. The broken models in the 7 revision cases were the KMFTR in 4, and HMRS and physio-hinge type I in one each. Revision for loosening of the stem was performed in a 7-year-old female with PNET who had undergone reconstruction of the distal femur with a Growing Kotz (Case 2). The model used for revision was the HMRS in 3 cases reconstructed with a KMFTR at the initial replacement excluding a proximal tibial case (Case 2). In Case 38, a PH type 1 with an 11-mm stem diameter was changed to the slightly thicker PH type 2 with a 12-mm stem diameter. In Case 39, the stem diameter (12 mm) of the new prosthesis was the same as that before breakage. However, the new stem, at 15 cm, was longer. In Case 2, a new component was prepared, considering that the tibial component of the HMRS employed for adults is too large. The new tibial component was designed with proximal and distal diameters of 15 and 10 mm, respectively, and a stem length of 14 cm, and the stem was entirely covered with a porous coating (Tables 1, 2).

Stem diameter

The stem diameters ranged from 8 to 14 mm (mean: 11.1 mm) in our 40 cases. In the non-revision cases, they ranged from 8 to 14 mm (mean: 11.2 mm). In the revision cases, the diameters ranged from 9 to 12 mm (mean: 10.2 mm), the diameter of the broken KMFTR was 10 mm in all 4 cases. The prosthesis was changed to one with a stem diameter of 12 mm in 3 of these 4 cases. In Case 39,

Table 1 List of cases with reconstruction of regions around the knee joint using prostheses

Case	Age	Gender	Pathological diagnosis	Model	Replacement site	Resected length (cm)	Stem diameter (mm)	Resection rat (%)
1	7	M	OS	Growing Kotz	DF	14	8	36.80
2	7	F	PNET	Growing Kotz	DF	21	9	53
3	11	M	OS	Growing Kotz	DF	13	11	33
4	16	F	OS	Growing Kotz	DF	13	10	31
5	10	F	OS	Growing Kotz	DF	15	9	45
6	12	M	OS	Growing Kotz	DF	17	10	44
7	8	M	OS	Growing Kotz	PT	12	10	50
8	12	M	Ewing sarcoma	Growing Kotz	PT	15	10	35
9	16	F	OS	Growing Kotz	PT	10	10	33
10	12	F	OS	Growing Kotz	PT	16	10	32
11	34	M	OS	HMRS	PT	16	12	47
12	60	F	OS	HMRS	DF	16	14	38
13	57	F	Chondrosarcoma	HMRS	DF	12	11	35
14	18	F	OS	HMRS	PT	12	12	34
15	57	F	Chondrosarcoma	HMRS (Rotating)	DF	12	12	26
16	27	F	OS	HMRS	DF	18	13	39
17	25	M	OS	HMRS	DF	17	13	34
18	24	M	OS	HMRS	PT	13	11	37
19	12	F	OS	HMRS	DF	18.5	12	42
20	20	M	MFH of bone	HMRS	PT	14.5	11	47
21	25	M	OS	HMRS (Rotating)	DF	12	12	27
22	27	M	OS	HMRS	DF	16	12	33
23	56	F	Chondrosarcoma	HMRS	PT	18	12	47
24	16	M	OS	HMRS	DF	12	10	30
25	13	M	OS	HMRS	DF	16	12	27
26	18	M	OS	HMRS	DF	14	12	43
27	20	M	OS	HMRS	DF	12	13	29
28	66	F	MFH of bone	KMFTR	DF	12	11	27
29	82	M	Chondrosarcoma	KMFTR	DF	16	12	34
30	27	M	OS	HMRS	PT	17	11	45
31	52	F	OS	Kyocera (cement)	DF	16	11	35
32	50	M	Chondrosarcoma	HMRS	DF	18	11	48
33	41	M	GCT	HMRS	PT	13	12	40
34	44	F	OS	KMFTR	DF	12	10	23
35	31	F	OS	KMFTR	PT	12	10	34
36	26	F	OS	KMFTR	DF	23	10	54
37	40	F	Synovial sarcoma	KMFTR	DF	14	10	48
38	15	M	OS	PH typel (cement)	DF	18	11	55
39	28	F	OS	HMRS	DF	22	12	48
40	66	F	MFH of bone	HMRS	DF	18	12	38

DF distal femur, *PT* proximal femur, *HMRS* Howmedica Modular Resection System, *KMFTR* Kotz Modular Femoral and Tibia Replacement, *PH type 1* physio-hinge type 1, *PH type 2* physio-hinge type 2

the stem diameter (12 mm) of the new prosthesis was the same as that of the broken one, but the new stem was longer (15 cm). In Case 2, the stem region of the tibial component of the Growing Kotz employed in the initial replacement had proximal and distal stem diameters of 12 and 9 mm, respectively, and a length of 10 cm. On revision, it was replaced with a new porous-coated tibial component with proximal and distal diameters of 15 and 10 mm, respectively, and a length of 14 cm. Screw breakage was noted in Cases 37 and 39.

Table 2 Revision cases managed by our department

Case	Age	Sex	Location	Time of revision (mon) (m)	Type	Diameter (mm)	Screw breakage	Type of new prosthesis	Diameter
Case 34	44	F	Distal femur	48	KMFTR	10	–	HMRS	12 mm
Case 35	31	F	Proximal tibia	84	KMFTR	10	–	KMFTR	10 mm
Case 36	26	F	Distal femur	10	KMFTR	10	–	HMRS	12 mm
Case 37	40	F	Distal femur	28	KMFTR	10	+	HMRS	12 mm
Case 38	15	M	Distal femur	108	PH type1	11	–	PH type 2	12 mm
Case 39	28	F	Distal femur	132	HMRS	12	+	HMRS	12 mm
Case 2	7	F	Proximal tibia	113	Growing Kotz	Proximal: 12 Distal: 9	–	Growing Kotz	Proximal: 15 m Distal: 10 mm

Resected bone length

The respective maximum and minimum lengths of resected bone including the tumor region were 12 and 23 cm, with a mean of 15.4 cm. Those in the 33 non-revision cases were 10 and 18.5 cm, respectively, with a mean of 14.6 cm. In the 7 revision cases, these lengths were 12 and 22 cm, respectively, with a mean of 17.7 cm (Table 1).

Resection rate of affected bone

The resected region accounted for 27–50% (mean: 36) in the 33 non-revision cases, and 23–53% (mean: 45%) in the 7 revision cases. Thus, the ratio of the resected region was greater, comprising nearly half of the affected bone, in cases undergoing revision for breakage or loosening (Table 1).

Conditions and backgrounds of patients at the time of breakage

The stem was broken in 6 patients. Five had experienced sudden pain in the femoral or knee joint regions, while walking. They visited our hospital, and breakage was identified on X-ray examination. In the other patient (Case 39), dull pain appeared in the proximal femoral region and had become severe about 4 months later. At this time, breakage was identified. Only this patient was actively engaged in activities such as dancing and mountain climbing, while the other 6 were not especially involved in athletic activities. Regarding the social backgrounds of these 7 patients, Cases 34–37 were housewives, Case 38 was a clerical employee, mainly working at a desk, Case 39 was a speech therapist, and Case 2 was a student.

