American Journal of Pathology, Vol. 151, No. 4, October 1997

Comparative Genomic Hybridization of Malignant Fibrous Histiocytoma Reveals a Novel Prognostic Marker

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DNA sequence copy number changes were studied by comparative genomic hybridization (CGH) along all chromosomes in 58 samples of malignant fibrous histiocytoma (MFH). The material consisted of 43 primary tumors (9 of myxoid and 34 of storiform-pleomorphic subtype), 13 local recurrences (2 myxoid and 11 storiform-pleomorphic), and 2 metastases (1 myxoid and 1 storiform-pleomorphic). Genetic aberrations, with a mean of 5.5 changes per sample (range, 0 to 22), were detected in 47 of 58 samples (81%). The minimal common regions of the most frequent gains were 1p31 (33%), 9q31 (29%), 5p14-pter (26%), 7q32 (24%), and 7p15-pter (22%). High-level amplifications were detected in 16 of the 58 samples (28%). High-level amplification of 13q31-qter was seen in four tumors (7%); other high-level amplifications were more sporadic. Losses of DNA sequences were less frequent than gains. The minimal common regions of the most common losses were 13q21 (21%) and 13q22 (21%). Statistically significant correlation was found between gain of 7q32 and the rates of worse metastasis-free survival ($P = 0.01$) and overall survival ($P = 0.004$). The gain of 7q32 retained its prognostic significance also in a multivariate analysis with tumor size and grade. Gain of 1p31 was associated with a trend to decreased overall survival. Gains of 5p14-pter and 9q31 and losses of 13q21 and/or 13q22 did not have any prognostic value; neither did the total number of aberrations, total number of gains, or total number of losses per sample. ***(Am J Pathol 1997, 151:1153–1161)***

Malignant fibrous histiocytoma (MFH) is the most frequent soft tissue sarcoma occurring in adults.[1–3] In a recent World Health Organization classification, MFH has been defined as "a pleomorphic spindle cell sarcoma usually occurring in adults and displaying no distinct line of differentiation."[4] Histologically, MFH is a heterogeneous group of sarcomas composed of a mixture of fibroblastic, histiocytic, and bizarre cells, often accompanied by variable amounts of inflammatory cells, collagen, and a myxoid substance in the stroma.[1] It is still unknown whether the tumor originates from histiocytes, fibroblasts, or undifferentiated mesenchymal cells. MFH has been divided into four subtypes.[4] The most common are the storiform-pleomorphic and myxoid subtypes. Giant cell and inflammatory subtypes are seen less frequently.[1]

Even though MFH is the most commonly diagnosed soft tissue sarcoma, relatively few comprehensive studies about prognostic factors have been published on this tumor. The strongest prognostic factors are tumor size and grade.[3,5–8] Additional prognostic information can be gained from tumor necrosis[3,9] and histological subtype.[6,8,9]

The karyotypic abnormalities in MFH are usually complex, with multiple numerical and structural rearrangements. No chromosomal aberrations specific to MFH have been identified so far, but telomeric associations, unidentified ring chromosomes, and dicentric chromosomes are frequently seen in MFH.[10,11] Cytogenetic signs of gene amplification, ie, homogeneously staining regions and double minute chromosomes, are also seen in MFH.[12,13] A 19p+ marker chromosome is a recurrent aberration in MFH.[14] This marker seems to correlate with an increased risk for local recurrence.[15] Furthermore, metastases in high-risk patients are more common with the 19p+ marker than without it.[15] The nature of the 19p+ marker is still unknown. The presence of ring chromosomes can indicate a reduced risk of relapse.[15]

Supported by the Sigrid Jusélius Foundation, the Finnish Cancer Society, and the Helsinki University Central Hospital in Finland and the National Council of Scientific and Technological Research (CONICET) and the National University of La Plata in Argentina.

Accepted for publication July 2, 1997.

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The participation of several oncogenes with potential pathogenic importance to soft tissue tumor development has been analyzed, for example, sarcoma amplified sequence gene *SAS*, human homologue of murine double minute 2 gene *MDM2*, cyclin-dependent kinase *CDK4*, and the gene for C/EBP homologous protein *CHOP* (all mapped to the 12q13-15 amplicon). All of these genes, except *CHOP*, have been shown to be amplified in more than one-third of the MFHs.[16–21] Alterations affecting tumor suppressor genes *p53* (17p13) and *RB1* (13q14) have been reported in approximately one-third of the tumors.[22,23] The prognostic significance of these gene alterations has not been determined.

