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Does comparative genomic hybridization reveal distinct differences in DNA copy number sequence patterns between leiomyosarcoma and malignant fibrous histiocytoma?

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Abstract Leiomyosarcoma (LMS) is the third most common type of soft tissue sarcoma after malignant fibrous histiocytoma (MFH) and liposarcoma. Comparative genomic hybridization (CGH) has shown similar DNA copy number imbalances in LMS and MFH. It has been suggested that both tumors may correspond to different differentiation states of a single tumor entity and that a large proportion of MFHs could correspond to undifferentiated LMS. We report CGH results from 102 MFH and 82 LMS cases, as well as a subsequent clustering analysis. The distribution pattern of DNA copy number changes could not differentiate LMS from MFH, suggesting that most MFHs could represent an ultimate state of tumor progression of LMS. Even if an oncogenic pattern common to LMS and MFH is valid, the genes relevant to smooth muscle cell differentiation may reside in one or more chromosomal imbalances that are not shared by both tumor types. Further explorative analysis identified a small cluster of tumors (9% of the samples: 2 LMS and 10 MFH) characterized by the presence of high-level amplifications at 1p33~p34.3, 17q22~q23, 17q25~qter, 19 p, 22 p, and 22q, and associated with a higher proportion of tumors located in the thigh ($P = 0.003$) and with male sex ($P = 0.079$).

1. Introduction

Leiomyosarcoma (LMS), the third most common type of soft tissue sarcoma after malignant fibrous histiocytoma (MFH) and liposarcoma, displays phenotypic features of smooth muscle differentiation [1]. Leiomyosarcomas are typically composed of spindle cells with blunt-ended nuclei and eosinophilic cytoplasm, usually expressing α-smooth muscle actin [2], desmin [3], and H-caldesmon [4]. In poorly differentiated cases the expression of these markers can be low or even lacking.

Malignant fibrous histiocytomas are composed of pleomorphic cells, sometimes showing fibroblastic or histiocytic features. Whether this sarcoma originates from histiocytes, fibroblasts, or undifferentiated mesenchymal cells is still unknown [5]. The clinicopathologic group of MFHs is heterogeneous, and its validity and existence have often been discussed [6–8]. The latest WHO classification of soft tissue tumors regards MFH as a synonym of undifferentiated sarcoma; MFH diagnosis is therefore based on exclusion criteria and reserved for tumors showing no other differentiation line [9].

Cytogenetic techniques have shown complex karyotypic abnormalities in both LMS and MFH. Many nonrandom numerical and unbalanced structural rearrangements common to both types of sarcoma, and signs of gene amplification have been revealed, but no single specific aberration has been identified [10].

Conventional comparative genomic hybridization (CGH) has been used to identify DNA copy number imbalances in >250 cases of LMS [11–23] and MFH [20,24–35]. Extensive genetic alterations have been detected in nearly all tumors analyzed. Both tumors show recurrent similarities of DNA copy number changes (http://www.helsinki.fi/cmg/cgh_data.html). Accordingly, it has been suggested that these tumors may correspond to

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different differentiation states of the same tumor entity, and that a large proportion of MFHs could correspond to poorly differentiated LMS [11,36,37].

To test this hypothesis, we used univariate and multivariate analyses in comparing results obtained by conventional CGH in a large series of 184 primary soft tissue sarcomas (102 MFH and 82 LMS).

2. Materials and methods

2.1. Leiomyosarcoma

The LMS material consisted of 82 primary samples from 82 Finnish patients from the Helsinki University Central Hospital. Characteristics of samples 1–80 have been reported previously [12,21]. All cases were histologically clear LMS showing smooth muscle differentiation. The tumors were positive for α-smooth muscle actin and negative for CD34. All except two showed at least focal positivity for desmin. The study series comprised 63 (77%; 23 males and 40 females) nonuterine LMS and 19 (23%) uterine LMS. The median age at diagnosis was 60 years (range, 20–91). Tumor sizes ranged from 0.7 to ⩾20.0 cm. None of the patients had received chemotherapy or radiotherapy before surgery (Appendix A) (http://www.helsinki.fi/cmg/cgh_data.html).

2.2. Malignant fibrous histiocytoma

The MFH material consisted of 102 primary samples from 102 Finnish patients from the Helsinki University Central Hospital. Characteristics of samples 1–43 have been reported previously [24]. When necessary, new immunostainings for cytokeratins, S-100 protein, desmin, and actins were performed. Cases with specific differentiation were excluded from the analysis. Tumors displaying myxoid stroma in at least half of the sampled area were classified as myxoid. The study series comprised 81 MFHs of the storiform–pleomorphic subtype (79%; 48 male, 33 female), 16 of the myxoid subtype (16%; 7 male, 9 female), 2 of the pleomorphic-myxoid subtype (2%; 1 male, 1 female), and 1 each of the giant cell subtype (1%; male), the inflammatory subtype (1%; female), and the spindle cell subtype (1%; female). The median age at diagnosis was 63 years (range, 23–91). Tumor sizes ranged from 2.0 to 26.0 cm. With one exception (sample 8), none of the patients had received chemotherapy or radiotherapy before surgery (Appendix A) (http://www.helsinki.fi/cmg/cgh_data.html).