ISOLS X-ray and functional evaluations

X-ray evaluation was performed at the final follow-up in all 40 cases, and all items pertaining to bone remodeling, interface, and anchorage were graded as excellent in about 70% of these patients. However, in the revision group, the interface before revision was graded as poor and fair in Cases 2 and 39, respectively, and anchorage was graded as fair in all cases. After revision, bone remodeling was graded as poor only in Case 38, in whom the bone cortex around the stem was thinned by more than 1/3. A radiolucent line was also noted in the interface, resulting in a grading of fair. Functional evaluation was performed before revision and at the final postrevision follow-up. The evaluation was 53–80% (mean: 70.1%) before and 63–86% (mean: 76.2%) after revision (Table 3).

Table 3 Functional and radiological assessments and the presence/absence of adjuvant therapy before and after replacement

Functional assessment			Radiographical assessment	
Case	Before replacement (%)	After replacement (%)	Before replacement Bone remodeling/ interface/ anchorage	After replacement Bone remodeling/ interface/ anchorage
Case 34	78	80	E/G/F	G/G/E
Case 35	75	78	E/G/F	G/G/E
Case 36	76	73	E/E/F	F/E/E
Case 37	73	74	G/G/F	G/G/E
Case 38	80	63	G/G/F	P/F/G
Case 39	60	80	G/F/F	G/G/E
Case 2	53	86	G/P/F	E/E/E

E excellent, *G* good, *F* failure, *P* poor

Table 4 Adjuvant therapy and details of revision surgery

Case	Adjuvant therapy	Blood loss (g)	Operation time (min)
Case 34	+	430	200
Case 35	–	370	260
Case 36	+	282	212
Case 37	+	330	240
Case 38	+	155	210
Case 39	+	600	220
Case 2	+	420	371

Details of revision (blood loss and operative time)

The shortest and longest operative times in the 7 cases were 3 h and 20 min and 6 h and 11 min, respectively, with a mean of 4 h and 7 min. The minimum and maximum blood losses were 155 and 600 g (mean: 369 g), respectively (Table 4).

Presence or absence of adjuvant therapy

Case 33 in the revision group was diagnosed with a grade 3 GCT of the bone, and underwent surgery alone. Pre- and postoperative chemotherapies were administered in the other 6 cases. In Case 2, radiotherapy (50 Gy) was additionally performed for local control, after the completion of preoperative chemotherapy (Table 4).

Discussion

Recent advancements in surgical approaches and chemotherapy for primary malignant bone tumors have increased survival rates. The usefulness of reconstruction methods for affected limbs, including prostheses, has also been confirmed. However, complications associated with prostheses, such as infection, loosening, and breakage, remain problematic. Regarding stem breakage, in 1994, Capanna et al. [6] reported that stem breakage occurred in 6 (6.3%) of 95 cases treated with modular uncemented tumor prostheses. In 2001, Mittenmayer et al. [5] reported that major complications occurred in 19 of 100 cases with uncemented tumor prostheses, 11 of these involved aseptic loosening, and septic loosening and implant fracture occurred in 4 each. In 2006, Gosheger reported that stem breakage occurred in 4 (1.6%) of 250 cases with uncemented tumor prostheses [7]. In our department, breakage occurred after 5 years and 6 months on average, with the earliest being 10 months and latest 11 years. The models used were the KMFTR in 4 and the HMRS and PH type 1 in one case each. The stem diameters of 10 mm in 4 and 11 mm in one case were relatively thin for distal femoral stems. After revision, the stem diameter was 10 mm in only one, being thicker in all other cases. Although increased activity of patients and stem loosening were considered to be the causes of stem breakage, the design of the prostheses may have contributed to breakage, because the prostheses generally had a hinge-type structure [8]. In 2005, Griffin et al. reported that the incidence of KMFTR stem breakage involving the proximal tibia rose when the stem diameter was small, and the length of resected bone increased. They also described cases of distal femoral replacement: the 5-year survival rates were 35, 85, and 71.2% in patients in whom the distal femoral stem sizes were 10–12, 13, and 14–16 mm, respectively. These observations showed that the stem diameter, rather than the resection length, was related to breakage in cases undergoing distal femoral replacement. We also focused on the stem diameter and the length of resected bone. We investigated the stem diameter and resection rate in all 40 cases. The mean stem size of 10.2 mm in the revision cases and 11.2 mm in the non-revision cases confirmed that a thin stem was used in the revision cases. Currently, 11–15-mm straight types and 12–15-mm curved types of diaphysis-fixing pieces are available for HMRS.

All stems with a 10-mm diameter are of the Growing Kotz and KMFTR types. In cases reconstructed with the HMRS, stems with a relatively small diameter, 11 or 12 mm, were used in 80%. This may reflect the Japanese physique. The mean resection rate was 34.7% in the non-revision and 45% in the revision cases. The length of resected bone was thus greater in the revision than in the non-revision cases. When the resection rate is almost 45% in the clinical setting, possible reconstruction methods other than the use of a tumor prosthesis include total femoral replacement and biological reconstruction [3, 9, 10]. However, these reconstruction methods are indicated in only limited cases. When a tumor prosthesis is used, attention should be paid to the bone resection rate.

Comparison by region, such as the femoral and proximal tibial regions, was not possible because of the small number of cases. However, we would not expect more stress to be loaded on a thin stem used for a region from which a large amount of bone was excised. Griffin et al. also reported that stem breakage occurred in 6 (6.1%) of 99 cases reconstructed with the KMFTR. These breakages occurred at 3 holes in the stem, indicating a structural problem. They stated that the ideal design of a prosthesis may be a strong and thick stem without holes to stop lateral movement, which facilitates bone ingrowth comparable or superior to that around the KMFTR stem. Aseptic loosening of the stem may be another cause of stem breakage. In our 40 patients, loosening was apparently present at the interface, being graded as poor in Cases 2 and 8 with a Growing Kotz. It was also graded as poor in Case 15, a

57-year-old female with distal femoral chondrosarcoma in whom a rotating hinge-type HMRS was applied. The incidence of aseptic loosening varies among reports, ranging from as low as 1 and 5% up to 26 and 29% [11–18]. In 2001, Mittermayer et al. reported that aseptic loosening occurred in 27% of cases with complications involving stems [5]. In 1990, they developed an anatomically curved stem, which fit in the femoral bone marrow cavity to avoid stress shielding generated by firm fixation around the stem. Then in 1996, they rotated the hinge-type HMRS. The incidence of aseptic loosening in cases receiving this type of prosthesis was approximately 10% during a 42–134-month follow-up period. Aseptic loosening is considered to be related to a patient's activity level. However, only in our Case 39 (28-year-old female) in the revision group had a high activity level, i.e., such a tendency was not apparent in our patients. Functional and X-ray evaluations following revision were favorable over the short, medium, and long term in various reports [6, 11, 14, 17]. A similar tendency was noted in our patients. However, a 40-mm leg length discrepancy remained after surgery in our Growing Kotz-revised cases, resulting in a functionally unsatisfactory outcome.