We used comparative genomic hybridization (CGH) to detect gains and losses of DNA sequences in soft tissue MFH. The CGH findings were further evaluated for their possible prognostic significance.

Materials and Methods

Tumor Specimens

The material consisted of 58 samples obtained from 55 patients. The samples were collected from the files of the Pathology Laboratory, Department of Oncology, Helsinki University Central Hospital. The histological slides were re-examined. Most of the cases had already at the time of the initial diagnosis been studied by immunohistochemistry and a few also by electron microscopy. When necessary, new immunostainings were performed, especially with antibodies against cytokeratins, S-100 protein, desmin, and actins. Cases that revealed specific differentiation were excluded. Tumors displaying myxoid stroma in at least one-half of the area sampled were classified as belonging to the myxoid subtype. Tumors fulfilling diagnostic criteria for giant cell and inflammatory subtypes were not seen. A four-grade system was applied. The main criteria for grading were proliferative activity and necrosis. All tumors except one were regarded as high-grade (III and IV) tumors. None of the patients except one (sample 1, Table 1) had received chemo- or radiotherapy before the operation. The 43 primary tumors were all of high grade: 17 grade III (8 of myxoid subtype and 9 of storiform-pleomorphic subtype) and 26 grade IV (1 myxoid and 25 storiform-pleomorphic). The 13 local recurrences were of grade II (1 myxoid), grade III (1 myxoid, 4 storiform-pleomorphic), and grade IV (7 storiform-pleomorphic). The two metastases were of grade IV (one myxoid and one storiform-pleomorphic). Of the 43 primary tumors, 24 were deep and 19 were subcutaneous. Histopathological and clinical characteristics of the samples are presented in Table 1. DNAs were extracted from 42 frozen tumor samples and from 16 paraffin-embedded tissue sections.

Treatment

All patients have been consecutively treated by the Soft Tissue Sarcoma Group at the Helsinki University Central Hospital between 1987 and 1996. The main principles for treatment have been published recently.[24] Briefly, after aspiration or coarse-needle biopsy, all tumors were operated with compartmental or wide resection, if possible. If histopathological analysis showed that the margins were close or intralesional, the patients received radiotherapy with a total dose of 50 to 60 Grey depending on the achieved surgical margins. Of the 43 patients with samples analyzed from primary tumors, three had been operated intralesionally and all had received postoperative radiation. Twenty-seven patients had been operated with a marginal margin, and nineteen of these had received postoperative radiation. Twelve patients were operated with a wide margin. None of them had received postoperative irradiation. In one case the surgical margins could not be determined with certainty.

Comparative Genomic Hybridization

CGH was performed using direct fluorochrome-conjugated DNAs for all samples according to a recently reported protocol.[25] Briefly, tumor DNAs were labeled with fluorescein isothiocyanate (FITC)-dUTP (DuPont, Boston, MA), and reference DNA was labeled with Texas Red-dUTP (DuPont) by nick translation to obtain DNA fragments ranging from 600 to 2000 bp. The hybridization mixture consisted of 400 ng of labeled tumor DNA, 400 ng of labeled reference genomic DNA, and 10 μg of unlabeled Cot-1 human DNA (Gibco BRL, Life Technologies, Gaithersburg, MD) dissolved in 10 μl of hybridization buffer (50% formamide, 10% dextran sulfate, 2X SSC). Hybridizations and post-hybridization washes were carried out as reported previously.[25]

Digital Image Analysis

Hybridizations were analyzed using an Olympus fluorescence microscope and the ISIS digital image analysis system (MetaSystems Hard & Software, Altlussheim, Germany) based on an integrated high-sensitivity monochrome CCD camera and automated CGH analysis software. Three-color images (green for tumor DNA, red for reference DNA, and blue for DAPI counterstaining) were acquired from 12 metaphases for each sample. Chromosomal regions were interpreted as overrepresented when the green-to-red ratio was higher than 1.17 (gains) or 1.5 (high-level amplifications, >10-fold) and as underrepresented when the ratio was lower than 0.85 (losses). In each CGH experiment, a negative control (blood DNA from a healthy donor) and a positive control (tumor with known DNA copy number changes) were included and run in parallel. All results were confirmed using a 99% confidence interval. Briefly, intra-experiment standard deviations for every position in the CGH ratio profiles were calculated from the variation of the ratio values of all homologue chromosomes within the experiment. Confidence intervals (CIs) for the ratio profiles were then computed by combining them with an empirical inter-experiment standard deviation and by estimating error probabilities based on the *t*-distribution.

Table 1. Histopathological and Clinical Characteristics of 58 Soft Tissue MFH Samples

Sample number	Sex	Age at diagnosis (years)	Tumor type, histological subtype	Grade	Location	Size (cm)
1	M	74	P, St-pl*	IV	Lower arm	20.0
2	M	29	P, St-pl	IV	Upper arm	4.5
3	F	85	P, St-pl	IV	Lower leg	7.0
4	F	83	P, St-pl	IV	Upper arm	4.0
5	F	65	P, Myx	IV	Lower leg	26.0
6	M	60	P, St-pl	IV	Thigh	14.0
7	M	60	P, St-pl	III	Thigh	2.0
8	F	77	P, St-pl	IV	Lower leg	7.0
9	F	63	P, St-pl	IV	Thigh	18.0
10	M	61	P, St-pl	III	Shoulder	4.0
11	M	70	P, St-pl	IV	Knee	13.5
12	M	40	P, Myx	III	Upper trunk	5.0
13	F	76	P, St-pl	III	Lower trunk	2.7
14	M	35	P, St-pl	IV	Head	7.0
15	M	70	P, St-pl	III	Lower leg	12.5
16	F	79	P, St-pl	IV	Lower leg	8.0
17	F	78	P, St-pl	IV	Thigh	9.0
18	F	77	P, St-pl	III	Lower arm	5.0
19	M	77	P, St-pl	IV	Upper arm	8.0
20	M	63	P, Myx	III	Thigh	6.0
21	M	79	P, St-pl	IV	Upper arm	6.5
22	M	53	P, St-pl	IV	Lower trunk	18.0
23	F	32	P, St-pl	III	Thigh	7.0
24	F	66	P, St-pl	III	Knee	4.5
25	F	74	P, St-pl	IV	Thigh	11.0
26	F	70	P, Myx	III	Lower leg	3.0
27	F	67	P, Myx	III	Lower leg	8.0
28	F	66	P, Myx	III	Upper trunk	6.0
29	F	31	P, St-pl	IV	Shoulder	4.0
30	F	35	P, St-pl	IV	Lower trunk	9.0
31	M	81	P, St-pl	III	Lower leg	2.5
32	M	60	P, St-pl	IV	Upper arm	7.0
33	M	42	P, St-pl	IV	Thigh	15.0
34	F	54	P, Myx	III	Lower leg	1.5
35	M	65	P, St-pl	IV	Lower arm	14.0
36	M	76	P, Myx	III	Lower arm	5.0
37	M	79	P, St-pl	IV	Lower arm	5.8
38	M	81	P, St-pl	IV	Upper arm	12.0
39	M	70	P, St-pl	IV	Lower arm	7.0
40	F	80	P, St-pl	III	Lower trunk	6.0
41	F	58	P, Myx	III	Shoulder	6.0
42	M	51	P, St-pl	IV	Upper arm	8.0
43	F	84	P, St-pl	IV	Shoulder	7.7
44	F	84	R, St-pl	IV	Upper arm	NA (7.0)†
45	M	57	R, St-pl	IV	Thigh	2.2 (NA)
46	M	64	R, St-pl	IV	Lower leg	3.2 (NA)
47	F	78	R, Myx	III	Lower leg	NA (1.0)
48	F	79	R, St-pl	III	Lower arm	NA (3.5)
49	F	67	R, St-pl	III	Knee	4.5 (NA)
50	F	66	R, Myx	II	Thigh	3.5 (NA)
51	F	78	R, St-pl	III	Lower leg	1.0 (2.0)
52	M	43	R, St-pl	IV	Upper trunk	NA (5.0)
53	F	71	R, St-pl	III	Lower leg	2.5 (NA)
54	M	81	R, St-pl	IV	Upper trunk	11.0 (NA)
55	M	84	R, St-pl	IV	Lower leg	NA (5.0)
56	M	51	R, St-pl	IV	Upper arm	10.0 (8.0)
57	F	66	M, Myx	IV	Groin	10 (26.0)
58	F	79	M, St-pl	IV	NA	NA (10.0)

M, male; F, female; P, primary tumor; M, metastasis; R, local recurrence; Myx, myxoid subtype; St-pl, storiform-pleomorphic subtype; NA, not available.
*Radiotherapy given before the operation.
†For metastases and recurrent tumors, the size of the primary tumor is given in parentheses.

Statistical Analysis

Correlation between the most frequent CGH aberrations and clinical features was analyzed in 43 patients with primary tumors studied by CGH. The median follow-up time was 21 months (range, 9 months to 7 years). The association between CGH aberrations and clinical features was tested by the χ^2 test (if the number of cases in all classes was greater than five), Fisher's exact test (grade and histological subtype), or the Mann-Whitney U-test (age at diagnosis and tumor size).

Figure 1. Summary of gains and losses of DNA sequence copy number in 58 soft tissue MFH samples analyzed by CGH. Losses are shown on the left and gains on the right. Each line represents a genetic aberration seen in one sample. High-level amplifications of small chromosomal regions are shown as thick lines.

Metastasis-free and overall survival rates were estimated using the Kaplan-Meier method. The most common aberrations were studied for their association with metastasis-free survival, overall survival, and local recurrence using the log-rank test. The associations among outcome in groups with and without DNA copy number changes were evaluated with the log-rank test. To avoid the problem with multiple comparisons, statistical testing was restricted to the five most common aberrations (four most frequent gains and a combination of the two most frequent losses), and the statistical significance level was set to 0.01 in accordance with the method of Bonferroni. The proportional hazards model was used to test whether there is a linear association between the risk of death or relapse and the total number of aberrations (total number of aberrations, total number of gains, and total number of losses per tumor).

Four other factors (grade, tumor subtype, tumor size, and patient's age at diagnosis) were tested for association with local control, metastasis-free survival, and overall survival. Tumor size and patient's age were tested as continuous variables and grade and tumor subtype as dichotomous. Factors with significant association to outcome were finally combined in a multivariate Cox analysis of metastasis-free and overall survival.

Results

Overview of DNA Sequence Copy Number Changes

Of the 58 samples, 47 (81%) had changes with a mean value of 5.5 aberrations per sample (range, 0 to 22). Eleven samples did not show any aberrations. Although nonrepresentative histology is a possible reason for CGH not detecting any changes, other explanations are, eg, balanced aberrations not affecting DNA sequence copy number, aberrations beyond the resolution capacity of CGH, and intratumor heterogeneity.

The number of DNA copy number changes was higher in the group of metastases and local recurrences (mean, 6.5 ± 1.7; range, 0 to 22) than in primary tumors (mean, 4.9 ± 0.6; range, 0 to 14). Gains were three times more frequent than losses (gains:losses = 2.9:1). All chromosomal regions with an increased or decreased DNA se-

quence copy number are summarized in Figure 1. Table 2 shows the copy number karyotypes and Table 3 shows the most frequent gains, high-level amplifications, and losses.

Gains and High-Level Amplifications

The minimal common regions of the most frequent gains were narrowed down to 1p31 (33%), 9q31 (29%), 5p14-pter (26%), 7q32 (24%), 7p15-pter (22%), 1q24 (21%), and 11q14 (21%). Other minimal common regions of gains are presented in Table 3.

High-level amplifications of small chromosomal regions were detected in 16 of the 47 samples with at least one alteration in CGH (34%). The most frequent high-level amplification affected 13q31-qter (four tumors, 7%; Table 3). Other high-level amplifications, seen in two tumors each, are listed in Table 3.

Losses of DNA

The minimal common regions of the most common losses were 13q21 (21%) and 13q22 (21%), adjacent regions in 13q. Other less frequent losses are listed in Table 3.

Correlations between Changes Detected by CGH and Clinical Features or Outcome

There were no statistically significant associations between the most common gains (1p31, 5p14-pter, 7q32, and 9q31) or losses (13q21 and/or 13q22) and any of the tested clinical features (tumor grade and size, histopathological subtype, or patient's age at diagnosis).

Statistically significant correlation was found between gain at 7q32 and worse metastasis-free survival (P = 0.01) and overall survival (P = 0.004). There was also a trend to an increased risk for local recurrence (P = 0.07). The 3-year metastasis-free survival was 70 and 38%, and overall survival was 70 and 23%, in patients without and with a gain at 7q32, respectively. Local control at 3 years showed 82 and 43% of the patients without and with the gain of 7q32. Gain of 1p31 was associated with a trend to a worse overall survival (P = 0.04; 65 and 49% at 3 years in patients without and with gain of 1p31, respectively) but not with metastasis-free survival (P = 0.18) or local control (0.38). A summary of the association between gains of 1p31 and 7q32 and metastasis-free and overall survival and local control is shown in Table 4. Metastasis-free and overall survival in patients with and without the gain of 7q32 are shown in Figures 2 and 3. Gains of 5p14-pter and 9q31 and losses of 13q21 and/or 13q22 did not have any prognostic value; neither did the total number of aberrations, total number of gains, and total number of losses per sample.

No association was found between the patient's age at diagnosis or tumor subtype and metastasis-free or overall survival or local control. Histological grade was significantly (P = 0.0009) associated with metastasis-free survival (3-year metastasis-free survival was 92% *versus* 42% for grade III and IV, respectively) but not to overall survival (P = 0.12) or local control (P = 0.94). Tumor size was significantly associated with both metastasis-free survival (3-year metastasis-free survival was 37% *versus* 89% for tumors ≥7 cm (median) or <7 cm, respectively; P = 0.003) and to overall survival (3-year overall survival was 36% *versus* 91%; P = 0.005) but not to local control (P = 0.08). The results of multivariate analysis on metastasis-free and overall survival, including tumor size, grade, and gain of 7q32, are shown in Table 5.

Discussion

The present CGH analysis of soft tissue MFH revealed that 81% of the samples showed gains and/or losses of DNA sequences involving at least one but typically several different chromosomal regions. On average, the tumors had five copy number aberrations per sample, gains being more frequent than losses. The most frequent gains affected 1p31 (33%), 9q31 (29%), 5p14-pter (26%), and 7q32 (24%). High-level amplifications were observed in 34% of the samples with aberrations. Similar complexity with a nonrandom pattern of abnormalities has been seen in conventional cytogenetic studies, but the complexity often makes it impossible to interpret the karyotypes.[10–13]

Our results pinpoint several novel chromosomal regions where frequent gains of DNA sequences were observed. Gains were found in both arms of chromosome 1, affecting 1p (33%) more often than 1q (21%). Gains of DNA sequences at 1q have been frequently detected by CGH in malignant glioma,[26] bladder cancer,[27] osteosarcoma,[28] chondrosarcoma,[25] and liposarcoma.[29] The minimal common region 1q24 detected in the present study overlaps with gains of 1q in other sarcoma types. 1p31 and 8q24 were frequently affected by gains (33 and 14%, respectively). These sites harbor the oncogenes *LMYC* and *MYC*.[30]

Gain of 5p14-pter was detected in 26% of the samples with two high-level amplifications. Gains of 5p have also been reported in liposarcoma,[29] malignant gastrointestinal stromal tumor,[31] colorectal carcinoma,[32] small-cell lung cancer,[33] and squamous cell carcinoma of head and neck.[34] The target genes in the copy number increases of 5p are not known at present.

Gain of 9q31 was also a relatively frequent event (29%). In previous CGH studies of other cancers, gains in 9q are extremely rare, suggesting that this gain might be specifically linked to the tumorigenesis of MFH. On the other hand, in some cases a gain of 9q was seen with a simultaneous loss of 9p, suggesting isochromosome formation. 9p21 contains tumor suppressors $p16^{INK4A}$ and $p15^{INK4B}$, frequently inactivated in different cancers[35] and also in a subset of sarcomas.[36,37]

CGH revealed an overrepresentation of chromosome 7 in 20 of the 58 samples (34%) with two minimal common regions narrowed down to 7q32 (24%) and 7p15-pter (22%). Trisomy 7 is a frequent additional aberration in several cancers,[38] and copy number increases of both 7p and 7q have been detected by CGH in several tumor

Table 2. DNA Sequence Copy Number Changes in 58 Soft Tissue MFH Samples Analyzed by CGH

Sample number	Copy number changes
1	+1p22-pter, +5p14-pter, +7, +8q23-qter, −9p23-pter, +9q21-qter, +15q15-q24, +22
2	No changes
3	+1p21-p31, +11q14-qter
4	−1q31-qter, −2q13-qter, −10q21-qter, −11q22-qter, −13q14-qter
5	+3q24-qter, +7q21-qter, +11/**11q13-q22**, +21
6	+1q22-q25, +8q21.3-qter, +9q22-qter, +Xpter-q13/**Xp21**
7	No changes
8	+1p31-pter, +3p14-p22, +5p14-pter, +8q21.1-q22, +15q14-qter
9	+1pter-q22, −1q32-qter, −2q21-qter, +3p12-qter, +4p, +5p, +6q26-qter, −8p12-pter, −8q21.3-q23, −10p12-pter, −13q14-q21, −18q
10	No changes
11	−2p14-p16, +6q21-qter, +12p11.2-pter, −13, −18q, +19q/**19q13.2-qter**
12	No changes
13	+1p22-pter, −2q32-q36, +4p, +17q12-qter
14	No changes
15	+1p22-p31, +2pter-q35, −4q31.2-qter, +6q24-qter, +7q31-qter, +8q13-q22, +9q21-q33, +10q22-24, +12, +15q25-qter, +17
16	+4q13-q25, +5, +9q21-q32, +10q24-qter, +13q21-qter/**13q22-qter**, +15q23-qter/**15q25-26**, +17p11.2-p12
17	+1p21-p31
18	+11q13
19	+1pter-q25, +5p14-pter, +6p12-p23, +9p13-qter, −12p, +13q31-qter, +17p, +19q/**19q12-qter**
20	+7, +15q12-qter
21	No changes
22	+7q, −13
23	+1p31-pter, −2p13-p21, −2q22-qter, +3p14-q13.1/**3p13-q11.2**, −4q28-q33, +6, +8pter-q13, −11q22-qter, +13q21-qter, +14q22-qter/**14q24-q31**, +X
24	+1p13-p31, +1q24-q25, −1q41-qter, +2, +3q13.1-q25, +4p13-pter, +5p13-pter, +14q22-qter
25	+1p32-q31, +3q13.2-qter, +7, +11p11.2-pter, +11q14-qter
26	+1p21-p31, +4cen-p15.3, +4q31.1-qter, +9q21-qter, +11q14-q22, +12p12-pter, +17cen-p12
27	No changes
28	+1p34.2-pter, +1p31-q24, +5/**5p14-p15.1/5q33**, +6q22-qter, +7/**7p12-p21**, +8q, −9p, +9cen-q32, +17p, +X/**Xp22.2-q13**
29	+1q23-q25, +3p13-p24, +4p, −4q, +5p14-pter, −6cen-q23, +7p15-pter, +8q, +9, −10p, −13q21-qter, +15q21-qter, +17p, +19q
30	+1pter-q31, +2q12-q31, +4p, +5p, +7p, +8p, +9q21-q31, −11q22-qter, +13q31-qter
31	No changes
32	+7p21-q22
33	**+8**
34	−1q31-qter, −3p12-pter, −6p, −8, −9p, −10, −11q23-qter, −13q21-qter, −15cen-q15, −16q21-qter, −22, −Xq21-qter
35	+12p/**12p12-pter**
36	No changes
37	+1q22-q31, +3q22-qter, +6pter-q12, +7q22-q32, −10cen-qter, +11q14-qter, +13q22-qter/**13q31-qter**, +15q14-q22
38	+5p14-pter/**5p15.3**, +6p22-pter, −6q21-q23, +7q32-qter, +9q22-qter, +11q13-q22, +15cen-q21, +19q
39	+1q12-q24, +2p12-q22, +4p15.3-pter, +6q23-qter, +7p11.2-qter, +11p11.2-q14, −13q21-q32
40	+6q22-qter, +10q23-q25, +11
41	−1q32-qter, +7, −8p12-pter, −8q22-q23, +9q13-q31, −10q21-q22, −11q21-qter, +12p13, **13q21-qter**, +19/**19p12-p13.2**, +Xp11.2-p21
42	+4cen-p15.3, +7
43	−1p21-p22, +2p12-q22, −3q13.1-qter, +11cen-q13, −11q23-qter, −12q15-q22, −13q22-qter, +15q22-qter, +18p, −18q
44	No changes
45	No changes
46	+11q14-q22
47	+11q13-q23, +17q12-q24
48	+14q21-qter, +20q11.2-qter
49	−4q, −12p12, −13q14-q31, +17p12-q21
50	+5p13-pter, +9q21-qter, +12q13-q21, **17p**
51	+3q24-q26.3, +4q26-q31.3, +8q13-qter, +12q14-q23
52	+1pter-q31, +7p, −9p, +9q31-qter, −12p, +Xp
53	+4p, −5pter-q23, +6q22-qter, +7p, −9p21-pter, +11cen-q13, −13q14-q32, +18p/**18p11.3,** +19q, +22/**22q11.2**
54	+1p22-pter, +2p11.2-p13, +4p13-p15.3, +5p13-pter, +8q13-qter, +9q21-qter, +10p12-p14, +13q21-qter, +14q11.2-q13, +15q22-q25, +17q21-qter
55	+1p22-pter, +1cen-q25/**1q12-24**, +5p/**5cen-p14**, +6/**6p23-pter**, +7/**7p21-q11.2**, −8p12-pter, −9p21-pter, +9q, −13q14-q31, +13q32-qter, +14, +15, +16p, +17/**++17p13-q12,** +18pter-q11.2/**18p11.2-p11.3**, +22, +Xp21-pter
56	+1p31-p36.1, −1q31-qter, −2q22-q31, +3p13-p21, +4p, −4q26-qter, +5p14-pter, −5q22-qter, +7p, +7q21-qter, +8p, −8q, +9p13-pter, +9q21-qter, −10p12-pter, −11p14-pter, +11cen-q14, +12p, −13q21-qter, +18p, +20q, +22/**22q11.2-q12**
57	+11pter-q23/**++11q13-q22,** +22, +Xpter-q23
58	+1p22-p36.1, +1q21-q31, −4q31.2-qter, +5p14-pter, +6p12-pter/**6p21.1-p24**, +8q12-qter, +9q12-qter, −11q14-qter, −13q12-q22, +13q22-qter/**13q31-qter**, +15q15-qter/**15q22-q26**, +16p11.2-pter

Gains of DNA sequences are marked with + and losses with −. High-level amplifications are in bold.

Table 3. The Most Frequent Gains, High-Level Amplifications, and Losses of DNA Sequence Copy Number Detected by CGH in 58 Soft Tissue MFH Tumor Samples

Gains		High-level amplifications		Losses	
Location	Number of samples (%)	Location	Number of samples (%)	Location	Number of samples (%)
1p31	19 (33)	13q31-qter	4 (7)	13q21	12 (21)
9q31	17 (29)	5p14	2 (3)	13q22	12 (21)
5p14-pter	15 (26)	6p23-p24	2 (3)	11q23-qter	7 (12)
7q32	14 (24)	7p12-p21	2 (3)	1q41-qter	6 (10)
7p15-pter	13 (22)	11q13-q22	2 (3)	4q31.2-q33	6 (10)
1q24	12 (21)	15q25-q26	2 (3)	9p23-pter	6 (10)
11q14	12 (21)	17p	2 (3)		
4p15.3	11 (19)	18p11.3	2 (3)		
11q13	10 (17)	19q13.2-qter	2 (3)		
6q26-qter	9 (16)	22q11.2	2 (3)		
8q21.3-q22	9 (16)	Xp21	2 (3)		
13q32-qter	9 (16)				
15q22	9 (16)				
15q25	9 (16)				
17p11.2-p12	9 (16)				

Locations indicate minimal common regions.

types.[29,33,34,39–42] The minimal overlapping region 7q32 is a novel finding in MFH, and so far the target genes affected by this gain are not known.

The only chromosomal region affected repeatedly by high-level amplification was narrowed down to 13q31-qter, amplified in four tumors (7%). This region was also affected by gains with the minimal common region of 13q32-qter. Partly or fully overlapping gains or amplifications have been reported in rhabdomyosarcoma,[43] colorectal carcinoma,[32] and ovarian cancer.[44] Other high-level amplifications were of a more random nature.

Table 4. Prognostic Significance of Gains of DNA Sequence Copy Number at 7q32 and 1p31 in Soft Tissue MFH

	Gain of 7q32		Gain of 1p31	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Metastasis-free survival	3.4 (1.2–9.3)	0.01	1.9 (0.38–5.1)	0.18
Overall survival	4.0 (1.5–10.9)	0.004	2.7 (1.0–7.1)	0.04
Local control	2.9 (0.29–10.1)	0.07	1.7 (0.50–5.9)	0.38

12q13-q15 contains several oncogenes known to be amplified in sarcomas and also in MFH.[17–21,45] In the present study, gains at this region were detected in only 5% of the samples. Furthermore, no high-level amplifications were seen. It is possible that oncogenes usually involved in this amplicon, eg, *SAS*, *MDM2*, and CDK4, were activated in the tumors of the present study through mechanisms other than amplification (eg, by point mutation, transcriptional gene activation, or chromosomal translocation).[17–21,29,46,47]

Losses of DNA sequences were most frequently observed at chromosome 13, with two adjacent minimal common regions (13q21 and 13q22). Alterations of tumor suppressor gene *RB1* (13q14) are frequent findings in cancers in general and also in MFH.[23,48] 13q14 was lost only in 14% of the samples with the most common losses affecting the more distal regions of chromosome 13. However, mechanisms other than physical deletions can impair the function of *RB1*.

Previously, 16 soft tissue MFHs have been studied by CGH by Forus et al.[49] Of these 16 tumors, 9 contained increases in DNA sequence copy number. The most frequent gains were narrowed down to 7q21-q31 (25%)

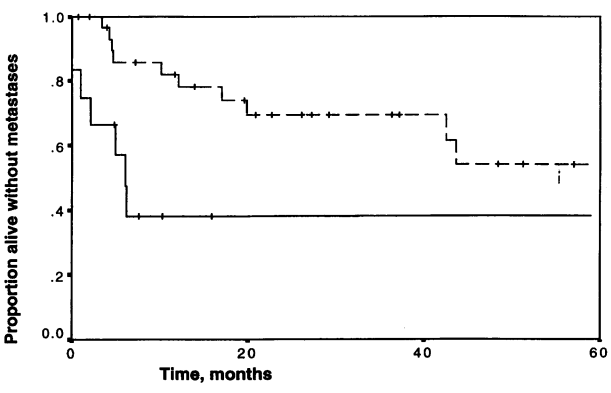

Figure 2. Metastasis-free survival in MFH patients with and without a gain at 7q32. The continuous line shows patients with a gain at 7q32, and the broken line represents patients without a gain at 7q32.

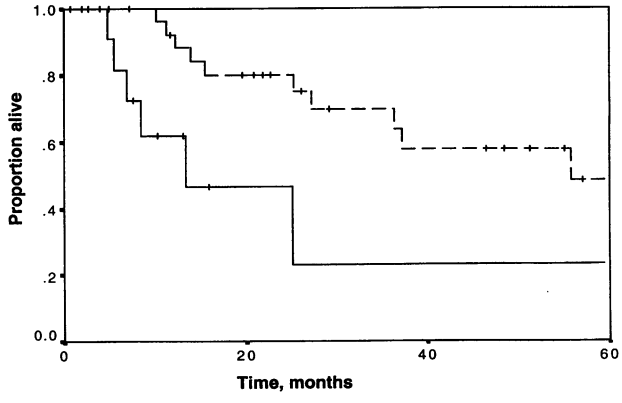

Figure 3. Overall survival in MFH patients with and without a gain at 7q32. The continuous line shows patients with a gain at 7q32, and the broken line represents patients without a gain at 7q32.

Table 5. Effect of Tumor Grade, Size, and Gain of 7q32 on Metastasis-Free and Overall Survival of Patients with Soft Tissue MFH: A Multivariate Analysis

	Metastasis-free survival		Overall survival	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
7q32 gain	3.3 (0.98–2.40)	0.05	3.7 (1.19–2.45)	0.02
Grade (III or IV)	8.9 (1.06–74.6)	0.04	1.2 (0.30–1.55)	0.81
Size (cm)	1.14 (1.03–1.26)	0.01	1.2 (1.07–1.37)	0.003

and 1q21-q22 (25%). Gain of 1p was observed in 19% of the samples.[49] All of these chromosomal regions were frequently affected by gains also in the present study but with slightly different or narrower minimal common regions.

In the present study the minimal common region of gain in 7q was q32. In statistical analysis this gain emerged as a novel prognostic marker. It was associated with a statistically significant worse metastasis-free survival ($P = 0.01$) and overall survival ($P = 0.004$) and with a trend to an increased risk for local recurrence ($P = 0.07$). It retained its prognostic significance in a multivariate analysis with the strongest clinical prognostic parameters (tumor size and grade). Gain of 1p31 was associated with a trend to worse overall survival. Total number of aberrations, total number of gains, or total number of losses per sample were not associated with outcome. Previous publications about prognostic significance of CGH findings are few. Isola et al[50] reported a correlation between total number of changes and total number of losses and outcome in breast cancer. High-level gain of 8q was significantly associated with recurrence.[50] In renal cell carcinoma, total number of losses and loss of 9p were associated with recurrence-free survival.[51] In uveal melanoma, monosomy 3 is a significant predictor of relapse-free and overall survival.[52] The findings of the present study seem to indicate that, rather than the number of genetic aberrations, specific genetic events are important for the outcome in MFH. Finally, our results show the power of CGH in screening for DNA copy number changes in tumors with complex karyotypic abnormalities.

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