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Microsatellite alterations in various sarcomas in Japanese patients

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Abstract: To determine the usefulness of microsatellite analysis in the differential diagnosis of various sarcomas, we investigated microsatellite alterations at 12 microsatellite loci by polymerase chain reaction and electrophoresis in 39 Japanese patients with sarcomas. The sarcomas were: osteosarcoma, Ewing's sarcoma, chondrosarcoma, liposarcoma, leiomyosarcoma, epithelioid leiomyosarcoma, rhabdomyosarcoma, synovial sarcoma, and malignant fibrous histiocytoma. We also examined ten leiomyomas to contrast with leiomyosarcoma. No microsatellite instability (MSI) or loss of heterozygosity (LOH) were found in Ewing's sarcoma, chondrosarcoma, epithelioid leiomyosarcoma, malignant fibrous histiocytoma, and leiomyoma. Only three patients, one each with liposarcoma, leiomyosarcoma, and synovial sarcoma, manifested MSI, whereas, osteosarcoma, liposarcoma, leiomyosarcoma, rhabdomyosarcoma, and synovial sarcoma manifested LOH, with an incidence of 43%, 14%, 86%, 20%, and 75%, respectively. Interestingly, three patients showed unusual patterns of LOH, probably due to intratumoral heterogeneity. Kaplan-Meier analysis revealed that LOH on 11p was predictive of poor prognosis in osteosarcoma. The low incidence of MSI indicates that MSI is not necessary for neoplastic transformation in sarcomas. However, the very high incidence of LOH in leiomyosarcoma indicates that microsatellite analysis may serve for the differential diagnosis of leiomyosarcoma versus leiomyoma. Microsatellite analysis may also predict prognosis in osteosarcoma.

Key words: bone and soft tissue tumors, mesenchymal tumors, microsatellite instability, loss of heterozygosity

Introduction

Microsatellites are short tandemly repeated DNA sequences scattered throughout the genome. Microsatellite instability (MSI), which is manifested as the occurrence of replication errors (RERs), is a molecular marker of genetic instability, reflecting germline mutations in the mismatch-repair (MMR) genes. MSI was initially described in sporadic[8,21] and hereditary colorectal tumors, and it was established that up to 86% of tumors in hereditary non-polyposis colorectal cancer (HNPCC) exhibit instability at one or more microsatellite loci.[1,2] In HNPCC kindreds, MSI was detected not only in most carcinomas but also in many of their precancerous adenomas,[2] indicating that MSI may be responsible for neoplastic transformation in HNPCC families. MSI was subsequently identified in nonneoplastic tissue of patients with germline mutations in MMR genes,[12] suggesting that MSI may be responsible for early genomic changes and that additional alterations may be necessary for malignant transformation.

MSI has been detected in many neoplasms, and it has been reported that MSI may be responsible for tumorigenesis in some neoplasms.[3,18,27] However, some neoplasms rarely manifest MSI, and whether their molecular genetic background is similar to that of HNPCC is unclear; they may represent as yet unclarified mechanisms of genetic instability different from the one seen in HNPCC.

MSI has also been detected with a very low frequency in bone and soft tissue tumors. To our knowledge, there have been only six reports of MSI in mesenchymal tumors; 2 of 18 soft tissue sarcomas manifested MSI among 12 loci examined;[28] 2 of 6 leiomyosarcomas manifested MSI in at least 1 of 9 loci examined;[16] 18 chondrosarcomas manifested no MSI among at least 10 loci examined;[15] 29 bone tumors manifested no MSI among 5–11 loci examined;[19] 22 giant cell tumors of bone manifested no MSI among 6 loci examined;[17] 14 of 26 embryonal and 1 of 6 alveolar rhabdomyosarcomas manifested MSI in at least 1 of 57 loci examined.[23] These reports suggest that MSI was unlikely to be a major

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component in bone and soft tissue tumorigenesis. It has also been suggested that MSI is a secondary phenomenon, possibly due to the loss of genes involved in MMR.[23]

Loss of heterozygosity (LOH) has been shown to be an important prognostic factor in a variety of malignant neoplasms. LOH of 13q (RB gene) and 17p (p53) was reported to be a poor prognostic factor in osteosarcoma,[6,25,30] but in other mesenchymal tumors, the relationship between LOH and the clinical course has not been examined sufficiently.