We experienced 6 cases requiring revision for stem breakage. The shortest and longest operative times in the 7 revision cases were 3 h and 20 min and 6 h and 11 min, respectively, with a mean of 4 h and 7 min, and the minimum, maximum, and mean blood losses were 155, 600, and 369 g, respectively. The levels of surgical stress may have been similar to that in the first wide resection with regard to the operative time and blood loss. Tang and Sim reported the revision procedures for stem breakage [19, 20]. The goals of distal femoral revision are to cut-off the femoral bone cortex using a Surge Airtome or drill following the shape of the stem. This requires great care to avoid breakage of the cut-out bone cortex upon removal of the broken stem. A new stem must also be inserted, followed by returning the cut-off bone cortex block to its original position. Concerning reaming, we ream the femoral medullary cavity to a diameter 1 mm larger than that determined by preoperative measurement in principle. However, when the medullary cavity is narrow, reaming is performed to the stem diameter selected based on preoperative measurement. When a trial stem can be inserted, the real stem is inserted. When a trial stem cannot be inserted, over-reaming by 1 mm is performed. In revision surgery, since a thicker stem is inserted, over-reaming by 1 mm is always performed. Firm fixation is then with a cable. At this point, it is also necessary to add autologous or artificial bone grafting to assure sufficient future strength [21] (Figs. 1, 2).

Based on the above observations, stem size, shape, and porous coating serve as countermeasures against stem breakage, as does bone grafting to the bone stump and

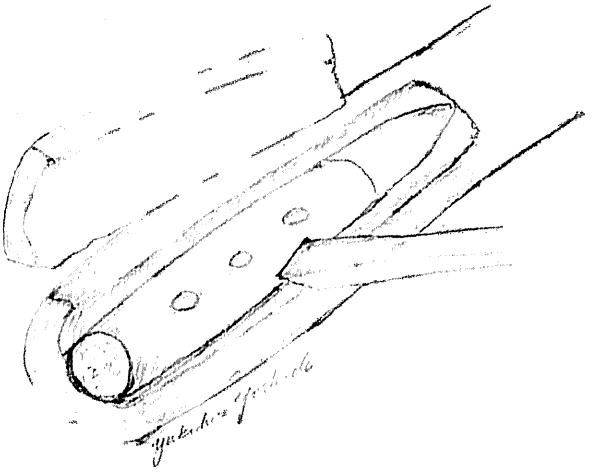

Fig. 1 The residual stem in the femur was carefully cut off using a Surge Airtome or chisel. It is important to carefully remove the broken stem because of intense bone ingrowth. Attention should also be paid to avoiding breakage of the fenestrated bone fragment and to return it to the original position after placement of the new stem

Fig. 2 After placement of the new stem, bone grafting is performed around the stem as shown. The use of a cable should also be considered for achieving stronger fixation

preservation of the periosteum [5, 11]. However, no ideal prosthesis has as yet been established, though many researchers have investigated and developed various promising models [11]. Based on this study, we consider the following points to be important for avoiding prosthetic stem breakage: (1) Minimizing the length of bone resected, i.e., it is desirable not to exceed one third of the affected bone by employing a limited operation, and (2) selection of a stem diameter of at least 12 mm. For the femur, the use of a

curved stem should be investigated in consideration of the anatomical shape of this bone.

Regarding limb salvage for malignant bone tumors in children, an elongation-type prosthesis can be lengthened to correspond to the predicted leg length discrepancy, when employed for wide resection of a periarticular tumor including the joint [22–30]. A characteristic of the elongation-type Growing Kotz is the porous coating on the diaphysis-fixing piece of the elongation region. In contrast, non-porous processing is added to the non-elongation region. However, this structure may create susceptibility to loosening. When a pediatric patient grows more than expected, particularly, in the transverse axis of the proximal tibia, loosening and burying of the stem start and slowly progress. This ultimately, compresses the bone cortex. Although the Growing Kotz can be elongated with growth, the prosthetic design, particularly the width of the tibial component, should be sufficiently investigated in consideration of the child's development.

Conclusion

Prosthesis use facilitates the early acquisition of stable functioning of the affected limb, but several complications have yet to be overcome. Breakage and loosening necessitate revision in some cases. Methods considered to reduce the risk of prosthesis breakage, include limiting resection of the affected bone to no more than 30% and adoption of as thick a stem as possible, i.e., with a diameter of at least 12 mm, fitting the anatomical shape of the femur. The unchanged function of the affected limb after revision and instructing of patients to avoid excessive exercise in daily activities are also important for maintenance of prostheses. Although only the elongation-type of Growing Kotz is covered by the national health insurance system in Japan, this prosthesis should also be selected with care, taking the child's future development into consideration.

Conflict of interest None of the authors has received any type of support, benefits, or funding from any commercial party related directly or indirectly to the subject of this article.

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Clinical report

Total femur replacement for Ewing's sarcoma after wide resection of the proximal femur: a long-term follow-up case study

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Background: Total femur replacement is a relatively rarely performed procedure for the reconstruction of an affected limb after resection of a malignant bone tumor.
Objective: Report total femur replacement in a 17-year-old male patient after wide resection of the right femur for involvement of the proximal segment of the bone by Ewing's sarcoma.
Results: The complications that often arose from the use of the tumor prostheses after the tumor resection, e.g., infection and migration/dislocation of the artificial bonehead, were overcome successfully. The patient has been under follow-up for a relatively long period of time (16 years) since the surgery. The operated limb function is now rated at 70% according to the rating system by Musculo-Skeletal Tumor Society (MSTS). The patient has almost completely regained his ability to walk and carries on with activities of daily living.
Conclusion: If appropriate measures are taken to deal with the complications, favorable function of the operated limb can be expected to be maintained for long periods after reconstruction using this technique.

Keywords: Total femur replacement, Ewing's sarcoma, complications

Total femur replacement is a relatively rarely adopted procedure from among the methods available for reconstruction of the affected limb after resection of a malignant bone tumor [1-6]. We performed total femur replacement in a 17-year-old male patient after wide resection of the right femur for involvement of the proximal segment of the bone by Ewing's sarcoma. The patient overcame complications that often arose from the use of the prosthetic joints after the tumor resection, e.g., infection and migration/dislocation of the artificial bone head. We report a case of the patient who has been under follow-up for a relatively long period of time (16 years) since the surgery.

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Case report

Around September 1991, a 17-year-old male adolescent was hit by a ball in the right leg near the hip joint and consulted with Bone-Setter. At that time, he was treated under the diagnosis of a pulled/torn muscle. Subsequently, he occasionally complained of pain around the right hip when walking. On September 17, 1992, the pain around the right thigh intensified, and he presented to our department. A plain X-ray revealed marked periosteal reaction, primarily at the diaphysis of the right femur. Based on a suspicion of malignant bone tumor, he was admitted to Department of Orthopedic Surgery, Nihon University Hospital on the same day for further examination and treatment.

