Loss of Heterozygosity at 12q14-15 Often Occurs in Stage I Soft Tissue Sarcomas and Is Associated with *MDM2* Amplification in Tumors at Various Stages

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Few studies have investigated the loss of heterozygosity and microsatellite instability in soft tissue sarcomas. Therefore, we analyzed samples of human soft tissue sarcomas to determine the status of the chromosomal region 12q14–15, which contains the *MDM2* gene encoding the well-known counterpart of the tumor suppressor p53. In addition, we determined whether an amplified *MDM2* gene was present in the samples. Of the 88 soft tissue sarcoma samples, 24 (27%) showed evidence of loss of heterozygosity of markers representing 12q14–15, and 12 (14%) showed evidence of microsatellite instability. Of the 72 samples analyzed by semiquantitative polymerase chain reaction, 15 (21%) possessed an amplified *MDM2* gene. Loss of heterozygosity ($P = .008$) and microsatellite instability ($P = .035$) were significantly more common in Stage I tumors than in higher stage tumors. This result indicated that these alterations occur early in soft tissue sarcoma progression and possibly define a subgroup of soft tissue sarcoma. Surprisingly, *MDM2* amplification in soft tissue sarcoma patients was associated with a prognosis better than that of patients without the amplification; however, this difference was not statistically significant ($P = .6$). Furthermore, of the tumors with an *MDM2* amplification, 40% (6/15) also experienced loss of heterozygosity at 12q14–15; in contrast, only 16% of tumors without an *MDM2* amplification (9/57) underwent a loss of heterozygosity. A concomitant occurrence of deletions and amplifications resulting from deficiencies in the nonhomologous end-joining pathway could in part explain this finding.

KEY WORDS: Gene amplification, Loss of heterozygosity, *MDM2*, Microsatellite instability, Nonhomologous end joining, Soft tissue sarcoma.

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The chromosomal region 12q13–q15 is often affected by translocations and amplifications in soft tissue sarcoma in humans (1–9). This region includes several genes that appear to be important in tumorigenesis: cyclin-dependent kinase 4 (*CDK4*), sarcoma-amplified sequence (*SAS*), glioma-associated oncogene homolog (*GLI*), C/EBP-homologous protein (*CHOP*; same as *DDIT3* and *GADD153*), and the mouse double minute 2 (*MDM2*) (1, 3, 10). The most studied gene in this region is likely *MDM2*; the well-known counterpart of the MDM2 protein is the tumor suppressor p53. *MDM2* was first localized to 12q13–q14 by analysis of human-hamster somatic cell hybrids by Oliner and coworkers (11). The location of *MDM2* was refined by fluorescence *in situ* hybridization to 12q14.3-q15 distal to CDK4 (12 and Fig. 1).

Several reports have described *MDM2* amplification in soft tissue sarcoma (8, 9, 13). Interestingly, the rate of *MDM2* gene amplification in gliomas has been correlated with the loss of heterozygosity at loci proximal and distal to the amplification site (4).
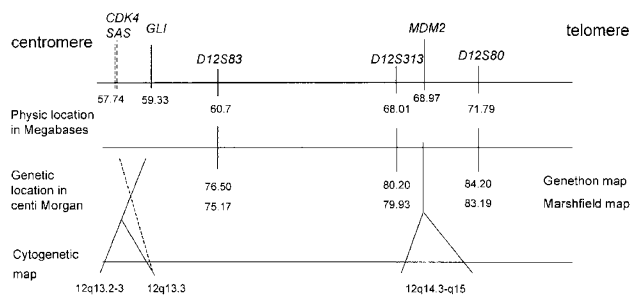
FIGURE 1. Location of the microsatellite markers and *MDM2*. Location of *CDK4* gene and *SAS* gene is crossing the location of *GLI* gene between the physical location and the cytogenetic map. Therefore, it is marked by a **dashed line.**

However, only one report has described the occurrence of loss of heterozygosity and microsatellite instability in 12q13–22 in soft tissue sarcoma, and the microsatellite markers that were investigated were not close to the *MDM2* locus (14). Therefore, we have performed studies to investigate the occurrence of MSI and loss of heterozygosity at 12q14–15 and their relationship to *MDM2* amplification in soft tissue sarcoma. The microsatellite markers used in our study were *D12S80* and *D12S83*, which flank *MDM2*, and *D12S313*, which resides between *D12S80* and *D12S83*.

MATERIALS AND METHODS

Patients' Characteristics and Soft Tissue Sarcoma Samples

This study involved 88 tumor samples from 88 adult patients who had soft tissue sarcoma and were treated between 1992 and 2000 at the Surgical Clinic 1, University of Leipzig, Leipzig, Germany, and at the Institute of Pathology, Georg-August-University in Göttingen, Germany. These tumor samples have been partially described in previous studies (15, 16). The tumor samples were obtained from 65 primary tumors and 23 local recurrences. Of these 88 samples, 24 were liposarcomas, 20 were malignant fibrous histiocytomas, 18 were leiomyosarcomas, 7 were synovial sarcomas, 6 were neurogenic sarcomas, 4 were fibrosarcomas, 4 were rhabdomyosarcomas, 2 were neuroblastomas, and 3 were other types (Table 1). The patients with primary tumors did not receive chemotherapy or radiotherapy. Of the 23 cases of local recurrences, 15 patients received radiotherapy, 19 patients received chemotherapy, and 14 received a combination of radio- and chemotherapy. All patients gave written informed consent to participate in the study.

Polymerase Chain Reaction for Detection of Microsatellite Instability and Loss of Heterozygosity at 12q14–15

We used a modified approach based on protocols described in www.ncbi.nlm.nih.gov/genome/sts (17). The polymerase chain reaction (PCR) mixture consisted of 1× PCR buffer, MgCl$_2$ (final concentrations: 2.5 mM when *D12S83* was analyzed, 1.5 mM when *D12S80* was analyzed, and 1.25 mM when *D12S313* was analyzed), 25 pmol of each of the appropriate primers (Table 2), 2.5 mM each dNTP, and 0.5 U *Taq* DNA Polymerase (Promega; Mannheim, Germany). The conditions for PCR included an incubation step at 95° C for 5 minutes and subsequently 35 to 40 cycles in which each cycle consisted of denaturation at 95° C for 1 minute, annealing at 53° C (*D12S313*) and at 55° C (*D12S83*, *D12S80*) for 30 seconds to 1 minute, DNA synthesis at 72° C for 1 minute, and elongation at 72° C for 5 to 8 minutes. The PCR products were run in 8% polyacrylamide gels that were subsequently silver stained as previously described (18). Results were considered uninformative when the normal tissue was homozygous for the marker of interest. Microsatellite mutations were defined by the presence of novel bands after PCR amplification of DNA from tumors; these bands were not present in products obtained by amplification of DNA from corresponding normal tissue. Loss of heterozygosity was indicated by a loss of a band or by a band whose intensity was very weak. Loss of heterozygosity was also indicated by the stronger intensity of a band representing one allele and the concomitant loss of the band representing the other allele (*i.e.*, gain and loss) (19).

Semiquantitative PCR for detection of amplified *MDM2*

Semiquantitative multiplex PCR was performed according to the method of Biernat *et al.* (20) but with a few modifications (8). In brief, we used primers for the *MDM2* gene and for the prothrombin gene to amplify specific sequences within 100 ng of genomic DNA extracted from the tumor samples and peripheral blood leukocytes of healthy volunteers (Table 2). The PCR mixture consisted of 1× PCR reaction buffer, 2.5 mM MgCl$_2$, 25 pmol of each primer pair, 200 μM each dNTP, and 2.5 U of HotStarTaq DNA Polymerase (Qiagen, Hilden, Germany). Thirty cycles of PCR were performed; each cycle consisted of a denaturation step at 95° C for 30 seconds, an annealing step at 58° C for 30 seconds, and a synthesis step at 72° C for 2.5 minutes. The PCR products were separated by electrophoresis on a 1.5% agarose gel, and the gel was stained with ethidium bromide. The stained gel was scanned, and the band

TABLE 1. Summary of Clinicopathologic Results and Percentages of Soft Tissue Sarcoma Samples that Experienced Loss of Heterozygosity, Microsatellite Instability at 12q14–15 or _MDM2_ Amplification

		Number of Tumor Samples with			
	Total	Microsatellite Instability (%)	Loss of Heterozygosity (%)	Gain (%)	_MDM2_ Amplification (%)
Total	88	12 (14)	24 (27)	3 (3)	15/72 (21)
sex					
Female	47	6 (13)	14 (30)	1 (2)	6/41 (15)
Male	41	6 (15)	10 (24)	2 (5)	9/31 (29)
Primary or recurrent tumor					
Primary tumor	65	10 (15)	19 (29)	3 (5)	10/51 (20)
Recurrence	23	2 (9)	5 (22)	0	5/21 (24)
Tumor type					
Liposarcoma	24	4 (17)	10 (42)	1 (4)	9/20 (45)
MFH	20	3 (15)	5 (25)	1 (5)	2/16 (12)
Leiomyosarcoma	18	3 (17)	6 (33)	1 (6)	2/14 (14)
Synovial sarcoma	7	0	2 (29)	0	0/6
Neurogenic					
Sarcoma	6	1 (17)	1 (17)	0	1/4 (25)
Fibrosarcoma	4	1 (25)	0	0	1/4 (25)
Rhabdomyosarcoma	4	0	0	0	0/4
Neuroblastoma	2	0	0	0	0/1
Others	3	0	0	0	0/3
Tumor grade					
I	21	5 (24)	10 (48)	0	6/16 (38)
II	37	5 (14)	7 (19)	3	5/31 (16)
III	30	2 (7)	7 (23)	0	4/25 (16)
Tumor stage					
I	17	5 (29)	9 (53)	0	4/14 (29)
II	32	4 (13)	6 (19)	3 (9)	7/29 (24)
III	23	2 (9)	5 (22)	0	1/23 (4)
IV	16	1 (6)	4 (25)	0	3/6 (50)
Tumor size					
T1	12	3 (25)	5 (42)	0	0/8
T2	76	9 (12)	19 (25)	3 (4)	15/64 (23)
Lymph node involvement					
N0	85	12 (14)	24 (28)	3 (4)	15/69 (22)
N1	3	0	0	0	0/3
Metastases					
M0	78	12 (15)	23 (29)	3 (4)	13/66 (20)
M1	10	0	1 (10)	0	2/6 (33)
Follow-up					
Alive	53	6 (11)	16 (30)	3 (6)	9/43 (21)
Dead	35	6 (17)	8 (23)	0	6/29 (21)
Av. length of observation in months (range)	36 (4–120)	37 (35–120)	44 (14–120)	32 (20–52)	26 (11–38)
Av. survival period in months (range)	23 (2–96)	37 (11–80)	29 (4–48)	—	37 (4–96)

Av., average.

TABLE 2. Primers Used for Multiplex Polymerase Chain Reaction and Semiquantitative Polymerase Chain Reaction

Target Microsatellite/Gene	Sequence of Primer	Reference
_D12S83_	sense 5′ TTT TTG GAA GTC TAT CAA TTT GA 3′	17
	antisense 5′ TAG CAG AGA AAG CCA ATT CA 3′	
_D12S313_	sense 5′ CAT CNC CAA TCT CTC CTC AGT 3′	17
	antisense 5′ CCA AAC TCA TTG ATG TCT TTA TT 3′	
_D12S80_	sense 5′ CCA GCC TGG AAT GAT ATG TA 3′	17
	antisense 5′ GAA TGT CAA TGG ACC AGA TG 3′	
MDM2	sense 5′-GCTGACTATTGGAAATGCAC-3′	37
	antisense 5′-ATTGGTTGTCTACATACTGGGC-3′	
Prothrombin	sense 5′-TCATCCTCAGTCCTAATGC-3′	8
	antisense 5′-AGACCCCAAGAAAGAAATGG-3′	

intensity was measured by densitometry (Image-Master VDS 3.0 Software; Amersham Pharmacia, Freiburg, Germany). Finally, we calculated the ratio of the intensity of the _MDM2_ bands to the intensity of the prothrombin band. The prothrombin (trombin) gene is located on chromosome 11p11–q12, which should not interfere with chromosomal location of mdm2 gene. We considered any ratio >3 to indicate amplification of the _MDM2_ gene. Genomic DNA extracted from peripheral blood leukocytes of healthy volunteers served as a control.

Statistical Analyses

We evaluated the association between tumor stage and the presence of microsatellite instability or loss of heterozygosity by using the χ^2 test, under the assumption that their percentage distribution in each tumor stage was equal. In Kaplan-Meier analyses, the relationship between survival and the presence of MSI, loss of heterozygosity, or gene amplification was evaluated. Statistical analyses were performed with SPSS 10.0 software (SPSS, Inc., Chicago, IL).

RESULTS

Analysis of Loss of Heterozygosity and Gains at 12q14–15

Of the 88 soft tissue sarcoma samples that we analyzed, 24 (27%) showed loss of heterozygosity at 12q14–15, and 3 (3%) showed a gain of DNA in that region. Loss of heterozygosity occurred in 42% (10/24) of liposarcomas, 33% (6/18) of leiomyosarcomas, 29% (2/7) of synovial sarcomas, 25% (5/20) of malignant fibrous histiocytomas, and 17% (1/6) of neurogenic sarcomas (Tables 1 and 3; Fig. 2). No loss of heterozygosity at 12q14–15 was found in rhabdomyosarcoma (4 cases investigated), fibrosarcoma (4 cases), neuroblastoma (2 cases), or other sarcomas whose classification could not be determined (3 cases). Gains of DNA at 12q14–15 were detected in only three tumor samples: a liposarcoma, a leiomyosarcoma, and a malignant fibrous histiocytoma (Table 1, Fig. 2). The percentage of Stage I tumors with a loss of heterozygosity (53%) was significantly higher than that of Stage II tumors (19%), Stage III tumors (22%), and Stage IV tumors (25%; $P = .008$; χ^2 test). In the Kaplan-Meier analysis, the median survival period for patients whose soft tissue sarcoma was a site of loss of heterozygosity at 12q14–15 (58 mo) was shorter than that for patients with unaffected soft tissue sarcoma (68 mo).

TABLE 3. Soft Tissue Sarcoma Samples that Experienced Loss of Heterozygosity, Microsatellite Instability in 12q14–15 or *MDM2* Amplification

Sample	Type	Primary (P) or Recurrent (R)	Staging	*D12S83*	*D12S313*	*D12S80*	*MDM2* Amplification
LZ3	LS myxoid	R	II	0	0	0	+
LZ6	NS	R	II	0	MSI	MSI	−
LZ9	LS well diff.	P	I	0	MSI	LOH	+
LZ10	LS pleom	P	II	0	0	MSI	−
LZ14	FS	P	I	0	0	MSI	−
LZ17	LS well diff.	P	I	LOH	0	MSI	−
LZ25	LS well diff.	P	I	0	0	0	+
LZ32	FS	R	II	0	0	0	+
LZ35	LMS	R	IV	LOH	LOH	LOH	−
LZ36	LS well diff.	P	I	0	LOH	0	+
LZ37	MFH	R	IV	LOH	0	LOH	+
LZ45	MFH	R	IV	0	0	MSI	n.d.
LZ46	NS	P	II	LOH	0	0	+
LZ48	LS well diff.	P	II	0	0	0	+
LZ54	LMS	P	III	0	0	LOH	+
LZ56	LS round cell	R	IV	0	0	LOH	n.d.
LZ59	LS well diff.	P	II	0	0	LOH	+
LZ63	LMS	P	II	0	LOH	0	n.d.
LZ68	MFH	P	III	0	0	MSI	−
LZ72	MFH	P	III	LOH	0	0	−
LZ76	LMS	P	II	0	0	0	+
LZ81	LS well diff.	R	I	0	LOH	0	+
LZ82	LS myxoid	P	II	0	0	0	+
LZ85	LS myxoid	R	IV	0	0	0	+
LZ86	LS well diff.	P	I	0	LOH	LOH	−
LZ94	SS	P	II	LOH	0	0	−
LZ99	MFH	P	IV	LOH	LOH	LOH	−
LZ100	MFH	P	IV	0	0	0	+
LZ101	LMS	P	II	0	0	Gain	−
LZ102	LMS	P	III	0	0	LOH	−
G2	LMS	P	I	0	LOH	MSI	n.d.
G4	LMS	P	II	LOH	LOH	MSI	n.d.
G12	MFH	P	II	0	Gain	LOH	n.d.
G14	LS myxoid/low diff.	P	III	0	LOH	0	n.d.
G26	MFH	P	III	LOH	MSI	0	n.d.
G29	LMS	P	II	0	LOH	MSI	n.d.
G35	LS myxoid	P	I	0	MSI	LOH	n.d.
G46	LS myxoid	P	I	0	LOH	0	n.d.

P, primary tumor; R, recurrence; FS, fibrosarcoma; LS well diff, well differentiated liposarcoma; LS low diff, low differentiated liposarcoma; LS pleom, pleomorphic liposarcoma; LMS, leiomyosarcoma; MFH, malignant fibrous histiocytoma; NS, neurogenic sarcoma; SS, synovial sarcoma; n.d., not determined.
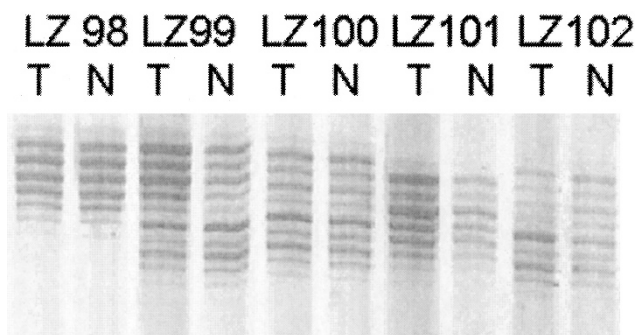
FIGURE 2. STS samples with loss of heterozygosity or gain in microsatellite marker *D12S80* visualized in a silver-stained polyacrylamide gel. LZ98 and LZ100 represent samples without changes. In Samples LZ99 and LZ102, loss of heterozygosity was detected (note a gain in intensity of **upper bands** and a loss of intensity in **lower bands** between normal tissue [N] and tumor tissue [T] in Sample LZ99). In Sample LZ101 a gain was identified.

Analysis of Microsatellite Instability at 12q14–15

We found instability at one microsatellite sequence marker in 12 (14%) of the 88 soft tissue sarcoma samples, but only in one was instability at two markers observed (Table 3). Microsatellite instability occurred in 4 (17%) of the 24 liposarcomas, 3 (17%) of the 18 leiomyosarcomas, and in 3 (15%) of the 20 malignant fibrous histiocytomas, but no MSI was found in synovial sarcoma (7 cases investigated), rhabdomyosarcoma (4 cases), neuroblastoma (2 cases), or other sarcomas whose classification could not be determined (3 cases; Table 1). The high percentages of MSI in fibrosarcomas (1 of 4; 25%) and neurogenic sarcomas (1 of 6; 17%) are based on a small number of cases investigated.

MSI occurred significantly more often in Stage I tumors (29%) than in Stage II tumors (13%), Stage III tumors (9%), and Stage IV tumors (6%; $P = .035$, χ^2 test). The Kaplan-Meier analysis showed that the median survival period for patients whose soft tissue sarcoma contained MSI at 12q14–15 was less than that for patients with unaffected soft tissue sarcoma (58 months compared with 68 months).

MDM2 Amplification

We performed semiquantitative PCR to determine whether the MDM2 gene was amplified in 72 of the 88 tumor samples. DNA from 16 tumor samples was inadequate for semiquantitative PCR. Amplification of *MDM2* was detected in 15 (17%) of the 72 samples of soft tissue sarcoma. In 45% of the liposarcomas, we found the amplification, whereas the amplification occurred in only 14% of leiomyosarcomas and 12% of malignant fibrous histiocytomas (Tables 1 and 3). The high percentage of neurogenic sarcoma and fibrosarcoma (each 25%; each 1/4) with the amplification are based on a small number of cases investigated. No amplifications were detected in samples of synovial sarcoma (6 samples), rhabdomyosarcoma (4 samples), neuroblastoma (1 sample), or other sarcomas whose classification could not be determined (3 samples). Patients whose tumors possessed an *MDM2* gene amplification had a longer median survival period (68 mo) than did those whose tumors lacked the amplification (32 mo; data not shown). There was no association between the occurrence of *MDM2* amplification and tumor stage.

DISCUSSION

The percentage of soft tissue sarcoma samples with loss of heterozygosity at the chromosomal region 12q14–15 (27%) was in the range of 3% to 57% reported for soft tissue sarcoma, with loss of heterozygosity at other chromosomal regions (6, 7, 14, 19, 21–23). However, the occurrence of loss of heterozygosity may depend on the type of tumor and the microsatellite markers that are analyzed. Loss of heterozygosity at 12q14–15 was most common in liposarcoma samples: 10 (42%) of the 24 samples were marked by loss of heterozygosity. This percentage corresponds to the findings of Schneider-Stock *et al.* (6, 7), who identified loss of heterozygosity in other chromosomal regions in 22% and 39% of liposarcoma samples in two studies. Such loss of heterozygosity was detected in myxoid liposarcoma and pleomorphic liposarcoma but not in well-differentiated liposarcoma. In contrast, six of the nine samples of well-differentiated liposarcoma in our study had undergone loss of heterozygosity at 12q14–15, and the single sample of pleomorphic liposarcoma that we investigated did not possess a loss of heterozygosity.

In the present study, loss of heterozygosity occurred in 33% of leiomyosarcoma samples (6/18), a percentage that is in within the range of other reports (22, 24). Few studies of the prevalence of loss of heterozygosity in other types of soft tissue sarcoma have been reported (14, 19, 23).

The number of soft tissue sarcoma samples in which MSI at 12q14–15 occurred (12/88; 14%) was relatively low. This finding is in accord with other reported results regarding the prevalence of MSI at all regions in soft tissue sarcoma. Therefore, it has been suggested that the occurrence of MSI in any analyzed region plays no major role in the genesis of soft tissue sarcoma and has no considerable impact on the prognosis of patients with soft tissue sarcoma (7, 23–26).

The percentage of soft tissue sarcoma samples with an MDM2 gene amplification in this study (21%) is very similar to that reported in the MDM2 amplification database (20%) (13). The amplification occurred most often in liposarcomas (45%); however, this percentage exceeds that in the data-

base (29%). This discrepancy might be due to the higher proportion of well-differentiated liposarcoma samples in our study. This subtype of liposarcoma has frequently associated with MDM2 amplification (6, 27, 28). The present paper is the first to report the presence of MDM2 amplification in leiomyosarcoma (2 samples); no other examples have yet been deposited in the MDM2 amplification database. However, in studies performed by comparative genomic hybridization, high-level amplifications of the 12q14–15 region could be found in leiomyosarcomas. Recently, in malignant fibrous histiocytoma, a subgroup was identified that carried these chromosomal aberrations, reminiscent of well-differentiated liposarcoma or leiomyosarcoma. The investigators suggested that malignant fibrous histiocytomas are a morphologic modulation in the tumoral progression of other sarcomas, particularly LMS (29, 30).

It was remarkable that the occurrence of loss of heterozygosity and of MSI was significantly more common in Stage I tumors ($P = .008$ and $.035$, respectively) than in tumors at other stages. Our result is supported by the earlier finding of loss of heterozygosity at regions other than 12q14–15 in a high number of low-grade soft tissue sarcoma of the extremities (19). This finding suggests that MSI and loss of heterozygosity at 12q14–15 may occur early in tumor development and supports the hypothesis that MSI and gene deletions are early events in tumor progression (31). The less frequent occurrence of loss of heterozygosity and MSI at 12q14–15 in tumors at Stages II, III, or IV indicates that Stage I tumors and tumors at the other stages may differ in their origin and behavior. The Stage I tumors with loss of heterozygosity and MSI at 12q14–15 may represent a separate group of the soft tissue sarcoma. Furthermore, two groups of tumors may exist: one with a diploid phenotype and a defective mismatch repair system that leads to MSI and a second group that exhibits aneuploidy, loss of heterozygosity, small mutations in tumor suppressor genes and oncogenes, and an absence of MSI (19). This hypothesis is supported by the findings of p53 mutations in the soft tissue sarcoma samples and the significant correlation between p53 mutation and poor prognosis of patients with soft tissue sarcoma (32 and our unpublished results). Of the 88 tumor samples in the present study, 50 were investigated to determine whether any p53 mutation was present. Six carried a p53 mutation (LZ1, LZ13, LZ23, LZ33, LZ35, LZ56), and the mutation was correlated with a prognosis that was worse than that of patients without the p53 mutation (data not shown).

There was a correlation between the occurrence of loss of heterozygosity at 12q14–15 and MDM2 amplification. In 6 (40%) of the 15 tumor samples with an MDM2 amplification, loss of heterozygosity at 12q14–15 occurred; however, only in 9 of the 57 tumor samples without an MDM2 amplification was loss of heterozygosity detected. Similar results concerning gliomas were reported by Reifenberger et al. (4). They found that 50% of gliomas with an MDM2 amplification also had loss of heterozygosity in 12q13–14, whereas only 16% of the gliomas without an MDM2 amplification had experienced loss of heterozygosity.

What might be the reason for a concomitant occurrence of MDM2 amplification and loss of heterozygosity at 12q14–15? Recent results concerning the nonhomologous end-joining (NHEJ) pathway may explain at least in part this phenomenon in soft tissue sarcoma. The NHEJ system repairs double-strand DNA breaks. The pathway consists of the following components: DNA ligase IV, Xrcc4, Artemis, and the DNA-dependent protein kinase (DNA-PK), which itself is composed of Ku70, Ku80, and the kinase catalytic subunit DNA-PKcs (33). Deficiencies in this pathway result in gene amplification, deletion, and translocation. Hoon Cho and coworkers (19) described the loss of heterozygosity of DNA-PK genes in low-grade soft tissue sarcoma. Moreover, the survival probability of patients with loss of heterozygosity of the DNA-PKcs gene was significantly lower than that of patients without loss of heterozygosity of this gene ($P = .016$). Our hypothesis is also supported by findings that the lack of Ku80 accelerates the formation of tumors, especially lymphomas and sarcomas, in $p53^{+/-}$ mice (34). Furthermore, mice heterozygous for the DNA ligase IV gene ($Ink4a/Arf^{-/-}$-background) develop more soft tissue sarcoma that possess clonal amplifications, deletions, and translocations than do mice homozygous for the wild-type gene (same genetic background). In 3 of the 12 $Lig4^{+/-}$ Ink4a/$Arf^{-/-}$-tumors, amplification of the Mdm2 gene was detected, but none of the eight $Lig4^+/Lig4^+$ Ink4a$^-$/$Arf^{-/-}$-tumors possessed this abnormality (33). Investigation of the components of the NHEJ system may give insight into the molecular basis of loss of heterozygosity at 12q14–15 and MDM2 amplification.

We confirmed our previously reported result that an amplification of MDM2 in soft tissue sarcoma is associated with a prognosis better than that associated with soft tissue sarcoma that lacks the amplification (8). However, this finding might be specific for liposarcoma. MDM2 amplification seems to be characteristic of well-differentiated liposarcoma (6, 27, 28), a subtype associated with a prognosis better than that associated with other subtypes of liposarcoma (35). Furthermore, Pilotti et al. (27) reported that amplicons at 12q13–22, which do not include MDM2, may contribute to transformation in non-retroperitoneal well-differentiated liposar-

coma. They support the role of CDK4 in opposing p53 function, particularly in this tumor type (27). However, the question of whether the better prognosis of soft tissue sarcoma patients with mdm2 gene amplifications is caused mostly by well-differentiated liposarcomas has to be investigated in larger patient groups.

There are also indications that the *MDM2* oncogene possesses a normal physiologic function in growth inhibition and apoptosis (36). Amplification of various genes is thought to occur late in tumor progression and might be an event secondary to deletion (31). This statement also seems true for the *MDM2* amplification, because this amplification did not occur more often in Stage I tumors than in tumors at other stages. In stages besides Stage I, *MDM2* amplification may balance the detrimental effects resulting from the loss of the *MDM2* gene that can be deleted at the beginning of tumor development. Another possibility is that *MDM2* amplification occurs as a part of an amplification of a longer chromosomal region, which can include other genes such as *CDK4*, *GLI*, *SAS*, or *CHOP* (1, 3 10). Furthermore, reports have described amplifications of the 12q13–15 region in the absence of an amplified MDM2 gene (1, 5), and the amplification of *MDM2* alone is less common than its amplification with other genes such as *SAS* or *CDK4* (1, 3, 26).

In summary, we found that loss of heterozygosity and MSI at 12q14–15 occur more often in Stage I tumors than in tumors at other stages. *MDM2* amplification in soft tissue sarcoma was associated with a longer median survival period than was the absence of *MDM2* amplification. Finally, *MDM2* amplification was associated with loss of heterozygosity at 12q14–15 in soft tissue sarcoma.

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