In the present study, we retrospectively analyzed the occurrence of microsatellite alterations such as MSI and LOH in mesenchymal tumors, in particular in bone and soft tissue tumors, to address the questions of (i) whether MSI plays an important role in tumor progression, (ii) whether LOH of microsatellite loci has any relationship with the clinical course, and (iii) to determine whether these molecular features aid in the differential diagnosis of various sarcomas or aid in distinguishing between benign and malignant tumors.

Subjects and methods

Sample acquisition

We obtained 85 specimens from 47 Japanese patients with sarcomas, retrieved from the files of Kagawa Medical University from April 1984 to August 1996, and one postmortem examination in July 1997. Twenty specimens were excluded from this study because DNA extraction was unsuccessful despite repeated attempts, probably due to prolonged formalin fixation or decalcification. Therefore, in this study we examined 65 specimens obtained from 39 patients; 7 patients with osteosarcoma, 3 with Ewing's sarcoma, 2 with chondrosarcoma, 7 with liposarcoma, 7 with leiomyosarcoma, 1 with epithelioid leiomyosarcoma, 5 with rhabdomyosarcoma, 4 with synovial sarcoma, and 3 with malignant fibrous histiocytoma. The specimens were classified as primary tumors ($n = 31$), locally recurrent tumors ($n = 14$), and distantly metastatic tumors ($n = 20$); the details are summarized in Table 1. The samples of ten patients with leiomyoma operated from July 1996 to February 1997, were also examined to contrast with leiomyosarcoma. All of the tumors and paired normal tissues were obtained from paraffin-embedded blocks, except for two metastatic tumors of one patient (OS[osteosarcoma]6) from whom half of each metastatic tumor specimens were frozen and stored at −50°C and the remaining half were formalin-fixed and paraffin-embedded after postmortem examination. Normal tissues were taken from the lymph nodes or other organs as controls wherever possible.

DNA Extraction

More than two sections were obtained at 4- to 8-μm thickness for each paraffin-embedded specimen. The thin section was stained with hematoxylin/eosin to serve as a guide, then microdissection was performed with the thicker section to separate the tumor from normal tissue. Sections 1-mm-thick were obtained from two frozen metastatic tumors. Each specimen was incubated in lysis buffer (1 ml 10 mM Tris-HCl [pH 8.3], 50 mM KCl, 2.5 mM $MgCl_2$ and 0.45% Tween 20) containing 10 mg/ml proteinase K at 55°C, all day long with shaking. After the incubation, proteinase K was inactivated at 95°C for 15 min. Each sample was then pelleted in a microfuge and 3 μl of the supernatant fluid was used for polymerase chain reaction (PCR).

Microsatellite markers

D6S459 (6), D6S460 (6), D9S104 (9p), D9S51 (9p), D9S66 (9q), D11S554 (11p), D11S905 (11p), D11S912 (11q), D11S916 (11q), D11S935 (11p), D17S261 (17p), and D17S785 (17q) were chosen as microsatellite markers to compare with previous studies of various tumors such as rhabdomyosarcoma (e.g., D9S51, D9S66, D9S104, D11S554),[23] neurofibromas (e.g., D11S905),[11] hereditary multiple exostosis (e.g., D11S554, D11S905, D11S916, D11S935),[7,29] skin cancers (e.g., D17S261, D17S785),[13,14] leukemia (e.g., D17S261),[24] and breast cancers (e.g., D11S554),[22] or because of close linkage to the *Cbfa1* gene, which is essential for osteoblast differentiation and bone development (e.g., D6S459).[10]

Polymerase chain reaction (PCR) amplification

PCR was carried out under standard conditions; 30 μl total volume, with 3 μl DNA supernatant fluid described above, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 200 μM each dideoxynucleotide, 0.75 units AmpliTag Gold DNA polymerase (Perkin Elmer, Foster, CA, USA), and 0.5 μM each primer (Research Genetics, Huntsville, AL, USA). Using the Program Temp Control System PC-800 (Astec, Fukuoka, Japan), reactions were started at 95°C for 10 min, followed by 10 cycles at 95°C for 50 s, 55°C for 40 s, and 72°C for 120 s, then followed by 25–30 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 90 s. Each sample was processed through 35 cycles, but some samples needed confirmation by repeated PCR amplification of both 35 and 40 cycles because of the ambiguity of the first results. All positive specimens were duplicated for confirmation by repeated PCR amplification.