On September 24, 1992, an incisional biopsy was performed under general anesthesia. Histopathological examination of the biopsy specimen revealed the diagnosis of Ewing's sarcoma. Then, the patient received preoperative chemotherapy using the RosenT-11 protocol (**Fig.1**).

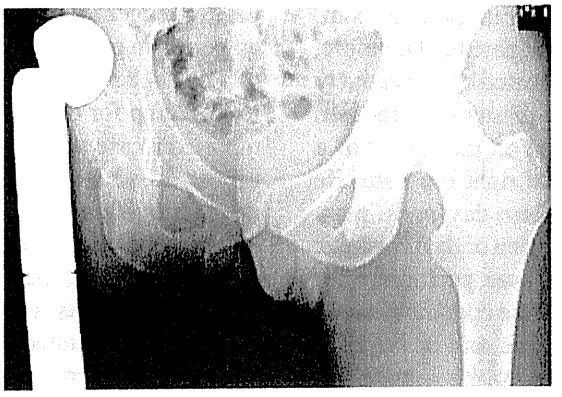

Fig. 1 Rdiographs of the dislocaton of the tumor prosthesis.Bipolar head is dislocated above the acetabulum.

On August 11, 1993, wide resection + total femur replacement was performed. During the operation, the straight muscle of the thigh was preserved, with its continuity from the proximal to the distal segment retained completely. The femur was resected en masse (along with the gluteus medius muscle and tensor muscle of the fascia lata in the proximal region and the lateral great muscle, 2/3 of the intermediate great muscle, and 1/3 of the medial great muscle in the distal region) (wide resection). The operated limb was reconstructed with a total femur prosthesis. The apparatus used for the reconstruction was the Howmedica modular resection system (HMRS). The proximal femur component (120 mm) was bound to the distal femur component (120 mm) with a connection piece (60 mm) and a stem extension piece (100 mm). A bipolar femoral head with a cup diameter of 52 mm was used. Postoperatively, chemotherapy was administered, again using the T-11 protocol.

On January 12, 1994, the patient was discharged from the hospital, wearing a long leg brace and using a T-shaped walking stick. The subsequent course was uneventful, with no evidence of local recurrence or metastasis during the follow-up. Around May 1997, a cystoma-like swelling was noted in the region of the right greater trochanter, suspected to be caused by bursitis.

In October 2002, a yellow- brown exudate was aspirated in large amounts from the cystoma-like part of the greater trochanter. Culture of the exudate grew methicillin-resistant *Staphylococcus aureus* (MRSA). Thus, a diagnosis of prosthetic joint infection caused by MRSA was made. On October 16, one-stage revision was performed. Then, after debridement, revision of total femur replacement was performed, while retaining the tibial component (**Fig. 2**). The local infection subsided thereafter, and the patient followed a favorable clinical course.

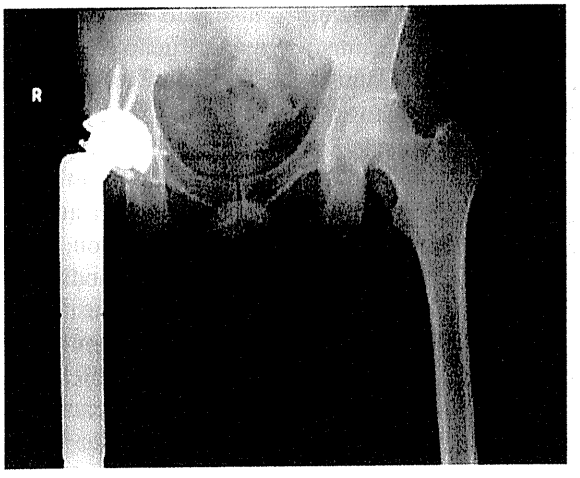

Fig. 2 Because the patient also complained of hip instability, installation of the acetabular component was carried out for revision total femur replacement.

On October 23, 2006, he fell in the bathroom, sustaining a fracture of the right patella. Therefore, he was hospitalized again and treated conservatively with the part immobilized by a plaster of Paris cast. On August 27, 2007, he slipped on the carrier of a truck and began to complain of right hip pain. He visited our department on the same day, and a plain X-ray revealed posterior dislocation of the prosthetic bonehead. Emergency surgery was performed for open reduction. Two weeks after the operation, a long leg brace was given for the patient, and he began to receive walk-training. He stayed at the hospital for about one month.

On July 16, 2009, 16 years after the first operation, he became aware of pain again in the right hip. At that time, hematological tests revealed evidence of inflammatory reaction, and a cystoma-like lesion was again seen in the region of the trochanter. However, culture of tissue specimens obtained from the swelling grew no bacteria. At the same time, a plain X-ray revealed migration of the bipolar head towards the acetabular side, and the patient complained of hip instability. On July 30, 2009, debridement was performed, and installation of the acetabular component was carried out for revision total femur replacement. In addition, a rectus abdominal myocutaneous pedicle flap was used to repair the defect, primarily in the region of the greater trochanter. The acetabular component used was the constraint type (cup diameter: 54 mm). The range of active movement of the hip joint was 20 degrees for abduction, 10 degrees for adduction, 10 degrees for external rotation, 10 degrees for internal rotation, and 65 degrees for flexion. The range of active motion of the knee joint was 30 degrees for flexion and 0 degree for extension. The strength of the quadriceps muscle of the thigh was rated at 3 by Manual Muscle Testing.

Assessment by the Musculo-Skeletal Tumor Society rating system (MSTS) yielded the following results: pain, 5; function, 3; emotional acceptance, 2; supports, 5; walking, 3; and gait, 3 (total, 70%). At present, 12 months after the operation, the patient is free of signs of local infection and hip instability. He can walk with the assistance of a T-shaped stick.

Discussion

Total femur replacement was first reported by Buchman [2]. Only a few reports of the procedure have been published since. Case reports involving 10-year or longer follow-up are rare. The mean follow-up period in previously reported cases is in the range of 1.5 to 4.8 years [3, 8]. Kalra et al. reported cases followed up for up to 28 years [6]. Present et al. [9] reported a case of total femur replacement followed up for 35 years. Complications arising from the prosthetic joints used after tumor resection include infection, breakage, loosening, and dislocation. In our case, we dealt with these problems in a timely manner, as appropriate. As a result, it has been possible to preserve the operated limb for as long as 16 years. There is still controversy over the optimum method of preventing and treating postoperative infections, etc. In our patient, we succeeded in controlling the infection through early detection and one-stage revision [10]. It is also important to cover the prosthetic joint with a myocutaneous flap rich in blood flow [11]. Nerubay et al. [12] reported an incidence of deep infection of 7%. Kalra et al. [6] used antibiotics to treat superficial infection and performed two-stage revision to deal with deep infection. When dealing with migration of the prosthetic bone head towards the acetabular side, it seems useful to install a control- type acetabular component as soon as possible and to replace the joint completely with a prosthetic one [13]. Bone head dislocation is one of the common complications arising after total femur replacement. This seems to arise from resection of the hip joint abductors, with the entire femur serving as a long lever arm. The incidence of this complication was reported by Kalra et al. [6] and Brend et al. [7] to be 11% and 12%, respectively. As a countermeasure against dislocation, they reported that reconstruction of the hip with a prosthesis after installation of an acetabular component would be desirable [14, 15]. The functional level, as assessed by the MSTS rating system, after reconstruction by this technique was reported to be 77.3% by Schindler et al. [16], 72.6% by Kalra et al. [6], and 72% by Cristian et al. [14]. Even in cases where complications arise, satisfactory function of the operated limb can be restored in all cases by taking appropriate measures.

In conclusion, total femur replacement is one of the useful methods of reconstruction in patients with malignant bone tumors requiring complete femur resection. If appropriate measures are taken to deal with the complications, favorable function of the operated limb can be expected to be maintained for long periods after reconstruction using this technique.

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Prognostic Significance of HLA Class I Expression in Ewing's Sarcoma Family of Tumors

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Background: Ewing's sarcoma family of tumors (ESFT) is one of the most malignant groups of tumors in young people. Human leukocyte antigen (HLA) class I displays endogenously processed peptides to CD8+ T lymphocytes and has a key role for host immune surveillance. In ESFT, the investigation concerning both HLA class I expression and T-cell infiltration has yet to be reported.

Methods: Biopsy specimens from 28 ESFT patients were evaluated by immunohistochemistry with the anti-HLA class I monoclonal antibody (mAb) EMR8-5 and anti-CD8 mAb, respectively.

Results: Expression of HLA class I was negative in 10 tumors and down-regulated in 22 tumors. The status of CD8+ T cell infiltration was closely associated with the expression levels of HLA class I. ESFT patients with down-regulated or negative expression of HLA class I showed significantly poorer survival than the rest of the patients.

Conclusions: Our results suggested that CD8+ T cell-mediated immune response restricted by HLA class I might play an important role in immune surveillance of ESFT, and we revealed for the first time that the status of HLA class I expression affects the survival of the patients with ESFT. *J. Surg. Oncol.* © 2010 Wiley-Liss, Inc.

KEY WORDS: **Ewing's; sarcoma; ESFT; HLA class I; CD8; T lymphocyte**

INTRODUCTION

Ewing's sarcoma family of tumors (ESFT), consisting Ewing's sarcoma and primitive neuroectodermal tumor (PNET), represents a subset of malignant small round blue cell tumors with characteristic chromosomal translocation occurring in bone and soft tissues with a peak incidence in children and young adults [1]. Although systemic adjuvant chemotherapy has significantly improved the prognosis of patients with ESFT, disease presenting with metastatic spread or relapses after primary treatment remains incurable in the majority of cases [2,3]. This emphasizes the need for alternative treatments including immunotherapy.

Recent clinical studies have shown efficacy of immunotherapeutic strategies against various malignant tumors, where anti-tumor cytotoxic T-lymphocytes (CTL) are induced by cancer vaccination [4,5]. As anti-tumor CTL responses are elicited by the recognition of immunogenic epitopes in the context of human leukocyte antigen (HLA) class I molecules on the tumors, it is important to evaluate the status of HLA class I molecules in the ESFT tissues [6,7]. However, there is only a scarce literature available about HLA class I expression in ESFT [8].

A monoclonal antibody (mAb) against HLA class I heavy chains, EMR8-5, has been confirmed to be valid for immunohistochemistry in formalin-fixed paraffin-embedded tissues [9–12]. Using this mAb, we investigated HLA class I expression in primary lesions of ESFT patients. Furthermore, we evaluated the correlation of HLA class I expression with various clinical and histological parameters including treatment outcomes of patients and infiltration of T lymphocytes in the tumor tissues.

MATERIALS AND METHODS

The present study was approved under institutional guidelines for the use of human subjects in research and patients' specimens were analyzed after having informed written consent from the patients or their families.

Patients and Samples

Twenty-eight patients with ESFT, who had been treated in Keio university hospital between 1979 and 2009, were enrolled in this study. Follow-up period after diagnosis is 76.5 months on average (range from 10 to 275 months). Demographic data of the patients are summarized in Table I. There were 15 male and 13 female patients. The average age at diagnosis was 26.3 years (range from 1 to 70 years). Nineteen of the primary tumors arose in bone, and 9 arose in soft tissue. Fourteen tumors were located in the trunk (spine or paraspinal region in 5, chest wall in 3, and pelvis in 6 patients) and 14 tumors in the extremity. Fusion genes including EWS/FLI-1 or EWS/ERG were determined by RT-PCR in 12 out of 16 cases in which frozen biopsy specimens were available [13].

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TABLE I. Clinical Characteristics of 28 ESFT Patients

Characteristics	Number
Age	
Range (average)	1–70 (26.3)
<30 Years	21
≥30 Years	7
Gender	
Male	15
Female	13
Tumor origin	
Bone	19
Soft tissue	9
Tumor site	
Extremity	14
Trunk	14
Spine or Paraspinal region	5
Chest wall	3
Pelvis	6
Tumor size	
Range (average)	4–20 (9.4)
<10 cm	12
≥10 cm	12
Stage	
IIB[a]	22
III[b]	6
Resectability of the tumor	
Resectable	23
Unresectable	5
Treatment	
Chemotherapy + surgery + radiotherapy	14
Chemotherapy + surgery	9
Chemotherapy + radiotherapy	5
Status of surgical margins	
Adequate	17
Inadequate	11

[a]Stage IIB: high-grade extracompartmental lesion, without metastasis.
[b]Stage III: any grade, with metastasis.

Size of the tumors was measured by magnetic resonance imaging in 24 patients. According to the Enneking's surgical stage [14], all the 28 patients were stratified into 22 stage IIB patients, and 6 stage III patients. Treatment consisted of chemotherapy, surgery, and radiotherapy in 14, chemotherapy and surgery in 9, chemotherapy and radiotherapy in 5 patients. Chemotherapy regimens used are VAC [15], VACA [16], CYVADIC [17], T11 [18], IFO-THP [19], VDC-IE [20,21], New A1 [22], A3 [22], and KS1 [21]. The chemotherapy regimens (KS1 and VDC-IE) containing both ifosfamide and etoposide have been used since 1995. Radiotherapy (30-65Gy) was implemented in 19 patients with unresectable tumors or those with inadequate surgical margin. The surgical margins were adequate (amputation or resection with wide margin) in 17, and inadequate (biopsy only or resection with marginal margin or intralesional margin) in 11 patients.

Immunohistochemistry

Monoclonal antibodies used were anti-HLA class I heavy chain antibody, EMR8-5 [9–12], anti-CD4 mAb (Dako, Glostrup, Denmark) and anti-CD8 mAb (Dako, Glostrup, Denmark).