2.3. Comparative genomic hybridization

The CGH was performed using direct fluorochrome-conjugated DNA for all samples, as described previously [38,39]. Tumor and reference DNA were labeled with fluorescein isothiocyanate–conjugated dCTP and dUTP (POTH-Dupont–Peckin-Elmer-NEN, Boston, MA), and Texas red-conjugated dCTP and dUTP (DuPont–NEN) by nick translation, respectively [40]. Hybridizations and post-hybridization washes were carried out as reported previously [39]. For analysis of samples we used ISIS digital image analysis software (MetaSystems, Altlussheim, Germany). Chromosomal regions were interpreted as overrepresented when the green-to-red ratio was >1.17 (gains) or >1.5 (high-level amplifications), and as underrepresented (losses) when the ratio was <0.85 [38]. The minimal common regions of DNA copy number changes in the whole series were determined using ProfileBase software 4.2 (http://www.progenetix.net).

2.4. Statistical analysis

For both univariate and higher-order statistical modeling of aberration patterns, a data set was constructed to reflect the presence or absence of aberrations in each primary tumor and in each chromosome band (387 bands, excluding the Y chromosome) (Appendix B). Cases without any DNA aberrations at any chromosome band were excluded.

Each of the 387 chromosome bands analyzed was examined for differences in the proportion of copy number aberrations between LMS and MFH or between clusters of samples. The significance of the observed differences was evaluated using the χ^2 test, with Yates' correction, unless the expected counts were ⩽5 for any cell, in which case Fisher's exact test was used. The resulting *P*-values were adjusted to account for the multiple transformation tests according to Hochberg and Benjamini [41]. A false discovery rate (FDR) of 10% was used as the cutoff to identify chromosome bands with statistically significant differences in proportions of aberrations. The clusters identified (see below) were assessed for differences in clinically relevant variables using Student's *t*-test (age, tumor size), the χ^2 test (sex, histology, location), or Fisher's exact test when the expected counts were 5 or less.

To maximize the effectiveness of the clustering algorithms, the data set was further reduced to include only aberrant chromosomal bands evidenced in ⩾20% of cases, disregarding tumor class.

Three different algorithms were used for modeling the data into two groups: self organizing maps (SOM), K-means clustering (K-means), and partitioning around medioids (PAM) implemented in the class, stats, and hopach packages, running under the R programming environment (http://www.r-project.org), version 2.4.1. Parameter settings were adjusted to obtain clusters with maximal intercluster difference and intracluster similarity. SOM was run with a 2×1 hexagonal topology and a neighborhood radius of 1, K-means was run with $k = 2$ and 100 random starting partitions, and PAM was set up with $k = 2$ and Euclidean distance for assessing sample similarity.

Clustering results were evaluated by multidimensional scaling (MDS), implemented in the R package MASS,

applied to the matrix of sample similarities computed using Euclidean distance. The clustering result exhibiting the largest distance between cluster averages and smallest variability, in that order of priority, was considered for further evaluation with regards to tumor class. Evaluation was performed by assessing the degree of overlap between cluster membership and tumor class through calculation of proportions.

3. Results

3.1. DNA sequence copy number changes revealed by CGH

3.1.1. Leiomyosarcoma

Of the 82 LMSs, 79 (96%) had changes with a mean value of 11.20 ± 0.72 aberrations per sample (range, 1–25). Three samples (4%) did not show any aberrations. Gains of DNA copy number changes were as frequent as losses (gains:losses = 1.0:0.9), with a mean value of 5.44 ± 0.44 (range, 0–17) and 4.96 ± 0.36 (range, 0–13) aberrations per sample for gains and losses, respectively. High-level amplifications were found in 43 of the 82 tumors analyzed (mean value 0.79 ± 0.12 aberrations per sample; range, 0–6) (Fig. 1). Appendix A gives copy number karyotypes of all LMSs analyzed, chromosomes most frequently affected by gains, and high-level amplifications and losses of DNA copy number imbalances with their minimal overlapping common regions.

3.1.2. Malignant fibrous histiocytoma

Of the 102 MFHs, 85 (83%) had changes with a mean value of 9.33 ± 0.75 aberrations per sample (range, 1–25). Seventeen samples (17%) did not show any aberrations. DNA copy number gains were 2.5-fold more numerous than losses (gains:losses = 1.0:0.4). with mean values of 4.83 ± 0.48 (range, 0–17) and 2.94 ± 0.29 (range, 0–11) aberrations per sample for gains and losses, respectively. High-level amplifications were found in 41 of the 102 tumors analyzed (mean value 0.83 ± 0.04 aberrations per sample; range, 0–6) (Fig. 2). Appendix A gives copy number karyotypes of all MFHs analyzed, chromosomes most frequently affected by gains, and high-level

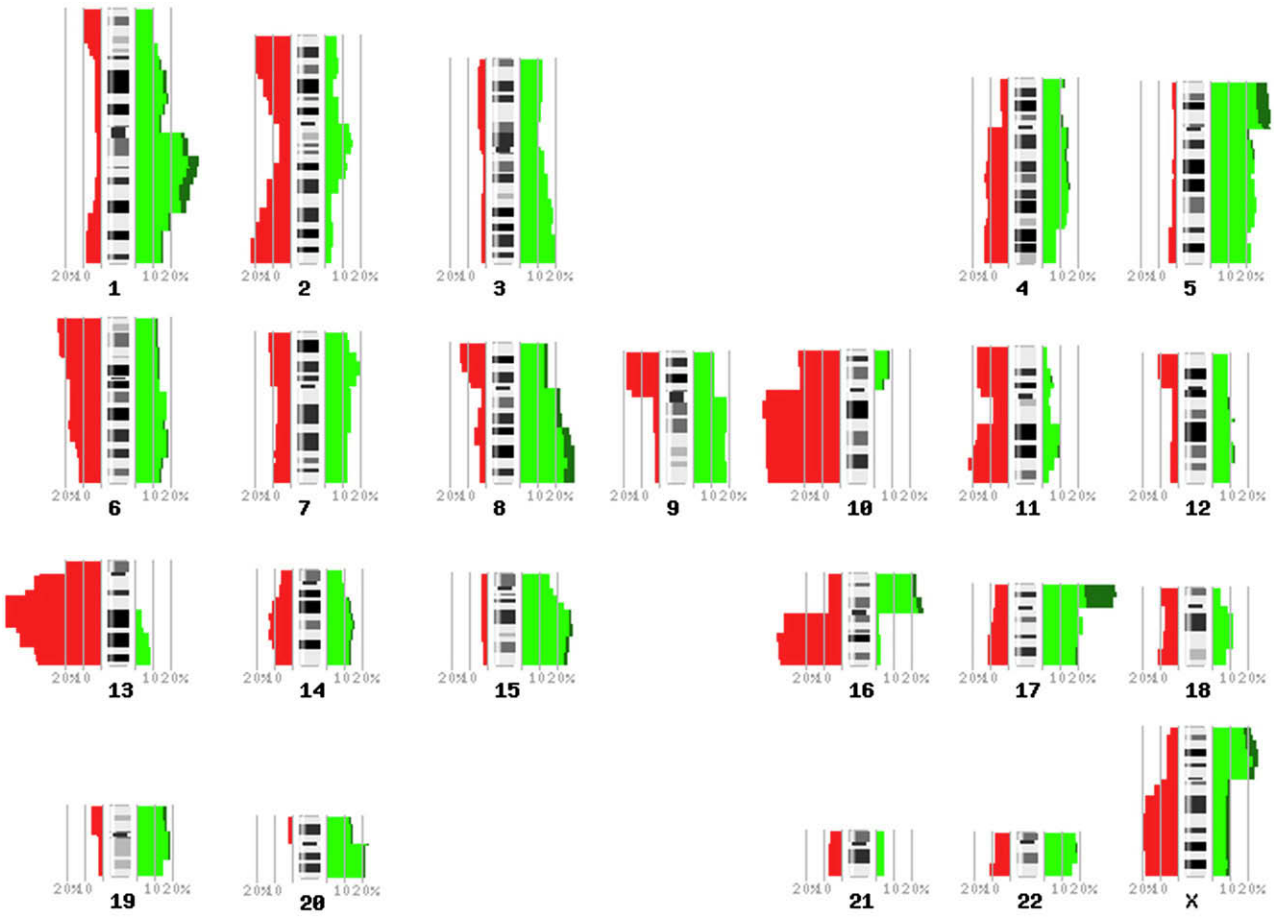

Fig. 1. Gains, losses, and high-level amplifications of DNA copy number changes in 82 primary leiomyosarcomas analyzed by comparative genomic hybridization (CGH). Losses are shown on the left side of each chromosome, and gains and high-level amplifications of small chromosomal areas on the right (light and dark areas, respectively).

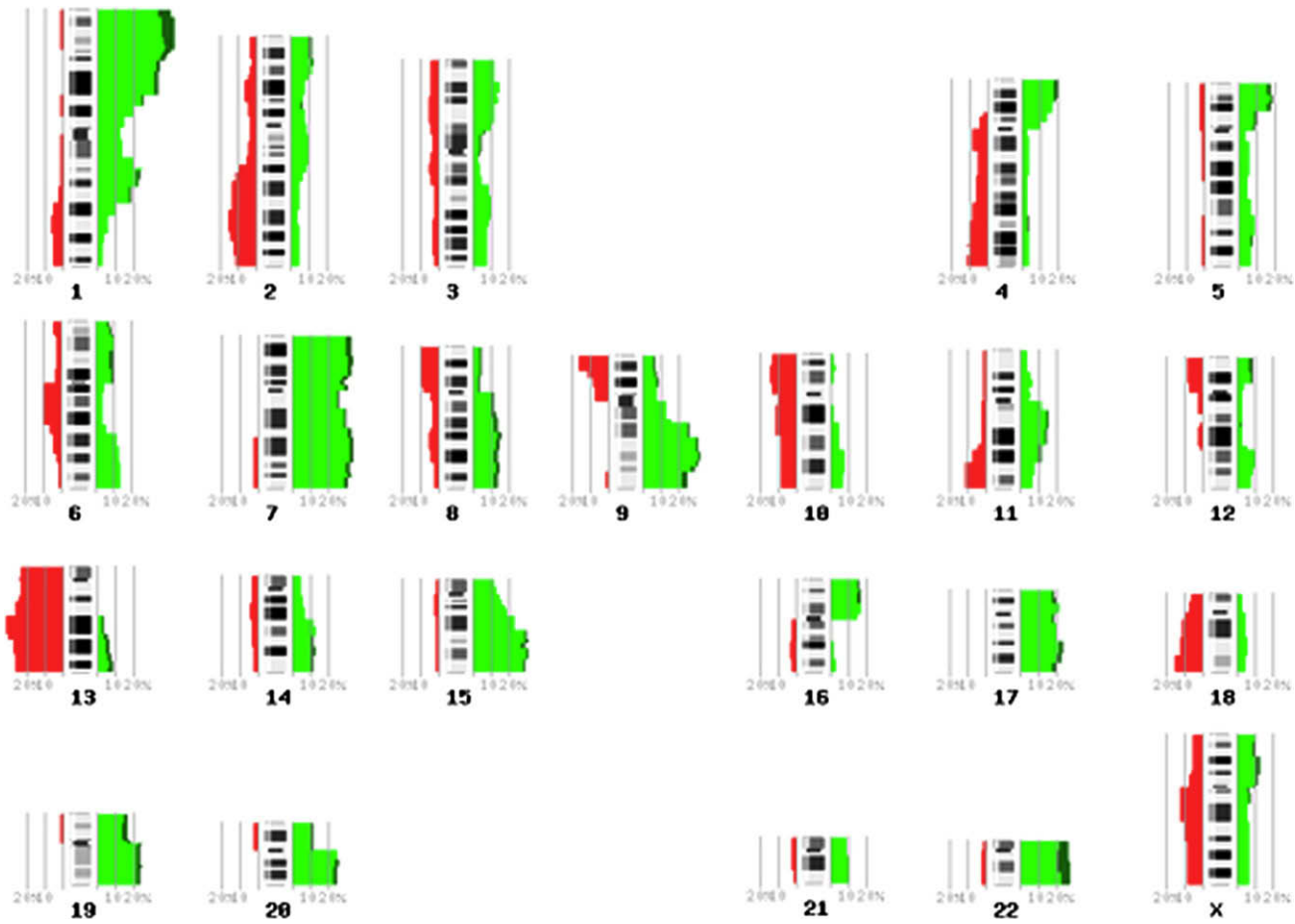

Fig. 2. Gains, losses, and high-level amplifications of DNA copy number changes in 102 primary malignant fibrous histiocytomas analyzed by CGH. Losses are shown on the left side of each chromosome, and gains and high-level amplifications of small chromosomal areas on the right (light and dark areas, respectively).

amplifications and losses of DNA copy number imbalances with their minimal overlapping common regions.

3.2. Statistical comparison of CGH patterns in LMS and MFH

Analyses were performed on the subset of cases exhibiting at least one aberration among the 387 chromosome bands examined, which reduced the number of samples from 184 to 164 (79 LMS and 85 MFH).

3.2.1. Specific differences at the single chromosome band level

We applied a 10% FDR cutoff value and identified a total of 110 chromosome bands affected differently in the two tumor entities (Fig. 3 and Appendix B); these were 41 gains and 69 losses. A more detailed survey of the results for the bands with different proportions in gains revealed three chromosomal regions (1p31~pter, 7p22~p15, and 7q21~q36) in which MFHs exhibited a significantly higher proportion of affected cases. The LMSs demonstrated a significantly higher proportion of tumors with gains in 1q32~qter, 4q26, 4q28, 5p13~q23, and 5q34. Examination of the results for bands with differences in proportions of losses uncovered a very different scenario, wherein all 11 regions with significant differences—including 1p36.1~pter, 2p22~p25, 6 p, 6q24~qter, 7 p, 10q, 11 p, 11q14~q21, 13q14, 16pter~qter, and 17q—showed a higher proportion of LMSs. Finally, only two chromosomal regions, 17cen~p11.2 and 17p13~pter (both more frequently observed in LMS than in MFH), demonstrated significant differences in the proportion of cases affected by high-level amplifications (Table 1 and Appendix B). Furthermore, only two chromosomal regions (1p36.1~pter and 7p15~pter) were identified with significant differences in proportions of cases with losses and gains simultaneously. Although each region was more frequently affected by gains or amplifications of DNA copy number sequences among MFHs, the proportion of cases with losses in the same region was significantly higher for LMSs.

3.2.2. Specific similarities at the single chromosome band level

Because there is no formal way to statistically test the similarity of two proportions, a filtering strategy was

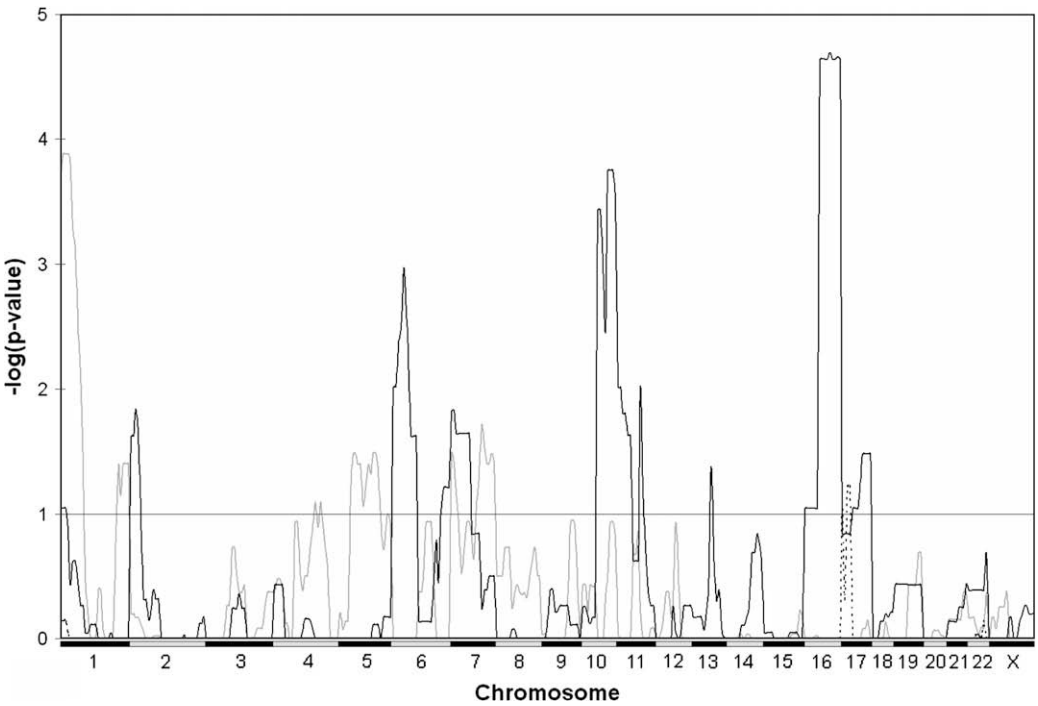

Fig. 3. Statistical significance for differences in proportion of cases with copy number changes between leiomyosarcomas and malignant fibrous histiocytomas as a function of chromosome location. *P*-values have been transformed using the $\log_{10}$ absolute value, yielding a range of positive values starting from 0 (for $P = 1$), with 1 equaling the cutoff level for statistical significance of the false discovery rate (FDR)–adjusted *P*-values ($P = 0.1$; highlighted with a horizontal line). The gray curve represents the copy number gains, the black curve losses, and the dotted black curve high-level amplifications of small chromosomal areas. Chromosomes are depicted from 1pter to Xqter.

devised based on the results of the statistical test for differences in the proportions. By requiring the unadjusted *P*-values for differences in proportions between LMS and MFH to be >0.5 simultaneously with regard to gains, losses, and high-level amplifications, the chromosome bands with smallest possible differences in proportion were identified. From this set, bands with copy number changes in $<20\%$ of cases were removed, leaving the loci with the most representative and prominent aberrations.

This approach identified a total of 29 chromosome bands distributed on seven chromosomes (2q13~q34, 3p22~p24, 4q32~qter, 12p12~pter, 13q11, 15cen~q15, and Xq13) (Appendix C) that were equally affected by gains and losses of DNA copy number changes in both LMS and MFH. High-level amplifications affecting simultaneously both tumor entities were found at Xq13 (affecting 1.3% of LMSs and 2.3% of MFHs), although the difference was not statistically significant (Appendix C).

3.2.3. Cluster analysis of overall differences

Cutoff values were defined to provide a more informative and less noisy data set for clustering, focusing on the most representative DNA copy number aberrations. Including alterations that were shared by $\geqslant 20\%$ of the cases, regardless of the sarcoma entity, brought the number of chromosomal bands down from 387 to 266. We applied three well-established and technically different clustering methods commonly used for discovering subclasses within complex high-dimensional data sets. The method yielding most stringent results (i.e., the one that showed the largest intercluster difference and smallest intracluster variation) was selected for further evaluation. Of the three methods, SOM performed markedly worse than the others (data not shown), whereas K-means and PAM were relatively close in terms of intercluster differences. There was, however, a significant difference in the homogeneity of the resulting clusters between these two methods, with PAM yielding smaller intracluster variability (data not shown).

The two clusters identified with PAM were highly disproportionate in size, the larger consisting of 148 cases and the smaller of only 16 cases. Because the numbers of MFHs and LMSs in the examined data subset were 85 and 79, respectively, the observed cluster sizes as such already indicate a lack of correspondence between cluster membership and tumor type. Indeed, overlaying cluster and tumor type information on two-dimensional MDS plots of the data clearly showed that the larger cluster included a roughly equal number of MFHs and LMSs (71 and 77, respectively) (Fig. 4). For the smaller cluster, however, the corresponding numbers were 14 for MFH (samples 51, 65, 82, 85, 87, 88, 91–93, 95, and 97–100) and 2 for LMS (samples 10 and 26) ($P = 0.0061$).

The smaller cluster, comprising MFH cases almost exclusively, was examined further to distinguish clinical

Table 1
The most frequent minimal common regions of DNA copy number changes present in at least 20% of the 184 primary tumors analyzed by conventional comparative genomic hybridization

Minimal common regions affected by alterations in DNA copy number sequences	Frequency of alterations,[a] %					
	Leiomyosarcoma			Malignant fibrous histiocytomas		
	Losses	Gains	Amp[b]	Losses	Gains	Amp[b]
Gains						
1p31~pter	3.7–9.8	9.8–15.9	0.0–2.4	0.0–1.0	31.4–37.3	2.0–7.8
1q32~qter	7.3–8.5	16.4–18.6	1.2–2.4	5.9–6.9	2.9–5.9	0.0
4q26	12.2	14.6	0.0	6.9	2.0	0.0
4q28	11.0	14.6	0.0	8.8	2.0	0.0
5p13~q23	1.2–2.4	22.0–29.3	0.0–7.3	0.0–2.0	3.9–8.8	0.0–1.0
5q34	4.9	22.0	0.0	1.0	6.9	0.0
7p15~p22	11.0–12.2	12.2–17.1	0.0–1.2	0.0	29.4–30.1	3.9
7q21~qter	7.3–9.8	12.2–14.6	0.0	0.0–2.0	28.4–31.4	1.0–2.9
Losses						
1p36.1~pter	9.8	9.8	0.0	1.0	35.3	7.8
2p22~p25	20.7	6.1–7.3	0.0	2.9–3.9	10.8–11.8	0.0–1.0
6 p	19.5–24.4	11.0–11.2	1.2	2.0–4.9	6.9–9.8	1.0–2.0
6q24~qter	12.2–13.4	13.4–17.1	1.2	1.0–2.0	12.8–13.7	0.0
7 p	11.0–12.2	12.2–19.5	0.0	0.0	26.5–30.4	2.0–3.9
10q	40.2–43.9	0.0	0.0	8.8–11.8	0.0–7.8	0.0
11 p	15.9–17.1	2.4–6.9	0.0–1.2	1.0	3.9–6.9	0.0
11q14~q21	17.1–18.3	9.8	1.2	2.0–4.9	10.8–13.7	1.0
13q14	53.7	0.0	0.0	26.5	0.0	0.0
16pter~qter	7.3–36.6	1.2–23.2	0.0–3.7	0.0–3.9	0.0–16.7	0.0–2.0
17q	8.5–11.0	18.3–22.0	0.0–1.2	0.0	17.7–21.6	1.0–3.9
Amplifications						
17cen~p11.2	7.3	24.4	15.9	0.0	20.6–21.6	0.0
17p13~pter	7.3	23.2	17.1	0.0	17.7	1.0

[a] Frequency of alterations in all samples (82 primary LMS and 102 primary MFH).
[b] High-level amplifications (Amp) of small chromosomal areas.

characteristics by analyzing the differences in mean age, tumor size, sex, histology, and location, compared with the principal cluster. Statistically significant differences ($P = 0.003$) were found in tumor location, with a higher proportion of thigh tumors among cases in the smaller cluster. A trend indicating a larger proportion of male patients within the smaller cluster was also observed ($P = 0.079$), with no significant differences in age or tumor size. With comparison of only MFH tumors between the two clusters, however, a significantly higher proportion of tumors classified as purely pleomorphic were found in the smaller cluster ($P = 0.036$). In an effort to determine the DNA copy number changes that contributed most to the segregation of samples into the two clusters, the differences in proportions of cases with a specific type of aberration between the cases in the smaller and larger clusters were tested (as for the MFH vs. LMS comparison). The results identified a number of regions with significant differences in the presence of copy number aberrations between the clusters (Fig. 5 and Appendix D), including several regions with a strikingly higher proportion of cases in the smaller cluster, affected by high-level amplified chromosomal bands narrowed down to 1p33~p34.3 ($P = 0.003$ to 4.08×10^{-8}), 17q22~q23 ($P = 0.02$), 17q25 ($P = 4.01 \times 10^{-6}$), 19cen~p13.3 ($P = 0.02$–0.005), 22 p ($P = 1.98 \times 10^{-10}$ to 4.70×10^{-12}), and 22q ($P = 1.55 \times 10^{-10}$ to 4.70×10^{-12}).

4. Discussion

We have reported CGH results from 82 LMSs and 102 MFHs. This is one of the largest series of primary soft tissue sarcomas published to date, using higher-order modeling data to evaluate whether true molecular cytogenetic differences exist in the pattern of DNA copy number changes between these two sarcoma entities. Univariate analysis of both entities clearly demonstrated similarities and differences in the chromosomal alteration patterns. Multivariate analysis did not segregate LMS from MFH, but differentiated a small group of 16 primary tumors: a few were LMS (2 samples) but most were MFH (14 cases).

CGH analysis revealed complex karyotypic changes in both MFH and LMS. Despite this complexity, we were able to show that neither the most frequent DNA gains nor the losses were unique either malignancy. This is in agreement with previous reports [11,12,14–18,21–24,26,29,30,33,34,36,42–46]. Furthermore, the differences in DNA copy number changes between LMS and MFH included some typical and nearly consistent DNA aberrations with prognostic value. In MFH, enh(7q32) is associated with worse metastasis-free survival and overall survival and enh(1p31) is associated with a trend to decreased overall survival [24]. In LMS, dim(10q) is associated with shorter overall

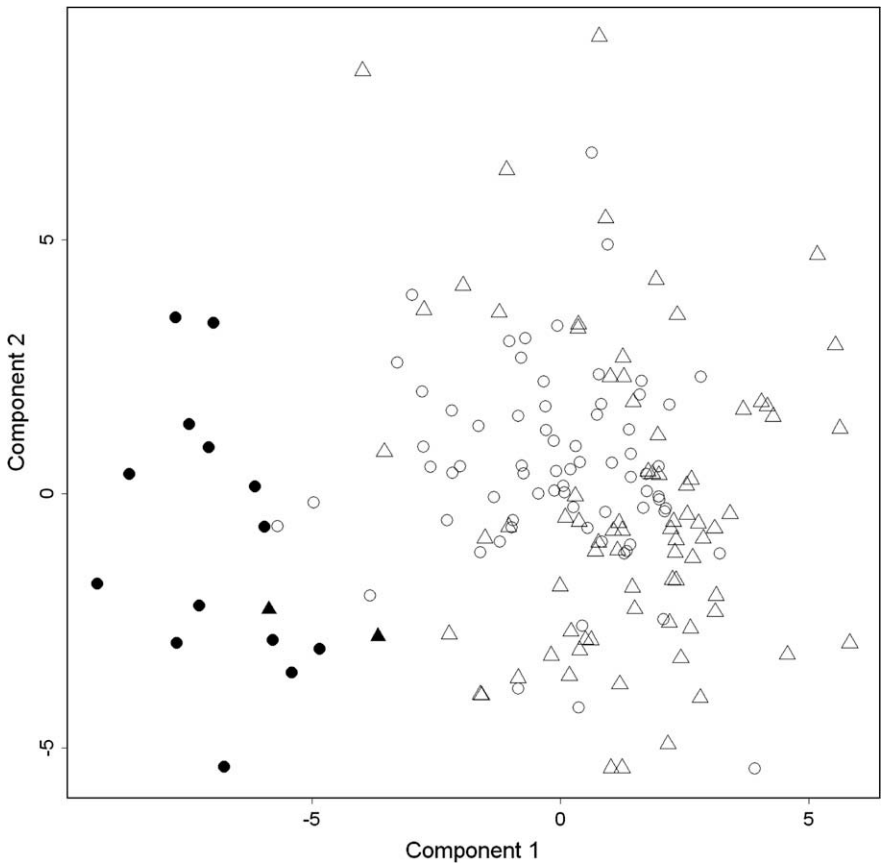

Fig. 4. Results from the partitioning around medioids (PAM) algorithm clustered the subset of 164 cases with at least one copy number change, with regards to copy number change patterns across the 266 chromosome bands that were affected in ⩾33 of the cases (20%). The data set has been reduced through multidimensional scaling based on Euclidean distance, and the two most significant components are displayed in the plot. Cluster-specificity (filled or open symbols) and tumor entity (circles, malignant fibrous histiocytoma; triangles, leiomyosarcoma) are overlaid, to enable comparison of proportions in the two clusters.

survival [15], and dim(13q14~q21) without dim(10q) is associated with longer survival [14].

Although clustering analysis failed to uncover any pathognomonic DNA copy number aberration to differentiate between LMS and MFH, we observed a clear division into two clusters. A small number of tumors (9.8%) differed significantly from the majority of the samples. In the smaller cluster, 14 of 16 tumors were MFH and only 2 were LMS, whereas the larger cluster included 77 LMS and 71 MFH. The smaller cluster was characterized by some striking highly amplified chromosomal regions, narrowed down to 1p33~p34, 17q22~q23, 17q25~qter, 19 p, 22 p, and 22q, and by a higher proportion of pleomorphic thigh tumors. Furthermore, a trend toward a larger proportion of tumors in males within the small cluster was observed. The amplified regions 1p33~p34, 17q22~q23 and 22 p were in MFH tumors alone, whereas 17q25~qter and 19 p were amplified in both MFH and LMS. Arm 22q was amplified in MFH samples, but only the band 22q11.2 was affected in LMS and in only one tumor (sample 26). This suggests that some phenotypic factors other than chromosomal aberrations are responsible for the segregation of this small group of tumors and underlie the observed copy number differences. We cannot rule out the cellular preponderance of pleomorphic type among the samples, or the location of tumors, or synergism among the three factors (i.e., among chromosomal aberration, location, and tumor subtype).

Several conventional CGH analyses and molecular approaches have suggested that MFH does not exist as an entity but rather conforms to a heterogeneous group of neoplasms. Increasing number of evidence supports the concept that MFH could represent a common morphologic form of neoplasm emerging as the result of tumor progression of different soft tissue sarcomas [6,8,11,32,34,36,37,42,43,47,48]. Recently, MFH tumors have been reported to include two subgroups. One is characterized by complex genomic alterations that strongly resemble those found in LMS, suggesting that a large proportion of these MFH tumors are LMSs that have been classified as MFH when undifferentiated. The other group of MFHs, characterized by recurrent gains or amplifications at 1 p and 6q, and localized preferentially in the abdomen and retroperitoneum, could most likely correspond to well-differentiated liposarcomas [47].

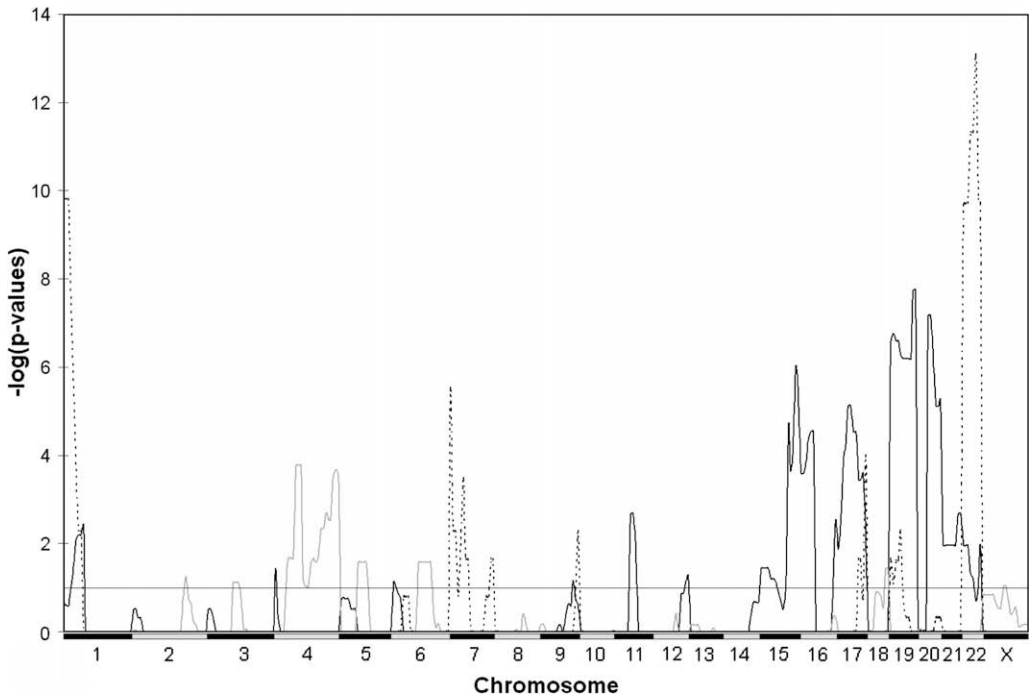

Fig. 5. Statistical significance for difference in proportion of cases with copy number changes between the two clusters identified using the PAM method. *P*-values have been transformed using the $\log_{10}$ absolute value, yielding a range of positive values starting from 0 (for $P = 1$), with 1 equaling the cutoff level for statistical significance of the FDR-adjusted *P*-values ($P = 0.1$; highlighted with a horizontal line). The black curve represents the copy number gains, the gray curve losses, and the dotted black curve high-level amplifications of small chromosomal areas. Chromosomes are depicted from 1pter to Xqter.

Our clustering analysis strongly suggests that LMS and MFH are closely related on the basis of their chromosomal aberrations, which agrees with the hypothesis proposed by Fletcher [48], who was the first to doubt the real status of MFH as a diagnostic sarcoma entity. Later, Hollowood and Fletcher [8] reported that only myxoid and angiomatoid MFHs could be regarded as specific and reproducible MFH entities. Furthermore, they recommended that the term myxoid MFH should be changed back to myxofibrosarcoma, as first proposed by Angervall et al. [49]. The remaining types of MFH represent only a morphologic feature during the progression of soft tissue sarcomas and other neoplasms (e.g., carcinomas and malignant lymphoma). Schürch et al. [50] demonstrated in a series of pleomorphic sarcomas that most of them expressed a myogenic line of differentiation (LMS or rhabdomyosarcoma) which often exhibited histomorphologic features similar to those in storiform−pleomorphic MFH.

Only 16 of our 164 tumors did not fit into the undifferentiated MFH−LMS cluster, consisting mainly of MFH classified as purely pleomorphic tumors (11/16). We cannot rule out the possibility that (i) these tumors have not yet expressed a myogenic line of differentiation, or (ii) the expression was poor, or (iii) that the tumors were misclassified and appeared segregated from the major cluster of tumors.

In summary, our results suggest that MFH does not form a specific sarcoma entity, but is more likely to represent a common oncogenic pathway in the development and progression of LMS. