

Gains of 13q are correlated with a poor prognosis in liposarcoma

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Liposarcomas are a phenotypical heterogenous group of tumors divided into four main subtypes: well-differentiated, dedifferentiated, myxoid/round cell, and pleomorphic. The aim of this study was to compare DNA sequence copy number changes of these subtypes as investigated by comparative genomic hybridization in 36 patients. Comparative genomic hybridization revealed genomic imbalances in tumors of 27 patients (mean 5.6 imbalances per tumor). The most frequent gains were within single regions of 1q, 12q, and 13q. We found a significant correlation of poor overall survival and gain of 13q21 ($P=0.0221$), 13q22 ($P=0.0341$), 13q31 ($P=0.0410$), and 13q32 ($P=0.0074$). The univariate Cox regression analysis revealed an increased risk of tumor-related death for patients whose liposarcomas possess with gains of 13q21 and 13q32 simultaneously ($P=0.010$; RR = 7.1; 95% CI 1.6–31.7). Furthermore, 12 high-level amplifications were found in tumors of seven patients. In four cases 12q14–q15 and in two cases 13q32–q33 were amplified. We identified in different liposarcoma subtypes characteristic genomic changes: Gains and high-level amplifications of 12q occurred in all 11 investigated well-differentiated liposarcomas, and these changes were often present simultaneously with gains of 1q (mean 5.5 changes). In the two dedifferentiated liposarcomas, gains of 1q in both liposarcomas, and a high-level amplification of 13q were striking. Only eight of the 17 patients with myxoid/round cell liposarcomas showed changes in DNA copy number (mean 3.4 imbalances). In four of these eight cases gains of 13q occurred. The six pleomorphic liposarcomas possessed the most frequent genomic imbalances (mean number 16.3) of all liposarcoma subtypes investigated. These imbalances were in almost all chromosomal regions detected predominantly as over-representations of chromosomes 1, 5p, 13q, and 22q. Summarizing, all subtypes but well-differentiated liposarcomas showed gains of 13q, which were associated with a poor prognosis.

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Liposarcomas are the most common soft-tissue sarcomas in adults and account for approximately 20% of all mesenchymal malignancies. The WHO classification divides liposarcomas into four main histological subtypes that are correlated with degree of malignancy: well-differentiated, dedifferentiated, myxoid/round cell, and pleomorphic liposarcomas.¹ Well-differentiated liposarcomas are tumors of low degree of malignancy. When located in the subcutis, they are usually cured by local surgical resection and rarely recur.² Comprising 40–45% of all lipo-

sarcomas, the well-differentiated liposarcomas are thus the largest subgroup of adipocytic malignancies. Cytogenetic characteristics of well-differentiated liposarcomas are rings as well as giant marker chromosomes that contain amplified genetic material derived from chromosome region 12q13–q15 and, alternatively, additional material from chromosomes 1, 4, and 16.^{3–6} Dedifferentiated liposarcoma is a high-grade sarcoma that arises in patients with pre-existing well-differentiated liposarcoma⁷ and shows the same cytogenetic aberrations as well-differentiated liposarcoma. Myxoid/round cell liposarcoma account for about 30% of all liposarcomas and share the typical reciprocal translocations t(12;16)(q13;p11) and t(12;22)(q13;q12), and trisomy 8, isochromosome 7q, and 6q deletions as secondary aberrations.⁴ Pleomorphic liposarcoma is defined as a high-grade liposarcoma and is

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associated with a very poor prognosis.⁸ Complex chromosomal changes without specific rearrangements have been detected in karyotypic analyses of pleomorphic liposarcomas.⁴

Investigations of liposarcomas by comparative genomic hybridization (CGH) have shown a broad scattered pattern of genomic imbalances.^{9–11} In this study, we analyzed altogether 36 cases of four liposarcoma subtypes by CGH to identify distinct DNA sequence copy number changes in the different subtypes and to evaluate their prognostic relevance.