Formalin-fixed paraffin-embedded sections of biopsy specimens from 28 ESFTs were deparaffinized and then boiled for 20 min in a microwave oven for antigen retrieval. The sections were blocked with 1% non-fat dry milk and stained with a streptavidin–biotin complex (Nichirei, Tokyo, Japan) as previously described [23]. The sections were then stained with hematoxylin. Positive reactivity of the EMR8-5 antibody was confirmed by staining of vascular endothelial cells and lymphocytes in sections of tumor specimens. Sections of a normal testis obtained from an autopsy specimen were used as an external negative control for immunostaining.

The reactivity of EMR8-5 was determined by staining of the plasma membranes of tumor cells. The expression status of HLA-class I was graded semiquantitatively. We classified 28 cases into three groups, high (positive cells ≥50%), low (50% > positive cells ≥5%) and negative (5% > positive cells) (Fig. 1).

Infiltrations of T cells into tumor tissues were also evaluated by semiquantitative scoring on a scale of +++ (diffuse infiltration), ++ (moderate infiltration), + (scattered or mild infiltration), and − (negative or rare) (Fig. 2).

Statistical Analysis

Disease-free survival and overall survival rates were estimated using the Kaplan–Meier plots. Both survivals were calculated from the date of initial treatment. A terminal point of disease-free survival was defined at the time of disease recurrence or progression, onset of a secondary neoplasm, death from disease, or the last review. A terminal point of overall survival was defined as the time of death or the last review. Prognostic significance of following variables on disease-free survival and overall survival was determined by univariate analysis using log-rank test [24]: age, gender, origin of tumor, tumor site, stage, surgical margin, chemotherapy regimen, status of class I HLA expression, status

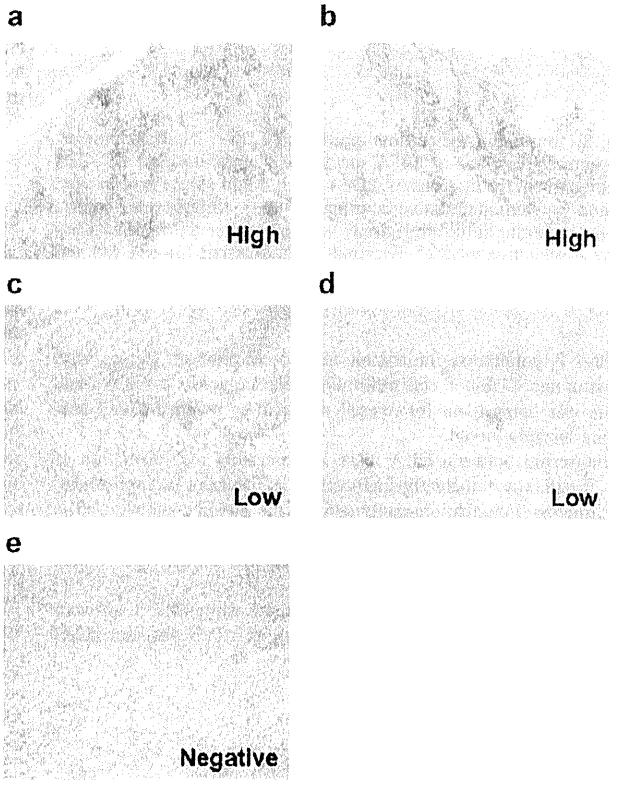

Fig. 1. Immunohistochemical grading of tumor specimens. Representative sections of ESFT specimens stained with the anti-HLA class I mAb EMR-5 are shown. **a**,**b**: "High" indicates a positive cell number of over 50%. **c**,**d**: "Low" indicates a positive cell number from 5% to 50%. **e**: "Negative" indicates that <5% of tumor cells were stained positively. Original magnification: (a), (b), (c), and (d), 200×; (e), 100×.

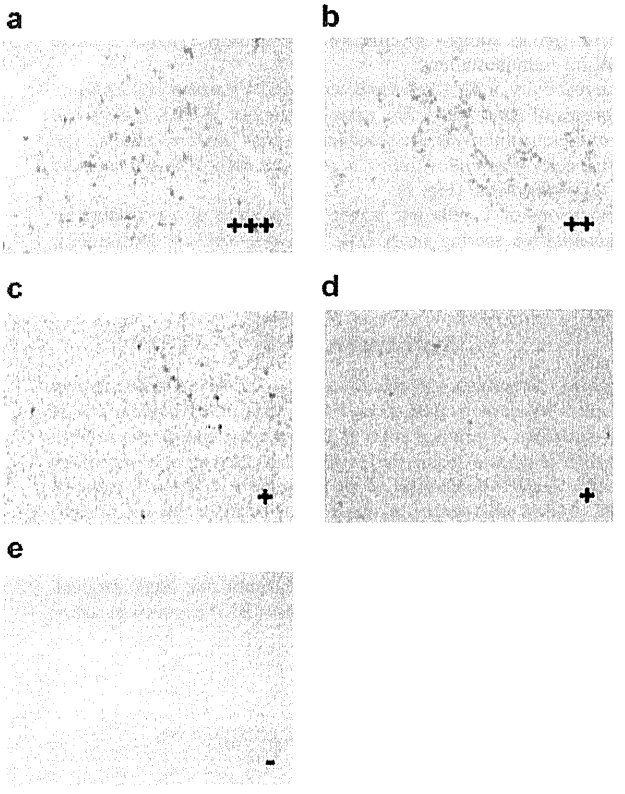

Fig. 2. Immunohistochemical grading of CD8+ T cell infiltration. Representative sections of ESFT specimens stained with an anti-CD8 mAb are shown. Infiltrations of CD8+ T cells into tumor tissues were evaluated by semiquantitative scoring. **a**: +++ (diffuse infiltration), (**b**) ++ (moderate infiltration), (**c,d**) + (scattered or mild infiltration), and (**e**) − (negative or rare). Original magnification: (a), (b), (c), and (d), 200×; (e), 100×.

of CD8+ T lymphocyte infiltration, and co-existence of HLA class I expression and CD8+ T cell infiltration. Subsequently a multivariate analysis was carried out for overall survival by using a Cox's proportional hazards model.

Relationship between HLA class I expression and infiltration of CD8+ T cells was statistically analyzed using Fisher's exact probability test. Clinicopathological characteristics of the patients showing HLA class I expression and CD8+ T cell infiltration were compared with those of the rest of the patients, and analyzed using Fisher's exact probability test.

All the statistical analyses were performed using JMP 8 software (SAS Institute Inc., Cary, NC). A probability of <0.05 was considered statistically significant.

RESULTS

Clinical Course of the Patients

Overall survival rate of whole 28 patients with ESFT was 53.1% in 5 years and 47.8% in 10 years, and disease-free survival rate was 38.5% in both 5 years and 10 years (Fig. 3). Local disease recurrence was seen in 11 cases. Distant metastasis was observed in 12 cases including 6 stage III cases. The sites of distant metastasis were lung in 9 patients, bone in 3, lymph nodes in 2 and other visceral organs in 1.

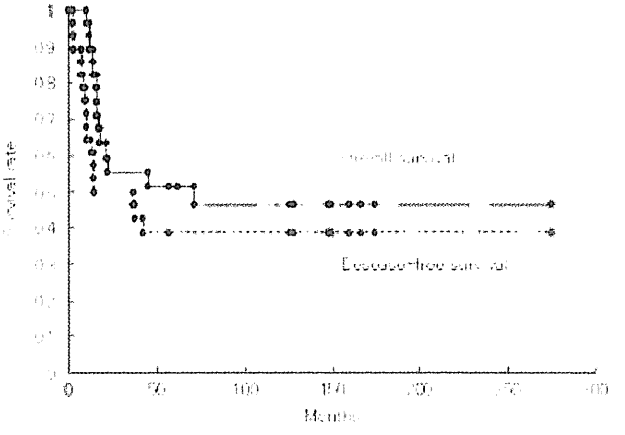

Fig. 3. Overall survival and disease-free survival of 28 patients with ESFT. Survival rates were estimated using Kaplan–Meier plots. The date of histological diagnosis was used as time 0.

Expression of HLA Class I in ESFT

To determine the phenotypic expression of HLA class I in ESFT, we stained 28 biopsy specimens of ESFT with anti-HLA class I mAb (EMR8-5). Of these, 18 specimens (64%) reacted positively with anti-HLA class I mAb where the plasma membranes of tumor cells were stained (Fig. 1). These positive cases were graded as high in 6 cases and low in 12 cases (Table II). Collectively the expression of HLA class I was lost (negative-grade expression) in 10 specimens (36%) and down-regulated (negative or low expression) in 22 specimens (79%) in ESFT.

Relationship Between HLA Class I Expression and T-Cell Infiltration in ESFTs

We then stained 28 ESFT specimens with anti-CD4 and anti-CD8 mAb. The infiltration of CD4+ T cells was seen in only two ESFT specimens (7%), whereas the infiltration of CD 8+ T cells was found in 20 specimens (71%) to various extents. Representative CD8+ T cell infiltration are shown in Figure 2. CD8+ T cell infiltration was +++ (diffuse) in 7, ++ (moderate) in 4, + (scattered or mild) in 9, and − (negative or rare) in 8 cases (Table II). The status of CD8+ T cell infiltration was closely associated with the expression levels of HLA class I ($P = 0.014$) (Table III). In the ESFT tumor tissues with positive expression of HLA class I, infiltrated CD8+ T cells tended to localize with tumor cells showing strong class I expression (Figs. 1a–c, 2a–c).

TABLE II. HLA Class I Expression and T-Cell Infiltration in 28 ESFT Specimens

High		Low	Negative
HLA class I expression			
6 (21%)		12 (43%)	10 (36%)
+++ (Diffuse)	++ (Moderate)	+ (Scattered or mild)	− (Negative or rare)
CD8+ T-cell infiltration			
7 (25%)	4 (14%)	9 (32%)	8 (29%)
CD4+ T-cell infiltration			
0	1 (4%)	1 (4%)	26 (92%)

TABLE III. Correlation Between HLA Class I Expression and CD8+ T-Cell Infiltration

	CD8+ T-cell infiltration		
HLA class I expression (n = 28)	++ or +++	− or +	*P*-value
High	5	1	0.014*
Low	5	7	
Negative	1	9	

P-value was calculated using Fisher's exact test.
*A probability of <0.05 was considered statistically significant.

Prognostic Significance of HLA Class I Expression in ESFT

Subsequently we analyzed the prognostic significance of 11 variables including expressions of HLA class I. As shown in Figure 4a, the overall survival of 18 patients with positive HLA class I expression was significantly better than that of the remaining 10 patients with negative HLA class I ($P = 0.037$). Notably, 10-year survival rate of patients with negative HLA class I was as low as 24%. In sharp contrast, all six patients with high expression of HLA class I remained alive (Fig. 4b) and continuous disease free. There were significant differences between patients with high expression of HLA class I and those with down-regulated HLA class I with respect to the overall survival (Fig. 4b; $P = 0.001$) and the disease-free survival ($P = 0.003$). Furthermore, 10 patients showing co-existence of positive HLA class I expression and moderate or diffuse CD8+ T cell infiltration had a better prognosis than the rest of the patients ($P = 0.024$) (Fig. 5). The overall survival rates of those 10 patients were 78.8% at 5 years and 10 years.

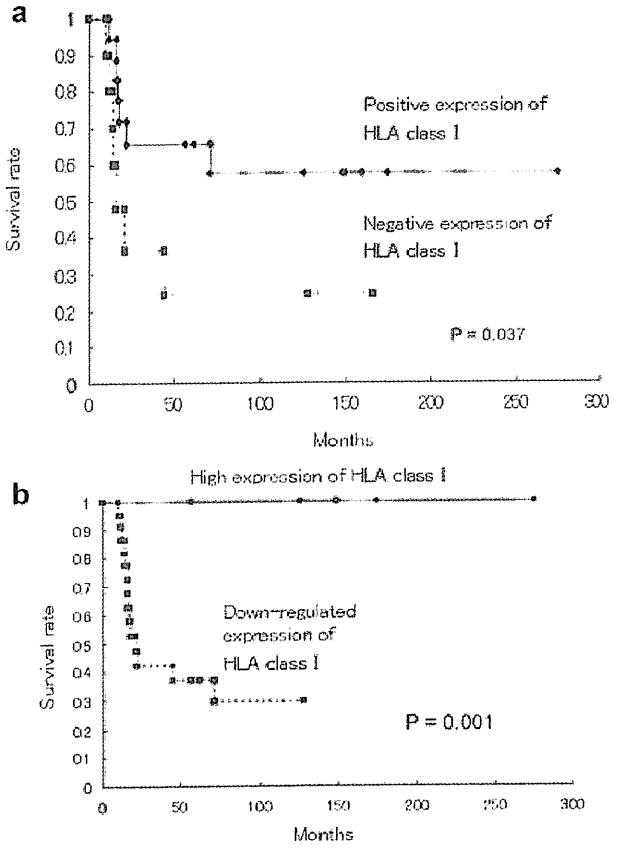

Fig. 4. Survival curves of 28 patients with ESFT stratified by HLA class I expression status. **a**: Overall survival curves. Patients were divided according to HLA class I expression status into two groups (positive (low or high) expression, n = 18; negative expression, n = 10). Survivals were estimated using Kaplan–Meier plots. *P*-value was calculated using log-rank test. **b**: Overall survival curves. Patients were divided according to HLA class I expression status into two groups (high expression, n = 6; down-regulated (negative or low) expression, n = 22).

Table IV summarizes the results of survivorship analysis. Tumor site (trunk), tumor size (≥10 cm), stage III, inadequate surgical margin, and down-regulated expression of HLA class had significant association with decreased disease-free survival. Soft tissue origin, tumor size (≥10 cm), stage III, and inadequate surgical margin, negative and down-regulated expression of HLA class I, and lack of co-existence of positive HLA class I expression and CD8+ T cell infiltration were significantly associated with decreased overall survival. None of other variables including age, gender, chemotherapy regimen, and the levels of CD8+ T cell expression showed significant association with disease-free or overall survival.

Prognostic impact of co-existence of positive HLA class I expression and CD8+ T cell infiltration was further examined by using a Cox's proportional hazards model. Given the limited sample size, the analysis included three covariate parameters in total. As shown in Table V, multivariate analysis revealed that stage III and co-existence of positive HLA class I expression and moderate or diffuse CD8+ T cell infiltration were independent prognostic factors, respectively.

Clinicopathological Characteristics of ESFTs Showing HLA Class I Expression and CD8+ T Cell Infiltration

Finally we assessed clinicopathological characteristics of 10 patients showing co-existence of positive HLA class I expression and CD8+ T-cell infiltration (Table VI). Of nine characteristics examined, tumor size (<10 cm) and absence of metastasis at the final follow-up were

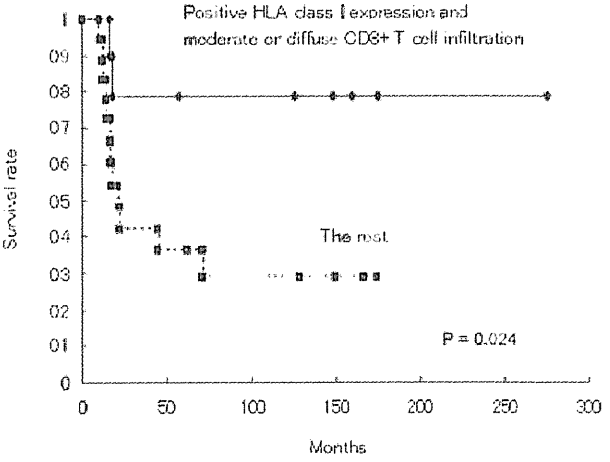

Fig. 5. Overall survival curves of 28 patients with ESFT. Patients were divided into two groups (patients showing co-existence of positive (low or high) HLA class I expression and moderate or diffuse CD8+ T cell infiltration, n = 10; patients with the other expression patterns, n = 18). Survivals were estimated using Kaplan–Meier plots. *P*-value was calculated using log-rank test.

TABLE IV. Univariate Analysis of Potential Unfavorable Prognostic Factors

Variables	Categories	No. patients	*P*-value	
			DFS	OS
Age (years old)	≥30 (vs. <30)	7	0.481	0.632
Gender	Female (vs. male)	13	0.515	0.679
Tumor origin	Soft tissue (vs. bone)	9	0.181	0.036*
Tumor site	Trunk (vs. extremity)	14	0.028*	0.237
Tumor size (cm)	≥10 (vs. <10)	12	0.020*	0.042*
Stage	Stage III (vs. stage IIB)	6	<0.001*	<0.001*
Surgical margin	Inadequate (vs. adequate)	11	0.008*	0.026*
Chemotherapy regimen	Not including IE (vs. including IE)	13	0.263	0.143
HLA class I expression	Negative (vs. positive)	10	0.132	0.037*
HLA class I expression	Down-regulated (vs. high)	22	0.003*	0.001*
CD8+ T-cell infiltration	− or + (vs. ++ or +++)	17	0.145	0.071
HLA class I/CD8+ T cell	Lack of co-existence of positive HLA class I expression and CD8+ T-cell infiltration (++ or +++)	18	0.067	0.024*

DFS, disease-free survival; OS, overall survival; IE, ifosfamide and etoposide.
P-value was determined by univariate analysis using log-rank test.*A probability of <0.05 was considered statistically significant.

significantly associated with co-existence of positive HLA class I expression and CD8+ T-cell infiltration. At the time of diagnosis, 22 patients were free from distant metastasis (Stage IIB). At the time of the final follow-up (76.5 months on average), all 9-stage IIB patients with co-existence of HLA class I expression and moderate or diffuse CD8+ T-cell infiltration remained free from metastasis. In contrast, 6 out of 13 stage IIB patients with lack of the co-existence had distant metastasis developed during the follow-up period.

DISCUSSION

By staining 28 biopsy specimens of ESFT treated in a single institute, we found down-regulation of HLA class I molecules in 79%, infiltration of CD4+ T cells in 7% and infiltration of CD8+ T cells in 74% of the primary tumors. Subsequent clinicopathological analysis revealed that down-regulation of HLA class I molecules was significantly associated with poor CD8+ T-cell infiltration, poor overall and event-free survival. Furthermore co-existence of positive HLA class I expression and moderate or diffuse CD8+ T-cell infiltration served as an independent significant favorable prognostic factor, and was associated with small tumor size and lack of having metastasis develop during follow-up. These findings indicated the prognostic role of HLA class I molecules in patients with ESFT, potentially through modulation of CD8+ T cell-mediated immune surveillance.

Previously Berghuis et al. [8] investigated HLA class I expression in 61 ESFT biopsy samples. Consistent with our findings, they found down-regulated expression of HLA class I in 79% of the samples. The frequency of absent HLA class I expression was as similar as 28% in their study and 36% in the present study. In contrast, in the study by Berghuis et al. [8], there is no significant impact of HLA class I expression levels on overall or event-free survival of the patients. This analysis was made on 35 out of 61 patients with ESFT. Therefore, the process of patient's selection may have influenced the prognostic significance of HLA class I expression, even though clinical pictures of these 35 patients have not been documented. Another

TABLE V. Multivariate Analysis for Overall Survival of 28 ESFT Patients

Variables	Categories	Risk ratio (95% CI)	*P*-value
Stage	Stage III	7.79 (2.09–33.18)	0.003*
Surgical margin	Inadequate	2.51 (0.83–8.48)	0.104
HLA class I/CD8+ T cell	Lack of co-existence of positive HLA class I expression and CD8+ T cell infiltration (++ or +++)	4.81 (1.28–31.44)	0.018*

95% CI, 95% confidence interval.
P-value was determined by Cox regression analysis.
*A probability of <0.05 was considered statistically significant.

TABLE VI. Clinicopathological Characteristics of 10 Patients Showing HLA Class I Expression and CD8+ T-Cell Infiltration

Characteristics	HLA class I expression and CD8+ T-cell infiltration (++ or +++)		*P*-value
	Yes (n = 10)	No (n = 18)	
Age (years)			
<30	7	14	0.491
≥30	3	4	
Gender			
Male	6	9	0.456
Female	4	9	
Tumor origin			
Bone	8	11	0.278
Soft tissue	2	7	
Tumor site			
Extremity	5	9	0.653
Trunk	5	9	
Tumor size (cm)[a]			
<10	7	5	0.014*
≥10	1	11	
Stage			
Stage IIB	9	13	0.277
Stage III	1	5	
Surgical margin			
Adequate	6	11	0.632
Inadequate	4	7	
Chemotherapy regimen			
Including IE	6	9	0.456
Not including IE	4	9	
Metastasis at final follow-up			
No	9	7	0.011*
Yes	1	11	

IE, ifosfamide and etoposide.
P-value was calculated using Fisher's exact test.
[a]Tumor sizes were evaluated in 24 patients.
*A probability of <0.05 was considered statistically significant.