Data analysis

All PCR products from the tumors and corresponding normal specimens were loaded in parallel on 3%–4%

Table 1. Summary of patients with various sarcomas and leiomyoma

Patient no.	Age (years)	Sex	Primary site	Normal tissue	Specimen[a]	Outcome (months of follow-up)
OS 1	13	Male	Femur	Amputation neuroma	P M	Dead, 23
OS 2	41	Male	Femur	Muscle	P	Dead, 16
OS 4	14	Male	Tibia	Muscle	P M	Alive with metastasis, 56
OS 5	14	Male	Femur	Lymph node	P	Alive, no evidence of disease, 35
OS 6	16	Male	Femur	Lymph node	P M1 M2	Dead, 14
OS 7	14	Female	Fibula	Lymph node	P M	Dead, 9
OS 8	47	Female	Retroperitoneum	Lymph node	M	Alive, no evidence of disease, 76
EWS 1	26	Male	Spine, extradural	Ligament	P R	Dead, 26
EWS 2	17	Male	Os coxae	Lymph node	P	Dead, 7
EWS 4	15	Female	Spine, extradural	Gastric mucosa	R	Dead, 24
CS 1	26	Male	Femur	Lymph node	P	Alive, no evidence of disease, 124
CS 2	44	Male	Os ilium	Lymph node	P	Alive, no evidence of disease, 76
LPS 1	47	Female	Thigh	Muscle	P	Alive, no evidence of disease, 136
LPS 2	50	Male	Thigh	Muscle	R M	Alive, with metastasis, 193
LPS 4	71	Male	Inguen	Lymph node	P	Alive, no evidence of disease, 50
LPS 5	56	Male	Popliteus	Lymph node	R	Alive, no evidence of disease, 47
LPS 6	70	Female	Elbow	Gastric mucosa	R1 R2	Alive, no evidence of disease, 42
LPS 7	73	Female	Thigh	Lymph node	P R	Alive, no evidence of disease, 42
LPS 8	61	Male	Forearm	Skin	P	Alive, no evidence of disease, 37
LMS 1	40	Male	Lower leg	Lymph node	P R	Alive, no evidence of disease, 124
LMS 2	54	Male	Lower leg	Lymph node	P	Alive with metastasis, 57
LMS 3	59	Male	Duodenum	Lymph node	P	Dead, unrelated causes, 28
LMS 4	48	Female	Uterus	Urethral mucosa	R	Dead, 16
LMS 5	64	Female	Duodenum	Endometrium	P R1 M1 M2 R3	Dad, 45
LMS 7	82	Female	Stomach	Gastric mucosa	P	Dead, 4
LMS 8	41	Female	Uterus	Vermiform appendix	M1 M3 M4 M5 M6	Dead, 84
ELMS 1	44	Female	Duodenum	Lymph node	P M1 M2 R	Alive, no evidence of disease, 45
RMS 3	79	Male	Thigh	Muscle	P	Unknown
RMS 4	16	Male	Anus	Muscle	P M	Dead, 11
RMS 5	1	Male	Lung	Lymph node	P	Dead, 44
RMS 6	56	Female	Urinary bladder	Lymph node	P	Alive, no evidence of disease, 74
RMS 7	23	Male	Scrotum	Gastric musocsa	M1 P M2	Dead, 45
SS 1	6	Male	Knee	Patella	P	Alive, no evidence of disease, 145
SS 2	39	Female	Buttock	Endocervix	R	Alive with metastasis, 48
SS 4	27	Male	Elbow	Muscle	P	Alive, no evidence of disease, 32
SS 5	37	Male	Knee	Lung	P	Dead, 13
MFH 1	81	Female	Popliteus	Muscle	P M	Dead, 6
MFH 2	83	Female	Thigh	Lymph node	P	Unknown
MFH 3	77	Female	Upper arm	Lymph node	P R	Alive with recurrence, 92
LM 1	48	Female	Uterus	Endometrium	P	
LM 2	44	Female	Uterus	Nasal mucosa	P	
LM 3	31	Female	Uterus	Skin	P	
LM 4	35	Female	Uterus	Ovary	P	
LM 5	53	Female	Uterus	Ovary	P	
LM 6	43	Female	Uterus	Vermiform appendix	P	
LM 7	71	Female	Duodenum	Lymph node	P	
LM 8	42	Female	Uterus	Ovary	P	
LM 9	46	Female	Uterus	Lymph node	P	
LM 10	32	Female	Uterus	Colonic mucusa	P	

OS, Osteosarcoma; EWS, Ewing's sarcoma; CS, chondrosarcoma; LPS, liposarcoma; LMS, leiomyosarcoma; ELMS, epithelioid leiomyosarcoma; RMS, rhabdomyosarcoma; SS, synovial sarcoma; MFH, malignant fibrous histiocytoms; LM, leiomyoma; P, primary tumor; R, locally recurrent tumor; M, distantly metastatic tumor

[a] Specimens are listed in order of operations, the number beside R and M indicates the number of operations

MetaPhor agarose gels (FMC, Rockland, ME, USA) in 1 × TBE (Tris-borate-EDTA) buffer with chilling and recirculating. The fine resolution capabilities of MetaPhor agarose are ideal for resolving short tandem repeats and are compatible with those seen in polyacrylamide gels.[26] The gels were visualized by ethidium bromide staining after the electrophoresis and photographed on an ultraviolet (UV) light box. In addition to the agarose gels, some PCR products were also loaded on 10%–20% gradient polyacrylamide gels, which were visualized by silver staining to compare with MetaPhor agarose gels. The visual analysis of tumor and normal patterns was carried out by at least two independent observers. LOH was scored as significantly decreased in intensity of one allele relative to the other, as determined from comparison of tumor and normal patterns. MSI was scored as extra bands in both informative and uninformative loci.

Statistical analysis

Survival was measured in months from the date of initial therapy to the date of death or last follow-up. All surgical resections of primary and locally recurrent tumors were considered curative rather than palliative. Kaplan-Meier analysis was used to estimate survival stratified by presence of LOH. Survival was measured in months after initial therapy, and the Cox-Mantel test was used to compare survival between the different groups of patients. P values of < 0.05 were considered statistically significant.

Results

No MSI or LOH were found in Ewing's sarcoma, chondrosarcoma, epithelioid leiomyosarcoma, malignant fibrous histiocytoma, and leiomyoma. In one patient each with liposarcoma, leiomyosarcoma, and synovial sarcoma, MSI was manifested in 1 of 12 loci examined (Fig. 1). By contrast, some patients with osteosarcoma, liposarcoma, leiomyosarcoma, rhabdomyosarcoma, and synovial sarcoma manifested LOH in at least one locus, with an incidence of 43% (3/7 patients), 14% (1/7 patients), 86% (6/7 patients), 20% (1/5 patients), and 75% (3/4 patients), respectively (Table 2). Overall, the incidence of MSI was 8% (3/39 patients) and that of LOH was 36% (14/39 patients) in the various sarcomas. The details of osteosarcoma, liposarcoma, leiomyosarcoma, rhabdomyosarcoma, and synovial sarcoma are described below.

Osteosarcoma (OS)

No patient manifested MSI, whereas three patients (5 specimens) manifested LOH (Table 2). LOH was found in D11S554 (3 patients) and D11S912 (1 patient), but was not found in D6S459, which is linked closely to the *Cbfa1* gene, which is essential for osteoblast differentiation and bone development.

The three patients who manifested LOH were all dead within 24 months after the initial therapy. Of the four patients who did not manifest LOH, one was dead within 24 months. Kaplan-Meier analysis revealed that