Materials and methods

Tumor Specimens

The investigated tumor material consisted of 36 liposarcomas (11 well-differentiated, two dedifferentiated, 17 myxoid/round cell, and six pleomorphic liposarcomas) from 36 patients (17 women, 19 men). The samples were collected from the Institute of Pathology at the Martin Luther University Halle-Wittenberg and from the Surgical Clinic 1 of the University of Leipzig (Germany) within a period of 15 years. We analyzed 27 primary tumors, eight recurrences, and one metastasis with histological grades 1 (16 cases), 2 (16 cases), and 3 (four cases), according to the Van Unnik grading system.¹² The material investigated consisted of 15 tumors with stage I, 12 tumors with stage II, four tumors with stage III, and five tumors with stage IV (UICC guidelines¹³). The 27 primary tumors were untreated before resection, of the eight recurrences only two patients (cases 20 and 30) received radiotherapy after primary tumor resection, and the one patient with metastasis (case 28) was treated by chemotherapy after primary tumor resection. In 20 cases, the liposarcomas were located in the lower extremity, in two cases in the upper extremity, in six cases in the external pelvis, in four cases in the retroperitoneum, in two cases in the thorax, and in two cases were found at multiple locations. The average age at diagnosis was 51 years (range 24–79). Of the 36 patients, 11 died from the disease and 25 are alive. The average survival time was 34 months (11–78 months), and the average observation time for patients who survived was 42 months (6–98 months).

CGH Analysis

Tumor DNA was isolated from 22 frozen samples and 14 paraffin-embedded samples. The reference DNA was isolated from the blood of healthy probands. CGH was performed as described by Kallioniemi *et al*¹⁴ but with some previously reported modifications.¹⁵ Briefly, tumor DNA was labeled with biotin-16-dUTP, and reference DNA was labeled with digoxigenin-11-dUTP (Boehringer Mannheim, Mannheim, Germany) by using nick

translation. Hybridization was performed for 3 days at 37°C. The biotinylated sequences were detected with avidin-fluorescein isothiocyanate (FITC) (Boehringer Mannheim), and probe sequences haptenized with digoxigenin were visualized with antidigoxigenin rhodamine (Boehringer Mannheim). Chromosomes were counterstained with DAPI (4,6-diamino-2-phenylindole) (Serva, Heidelberg, Germany).

Analysis of Digital Images Resulting from CGH

The hybridization was analyzed by the ISIS digital image analysis system (MetaSystems, Altlußheim, Germany).¹⁵ Chromosomal regions with a green to red ratio above 1.25 were considered to be over-represented (gains), whereas regions with a ratio below 0.75 were considered to be under-represented (losses). The cutoff value for high-level amplifications was 1.5 according to Kallioniemi *et al*.¹⁴ The results were confirmed by calculating the double standard deviation of the individual ratio profiles for each chromosome within the same experiment. Heterochromatic regions were excluded from analysis.

Statistical Analysis

We used SPSS 11.0 software (SPSS Inc., Chicago, IL, USA) to perform statistical analyses, which included the χ^2 -test, the Kaplan–Meier method, the univariate Cox regression analysis, and the log-rank test to determine statistical significance. The level of significance was set at $P < 0.05$.

Results

CGH Analysis

Genomic imbalances were detected in tumors from 27 of the 36 investigated patients with a mean value of 5.6 imbalances per tumor (range 1–23). The DNA sequence copy number changes are given in Table 1. The chromosomal regions that were most frequently affected are shown in Figure 1.

The minimal common regions of the most frequent gains in the 36 liposarcomas were 12q14–q15 (15 cases); 12q21 (13 cases); 12q13 (12 cases); 1q23 and 12q22 (11 cases each); 1q21–q22 and 1q24–q25 (10 cases each); 1q31 and 13q31 (nine cases each); 13q22 and 13q32 (eight cases each). A total of 12 high-level amplifications were observed in tumors of seven patients. The region 12q14–q15 was amplified in four samples, and the region 13q32–q33 in two samples. The other high-level amplifications occur once.

Losses were detected strikingly less frequently than gains. Only the chromosomal region 16q12.1–qter was characterized by losses in three cases, the other regions of losses occurred only once or twice.

Table 1 Copy number changes in 36 liposarcomas analyzed by CGH^a

Case	Histologic subtype	Copy number changes
1	WDLS	+Xq22-q26, +1q21-q32/ 1q23-q25 , +6p21.1-pter, +8q13-q23, +9, +12q13-q21/ 12q14-q15
2	WDLS	+1q21-q31, +4p12-p14, +4q28-q33, +5q14-q33, +7p13-p15, +8p12-p22, +9p, +11p, +12p , +12q12-q23/ 12q14-q21 , +14q12-q24, +18p, +21q21-qter
3	WDLS	+Xq21-qter, +1q21-q41, -2q23-qter, +5p12-p14, +6q12-qter/ 6q22-q24 , +7p, +7q31-q34, +8p12-pter, -8q21.1-q23, +9p, +10q11.2-q21, -10q23-q25, +12q13-q15, +14q13-qter, +15q, -16q12.1-qter, +18p, +18q12-q21, +20q, +21q21-qter
4	WDLS	+12q12-q22/ 12q14-q15 , +19q
5	WDLS	+2q24-q35, +12q13-q24.1/ 12q14-q15
6	WDLS	+2p16-pter, +5q21-q23, +12p, +12q14-q21, +20q12-qter, +21q22
7	WDLS	+4q22-qter, +6q12-q24, +12q13-q22, +16q21-qter
8	WDLS	+12q14-q22
9	WDLS	+1q21-q25, +12q13-q22
10	WDLS	+12q13-q22, +18q21-q22
11	WDLS	+7q11.2-q31, +12q12-q15
12	DDLS	+1p, +1q21-q31, +4p, +5p, -9p, -12p, -13q12-q14, +13q21-qter/ 13q31-q33 , -16q12.1-qter, +17p, +19q, +20q, +21
13	DDLS	+1q21-q23, +4q21-q31.1, +6q16-q22, +7q22-qter, +12q12-q15
14	M/RCLS	No changes
15	M/RCLS	+Xq22-qter, +12q21-q24.1
16	M/RCLS	-3p12-p21.1
17	M/RCLS	+3p12, +3p24-pter, +3q12-q13.3, +4q12-q22, +6q13-q16, +13q21-q31
18	M/RCLS	+5q21-q32, +6q16-qter, +13q, +18q
19	M/RCLS	+7q22-qter, +8p12-pter, +13q21-qter, +18q12-qter, -22q13
20	M/RCLS	+6q12-q24, +11q14-q23, +13q14-q32, +18q12-q22
21	M/RCLS	No changes
22	M/RCLS	No changes
23	M/RCLS	No changes
24	M/RCLS	No changes
25	M/RCLS	No changes
26	M/RCLS	-8p12-p22, +12q12-q22
27	M/RCLS	No changes
28	M/RCLS	No changes
29	M/RCLS	+Xq23-qter, +8p, +20q
30	M/RCLS	No changes
31	PLS	+Xq12-q21, +2q11.2-q32, +3p12-p14, +3q24-qter, +5p12-p14, +5q11.2-q14, +8q11.2-q22, +12q14-q22, -13q21-qter, +14q11.2-q21, +15q11.2-q15, +17p, +18p, +18q11.2-q12, +22q
32	PLS	+1p, +1q21-q31, +5p, +6p21.1-pter, +7p, +10q, +13q22-qter, +17q21-qter, +18q12-qter, +20q, +22q
33	PLS	+Xp11.2-p21, +Xq21-q27, +1p12-p32, +1q21-q32, +2p13-pter, +2q13-q22, +3p12-p21, +5p, +5q13-q23, +7p11.2-p15, +7q, +8p12-pter, +8q21.1-qter, -9, -10p, +11q, +12q12-q24.1, +13q14-q32, +14q21-q24, -16, -17p, +21, +22
34	PLS	+1p, +1q21-q31, -2p12-pter, +3p21-pter, +4q22-q31.3, +5q31-qter, +6q21-qter, +8, +9p13-pter, -11, -13q12-q14, +13q31-qter, -15q15-qter, -16p, +17p, +19p, +20q
35	PLS	+1p21-p31, +1q23-q31, +2q14.1-q21, +3p21-pter, +4q22-q33, +6p, +7p14-pter, +9p13-pter, -10q11.2-q23, +14q22-qter, +20q12-qter
36	PLS	-Xq21-q26, +1p13-pter/ 1p33-p35 , +1q21-q31, -1q41-qter, -2q14.1-qter, +3q12-q22/ 3q13.1-q13.3 , -4q31.1-qter, +5p12-p15.2, +5q14-q23, +6p12-pter, -6q14-qter, +8p, +9q13-qter, +11p, +11q12-q13, -11q14-qter, -12p, -12q12-q21, +13q13-qter/ 13q32-q33 , +14q12-qter/ 14q31 , +22q

^aWDLS, well-differentiated liposarcoma; DDLS, dedifferentiated liposarcoma; M/RCLS, myxoid/round cell liposarcoma; PLS, pleomorphic liposarcoma. Gains of DNA sequences are marked with +, losses with -; high-level amplifications are indicated in bold.

Correlations between Frequent Genomic Imbalances, Clinicopathological Parameters and Overall Survival

A significant correlation of poor overall survival and gain of 13q21 ($P=0.0221$), 13q22 ($P=0.0341$), 13q31 ($P=0.0410$), and 13q32 ($P=0.0074$) were found in Kaplan–Meier analysis. The patients without gains of 13q had a mean survival time of 78 months, whereas the patients with gains of 13q survived on average 35 months. Frequent gain of single regions of chromosomes 1q, 12q, and 20q (and also of 2q, 3p, 5, 6p, 7p, 8, 14q, and 22q in pleomorphic liposarcoma-

detected) did not have any significant influence on patient prognosis (data not shown). In addition, we observed a prognostic impact on overall survival of tumor stage ($P<0.00001$) and tumor grade ($P=0.0341$). Tumor subtype and age of patients did not have any significant effect on patient prognosis.

The univariate Cox regression analysis revealed an increased risk of tumor-related death for patients with gains of 13q21 ($P=0.037$; RR=4.2; 95% CI 1.1–16.4), gains of 13q32 ($P=0.020$; RR=6.0; 95% CI 1.3–27.1), and for patients whose liposarcomas

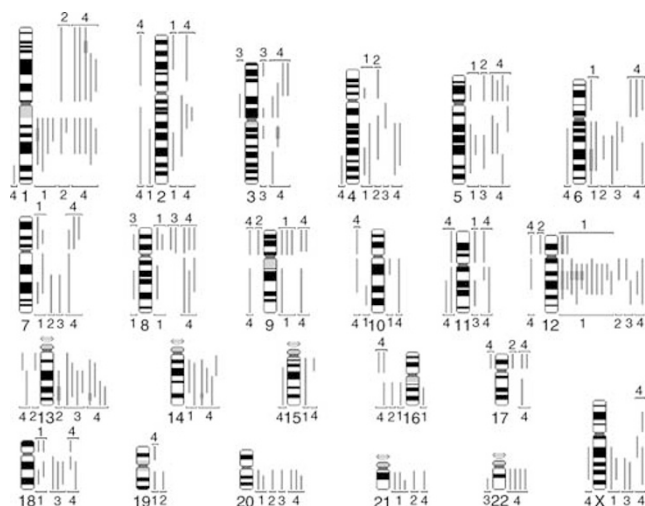


Figure 1 Summary of gains and losses found by CGH in liposarcomas from 36 patients. Losses are shown to the left and gains to the right. High-level amplifications are shown as thick lines. Clamp 1, well-differentiated liposarcomas; clamp 2, dedifferentiated liposarcomas; clamp 3, myxoid/round cell liposarcomas; clamp 4, pleomorphic liposarcomas.

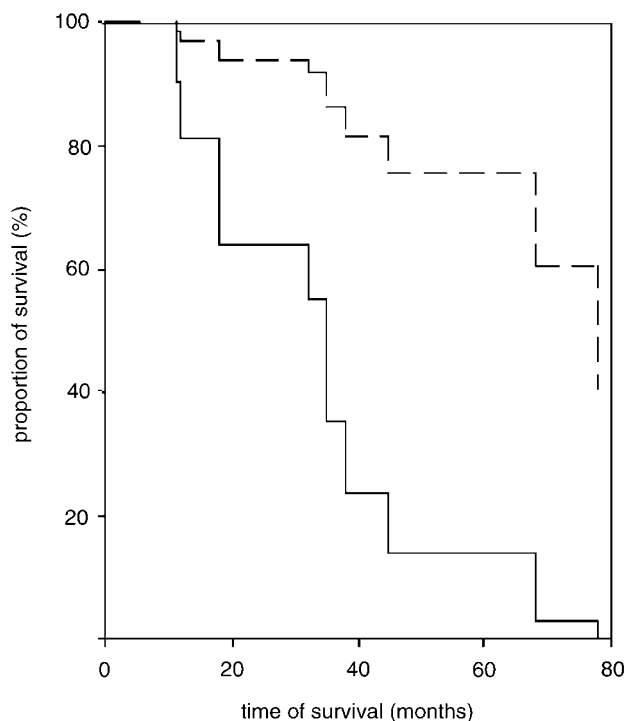


Figure 2 Univariate Cox regression analysis shows a significant correlation between survival of liposarcoma patients and simultaneous gain of 13q21 and 13q32 in their tumors. The broken line represents patients with tumors without gains of 13q, and the continuous line represents those with gains of 13q ($P=0.010$; $RR=7.1$).

possess with both gains of 13q ($P=0.010$; $RR=7.1$; 95% CI 1.6–31.7) (Figure 2).

To investigate the correlation of prognostic relevant gains of 13q and the different groups of tumor

Table 2 Occurrence of gains at single regions of 13q in tumors with different subtypes (χ^2 -test)

	Total number of cases	+13q21	+13q22	+13q31	+13q32
WDLS	11	0	0	0	0
DDLS	2	1	1	1	1
M/RCLS	17	4	4	4	3
PLS	6	2	3	4	4
<i>P</i> -value		0.190	0.081	0.019	0.011

WDLS, well-differentiated liposarcoma; DDLS, dedifferentiated liposarcoma; M/RCLS, myxoid/round cell liposarcoma; PLS, pleomorphic liposarcoma.

stages, tumor grades, and tumor subtypes we used the χ^2 -test. There were positive correlations of gains at 13q31 and 13q32 with higher tumor stage ($P=0.035$ and 0.008), higher tumor grade ($P=0.013$ and 0.003), and pleomorphic liposarcoma ($P=0.019$ and 0.011). Table 2 shows the distribution of cases with gains of the single regions of 13q in the tumors with different subtypes.

Frequent Genomic Imbalances in Different Subtypes of Liposarcomas

Well-differentiated and dedifferentiated liposarcomas

The mean number of genomic imbalances in 11 well-differentiated liposarcomas was 5.5 (range 1–20). All well-differentiated liposarcomas showed gains of chromosome arm 12q: 12q13 (nine cases), 12q14–q15 (11 cases), 12q21 (10 cases), and 12q22 (eight cases). We could observe seven high-level amplifications in five well-differentiated liposarcomas. The four high-level amplifications at 12q14–q15 (one of which extended to 12q21) occurred only in well-differentiated liposarcomas. Furthermore, one high-level amplification at 1q23–q25, one at 6q22–q24, and another at 12p were detected. Losses were found only in two cases (Figure 1, clamps 1; Table 1).

One (case 13) of the two dedifferentiated liposarcomas possessed five regions of gain. In the other tumor (case 12), nine regions with gain, one high-level amplification at 13q31–q33, and four regions with loss were detected. Only a gain of 1q21–q23 was observed in both samples of dedifferentiated liposarcomas (Figure 1, clamps 2; Table 1).

Myxoid/round cell liposarcomas

Genomic imbalances were observed less frequently in myxoid/round cell liposarcomas than in other subtypes of liposarcoma. Only eight of the 17 myxoid/round cell liposarcomas showed changes in DNA copy number (mean 3.4 imbalances per tumor; range 1–6). Gains were identified most frequently in 13q21–q31 (four cases); 6q16, 13q32,

and 18q12–q22 (three cases each). No high-level amplifications, and only one loss each were found in three myxoid/round cell liposarcomas (Figure 1, clamps 3; Table 1).

Pleomorphic liposarcomas

In the six pleomorphic liposarcomas, the mean number of genomic imbalances was 16.3 (range 11–23). Changes occurred in all chromosomes. We found in all six pleomorphic liposarcomas gains, mostly in the regions 1p21–p31, and 1q23–q31 (five cases each), 1p13, 1p32, 1q21–q22, 5p12–p14, 13q31–q32, and 22q (four cases each). In three of the six pleomorphic liposarcomas, gains were limited to the regions 1p12, 1p33–pter, 2q14.1–q21, 3p21, 5p15.1–p15.2, 5q14, 6p21.1–pter, 7p14–p15, 8p12–pter, 8q21.1–q22, 13q22, 13q33–qter, 14q21–q24, and 20q12–qter. One pleomorphic liposarcoma contained four high-level amplifications at 1p33–p35, 3q13.1–q13.3, 13q32–q33, and 14q31. Losses were observed in five pleomorphic liposarcomas (mean 3, range 1–8) (Figure 1, clamps 4; Table 1).

Discussion

The aim of this study was the comparison of genomic imbalances in the four main subtypes of liposarcoma investigated by CGH. The most frequent DNA copy number changes were found as gains of single chromosomal regions of 1q, 12q, and 13q. The number of detected losses is very low. Whereas gains of 1q and 12q were reported previously,^{3,9–11,16–18} the frequent gain of 13q is not reported in the 111 liposarcomas investigated by CGH until now. The present study showed that gains of 13q are correlated significantly with a poor prognosis, and this finding has been described in liposarcoma subtypes for the first time. Comparable findings of copy number gains of single regions of 13q has been reported also in other sarcomas, for example, in malignant fibrous histiocytomas,¹⁹ and alveolar rhabdomyosarcomas.²⁰ However, these authors could not find any prognostic relevance of gains of 13q regions. Using a univariate Cox regression analysis we showed that the patients whose liposarcomas possess with gains at 13q, have an increased risk of tumor-related death. We found a significant positive correlation of gains at 13q31 and 13q32 with higher tumor grades and tumor stages. About relevant target genes we can only speculate. The pancreatic cancer-derived gene *PCD1* (13q21.33) is highly expressed in colon, breast, liver, lung, pancreas, stomach, and prostate tumor tissues.²¹ However, with RT-PCR we could not detect an overexpression of *PCD1* in liposarcomas with a gain of 13q (data not shown).

The copy number changes in the analyzed 36 liposarcomas varied widely, but the single subtypes

of this tumor group have characteristic genomic differences.

In the 11 investigated well-differentiated liposarcomas the most frequent gain was that of 12q; we also found four high-level amplifications of 12q14–q15. These high-level amplifications were found exclusively in well-differentiated liposarcomas. Four tumors with gains of 12q also had gains of 1q. This coamplification of both regions of 1q and 12q was characteristic for well-differentiated liposarcomas. As target genes in the region of 12q, for example, the amplicons *SAS*, *CDK4* (12q13–q14), and *MDM2* (12q14.3–q15) are known in sarcomas. Previously, we have investigated nine of the 11 well-differentiated liposarcomas of the present study by semiquantitative PCR for *MDM2* amplification.²² Of the nine cases, we found in four cases both high-level amplifications of 12q14–q15 by CGH analyses and an amplification of *MDM2* by semiquantitative PCR.²² In four out of the other five cases, gains of 12q13–q15 were detected, which carried in two cases an *MDM2* amplification as well. At the time this paper was written, 41 well-differentiated liposarcomas had been analyzed by CGH. Gains, high-level amplifications, or both, of subregions of 1q and 12q are the imbalances that have been described most frequently.^{2,3,9,10,16,17} The results of our analyses of 11 well-differentiated liposarcomas confirmed the findings of these earlier reports.

In the two analyzed dedifferentiated liposarcomas, the remarkable findings were the over-representation of 1q regions in both cases and a high-level amplification of 13q. Other CGH analyses revealed over-representation of single regions of 12q in each of 30 cases studied to date^{10,11,17,18} and frequent gains of 6q regions and 20q13.¹¹ In our study a gain of 6q16–q22, 12q12–q15, and 20q was identified in one case each.

In nine of 17 myxoid/round cell liposarcomas we detected no genomic imbalance. In the other eight tumors, the most frequent change was a gain of subregions of 13q (four cases). We detected neither gains of 1q nor high-level amplifications of 12q as in well-differentiated liposarcomas. The absence of any high-level amplification was characteristic for myxoid/round cell liposarcoma. Other CGH analyses of 16 cases of myxoid/round cell liposarcomas showed over-representation of chromosome 8 as the most frequent aberration.^{3,9,17} However, our analysis of 17 myxoid/round cell liposarcomas revealed an over-representation of 8p12–pter in only two cases. Altogether we suggest that the development of myxoid/round cell liposarcomas is characterized more by structural aberrations that occur at the chromosome level but do not disrupt the genomic balance.

Our CGH analyses of six pleomorphic liposarcomas revealed genomic imbalances in all chromosomes with the highest mean aberration number of 16.3 per tumor. These results confirmed earlier findings of 24 pleomorphic liposarcomas

analyzed.^{9,11,16,17} Most frequently, we found DNA sequence copy number gains of regions of the chromosomes 1, 5p, 13q, and 22q, also described in other sarcomas.²³ In the CGH analysis described by Rieker *et al*,¹¹ the gain of 20q13 was the most frequent genomic imbalance in pleomorphic liposarcomas. In the present study, in half of the pleomorphic liposarcoma samples, a gain of 20q12–qter was detected by CGH as well.

In conclusion, we have shown that there are a few distinct cytogenetic differences between subtypes of liposarcoma. In general, the pleomorphic liposarcomas possessed the most frequent genomic imbalances, which occurred in almost all chromosomal regions. However, pleomorphic liposarcomas can be distinguished from well-differentiated liposarcomas and also from dedifferentiated liposarcomas by the lack of high-level amplifications in 12q13–q15, the loci of *SAS*, *CDK4*, and *MDM2*. These oncogenes are amplified exclusively at a high level in well-differentiated and dedifferentiated liposarcomas. However, well-differentiated liposarcomas differ from dedifferentiated liposarcomas by the absence of detectable gains and high-level amplifications of 13q. It is intriguing that a gain of 13q21–q32 is correlated significantly with a poor prognosis in different liposarcoma subtypes. The myxoid/round cell liposarcomas generally lacked frequent or striking genomic imbalances detected by CGH analyses. We observed no gains of 1q and no high-level amplifications of 12q. Predominantly, myxoid/round cell liposarcomas were characterized by the known reciprocal translocations.

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