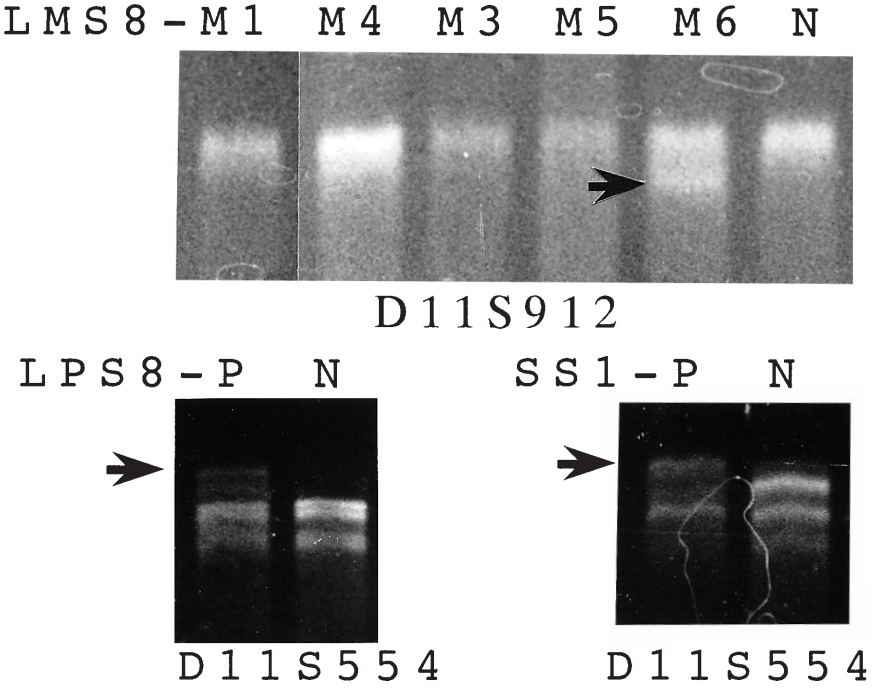

Fig. 1. Three patients, with liposarcoma (*LPS8*), leiomyosarcoma (*LMS8*), and synovial sarcoma (*SS1*) manifested microsatellite instability (MSI), *indicated by arrows*. Two primary tumors in LPS8 and SS1 manifested MSI, and SS1 manifested both MSI and loss of heterozygosity (LOH) at the same time, whereas LMS8 which had metastasized six times, manifested MSI in only one metastatic tumor (*M6*) taken from the last operation. *M*, metastatic tumor; *N*, normal tissue; *P*, primary tumor

Table 2. Microsatellite alterations in sarcomas

Specimen	LOH[a]	MSI[b]	Specimen	LOH[a]	MSI[b]
OS 1-P	**1/6**	0	LPS 1-P	0/6	0
OS 1-M	0/6	0	LPS 2-R	0/8	0
OS 2-P	0/9	0	LPS 2-M	0/8	0
OS 4-P	0/9	0	**LPS 4-P**	**2/8**	0
OS 4-M	0/9	0	LPS 5-R	0/11	0
OS 5-P	0/10	0	LPS 6-R1	0/8	0
OS 6-P	0/10	0	LPS 6-R2	0/8	0
OS 6-M1	**1/10**	0	LPS 7-P	0/7	0
OS 6-M2	**2/10**	0	LPS 7-R	0/7	0
OS 7-P	**1/5**	0	**LPS 8-P**	0/10	**1**
OS 7-M	**1/5**	0			
OS 8-M	0/7	0			
			LMS 1-P	0/8	0
			LMS 1-R	**1/8**	0
RMS 3-P	**2/8**	0	**LMS 2-P**	**1/7**	0
RMS 4-P	0/10	0	**LMS 3-P**	**1/6**	0
RMS 4-M	0/10	0	LMS 4-R	0/7	0
RMS 5-P	0/6	0	LMS 5-P	0/9	0
RMS 6-P	0/7	0	LMS 5-R1	0/9	0
RMS 7-M1	0/8	0	**LMS 5-M1**	**1/9**	0
RMS 7-P	0/8	0	LMS 5-M2	0/9	0
RMS 7-M2	0/8	0	LMS 5-R3	0/9	0
			LMS 7-P	**3/7**	0
			LMS 8-M1	**1/9**	0
SS 1-P	**2/6**	**1**	**LMS 8-M3**	**1/9**	0
SS 2-R	**3/9**	0	**LMS 8-M4**	**1/9**	0
SS 4-P	0/7	0	**LMS 8-M5**	**1/9**	0
SS 5-P	**1/9**	0	**LMS 8-M6**	**1/9**	**1**

Positive specimens are shown in bold type
LOH, Loss of heterozygosity; MSI, microsatellite instability
[a] number of loci with LOH/informative loci
[b] number of loci with MSI

LOH in D11S554 (11p) was predictive of poor prognosis ($P < 0.05$) (Fig. 2).

Of the three patients who manifested LOH, two showed unusual patterns of LOH (Fig. 3). In one patient (OS1), the primary tumor manifested LOH in D11S554 but the metastatic tumor did not. In the other patient (OS6), one metastatic tumor (M1) did not manifest LOH but the other (M2) did, although these two metastatic tumors were excised from different organs at the same time at postmortem examination.

Liposarcoma (LPS)

One patient (LPS8) manifested MSI, in D11S554 (Fig. 1). Another patient (LPS4) manifested LOH (Table 2) in D9S104 and D11S905, and this patient was alive without disease, so that we found no relation between LOH and survival.

Leiomyosarcoma (LMS)

Only one specimen (LMS8-M6) manifested MSI in D11S912 (Fig. 1); this patient was operated six times for metastases to intestine. MSI was found only in the specimen taken from the last operation, although the incidence of LOH was the same in all the specimens (1/9) and did not increase on retrospective examination (Table 2).

By contrast, LOH was found in D6S459 (1 patient), D6S460 (1 patient), D9S104 (1 patient), D11S554 (3 patients), D11S905 (1 patient), and D11S935 (1 patient), with a very high incidence of 86% (6/7 patients) or 63% (10/16 specimens). We could not find a definite

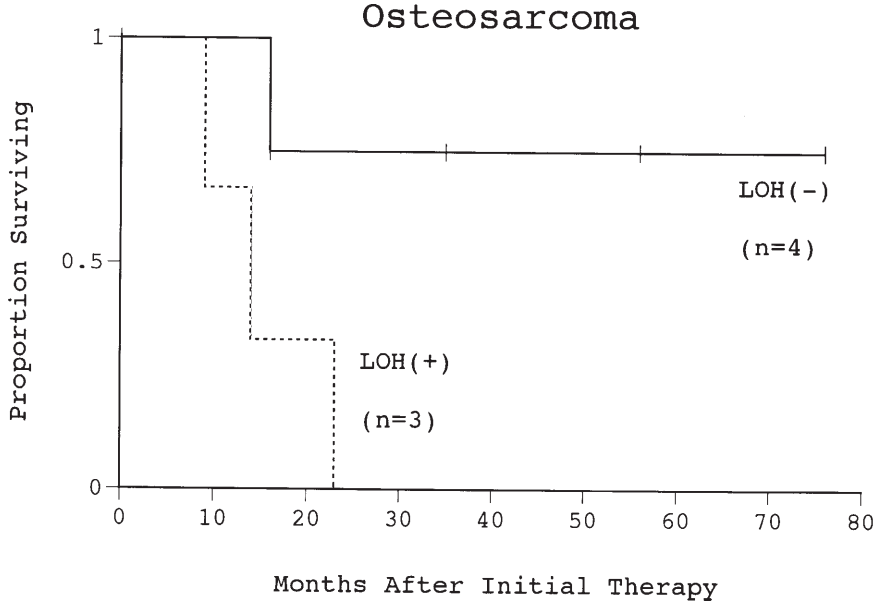

Fig. 2. All three patients with osteosarcoma who manifested LOH were dead within 24 months after initial therapy. By contrast, of the four patients who did not manifest LOH, only one was dead within 24 months. Kaplan-Meier analysis revealed that LOH of microsatellite loci was predictive of poor prognosis ($P = 0.048$)

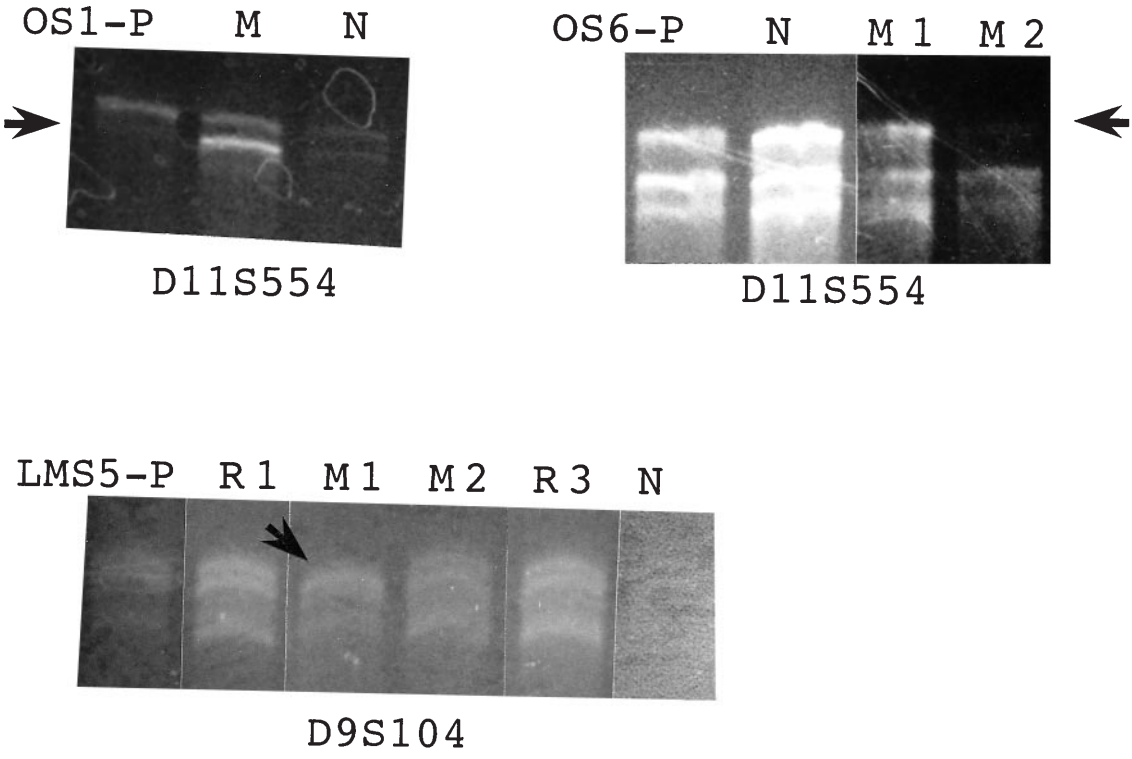

Fig. 3. In two of the three patients with osteosarcoma who manifested LOH (*OS1* and *OS6*) and one of the patients with leiomyosarcoma who manifested LOH (*LMS5*) unusual patterns of LOH were shown (*indicated by arrows*), probably due to intratumoral heterogeneity. In OS1, P manifested LOH but M did not; in OS6, M1 did not manifest LOH, but M2 did; In LMS5, M1 manifested LOH but the other tumors did not

correlation between LOH and survival, but one patient (LMS7) who manifested LOH in many loci died earliest of all patients after initial therapy.

One patient (LMS1) showed an unusual pattern of LOH; the primary tumor did not manifest LOH but the recurrent tumor did. Another patient (LMS5) also showed an unusual LOH pattern; one metastatic tumor (M1) showed LOH but five specimens of primary, recurrent, and metastatic tumors did not (Fig. 3). These patterns were similar to those of osteosarcoma.

Rhabdomyosarcoma (RMS)

MSI was not found in any of these patients' specimens. One patient (RMS3) manifested LOH in D11S935 and D17S785, but he dropped out of follow-up and no further investigation was possible.

Synovial sarcoma (SS)

One patient (SS1) manifested both MSI and LOH in D11S554 at the same time (Fig. 1) and only LOH in D6S459. In addition to this patient, two other patients manifested LOH in D11S554 (2 patients), D11S912 (1 patient), and D11S935 (1 patient). Of these three patients with LOH, one died of this sarcoma and two were alive for more than 48 months, so we could not find a definite correlation between LOH and survival.

Discussion

Since the multistep tumorigenesis theory has been generally accepted for many neoplasms, at least two different mechanisms of tumorigenesis have been described from the aspect of germline mutations; loss of tumor suppressor genes plays an important role in tumorigenesis, while defective MMR genes result in an increased mutation rate. The latter mechanism has been extensively described in HNPCC kindreds.[1,2,8,21] With this mechanism, RERs may be early events in tumorigenesis and MSI may be responsible for neoplastic transformation.

However, most previous studies detected MSI at a very low frequency in mesenchymal tumors, so they suggested that MSI was unlikely to be a major component in the development of mesenchymal tumors.[15,17,19,28] A study that detected MSI in rhabdomyosarcoma with a significant correlation to increased LOH suggested that

MSI was a secondary phenomenon, possibly due to the loss of genes involved in MMR.[23]

In this study, we also detected MSI with a low incidence, of 8% (3/39 patients) in various sarcomas, suggesting that MSI is not necessary for neoplastic transformation, but may be a secondary phenomenon. Among the three patients with MSI, the specimens of two patients, with liposarcoma and synovial sarcoma, respectively, were primary tumors, which did not recur anywhere, so we could not study them retrospectively. In the remaining one patient, with leiomyosarcoma, the tumor had metastasized six times, and manifested MSI in only one metastatic tumor taken from the last operation, without correlation to increased LOH; the incidence of LOH was the same in all the specimens. Therefore, it is likely that MSI may be a "byproduct" of tumor progression rather than a secondary phenomenon in some sarcomas.

We found that the sarcomas we examined manifested LOH of microsatellite loci at an incidence of 36% (14/39 patients). There was no unique locus of LOH which corresponded to a particular type of sarcoma, so we found no benefit of this molecular analysis in the differential diagnosis of various sarcomas. However, chromosomal arm 11p was the most frequently affected of the several arms we examined and this tendency was marked in osteosarcoma, leiomyosarcoma, and synovial sarcoma, suggesting that chromosomal arm 11p may harbor a tumor-suppressor gene or genes associated with the development or progression of some sarcomas.

Although the incidence of LOH varied among the sarcomas we tested, LOH was most frequently detected in leiomyosarcoma; six of seven patients (10 of 16 specimens) manifested LOH. By contrast, ten patients with leiomyoma manifested no MSI or LOH. The criteria for diagnosing leiomyosarcoma versus leiomyoma are not clear-cut. Although standard criteria for the differential diagnosis focus on the number of mitoses expressed per ten high-powered microscopic fields, mitotic activity alone is a poor predictor, and additional parameters, such as size, cellularity, atypia, and necrosis, are necessary for the diagnosis.[4,5,9] From the aspect of molecular biology, γ-smooth muscle isoactin gene expression was recently reported to be a unique molecular marker for the differential diagnosis.[20] Now, we propose that microsatellite analysis also serves as a novel molecular parameter for the differential diagnosis of leiomyosarcoma versus leiomyoma.

Interestingly, three patients showed unusual LOH patterns; the primary tumor manifested LOH, but the metastatic tumor did not in a patient with osteosarcoma; in another patient with osteosarcoma, one of the two metastatic tumors excised at the same time manifested LOH but the other did not; in a patient with leiomyosarcoma one of the five specimens, including primary, recurrent, and metastatic tumors, manifested LOH and the others did not. Intratumoral heterogeneity is well known and these results suggest that a mutator phenotype detected by LOH of microsatellite loci represents one of a number of heterogeneous tumor clones in these patients. In addition to these three patients, another patient with leiomyosarcoma showed an unusual LOH pattern; the primary tumor did not manifest LOH, but the recurrent tumor did. This also may be due to intratumoral heterogeneity.

We also examined whether LOH of microsatellite loci had any relationship with the clinical course. In this study, there was no apparent correlation between LOH and survival, except for osteosarcoma, in which LOH in D11S554 (11p) was probably a predictor of poor prognosis. Previous studies reported that LOH of the *RB* gene (13q) or p53 (17p) was a poor prognostic factor in osteosarcoma.[6,25,30] We also associate LOH of microsatellite loci on chromosomal arm 11p with poor prognosis in osteosarcoma. Now, further studies with a larger number of microsatellite loci, including 13q and 17p, are underway.

In conclusion, MSI is not necessary for neoplastic transformation and it may be a "byproduct" of tumor progression in some sarcomas. However, microsatellite analysis may be useful for investigating intratumoral heterogeneity. It is possible that microsatellite analysis could play an important role not only in the differential diagnosis of leiomyosarcoma versus leiomyoma but also in the prediction of prognosis in osteosarcoma.

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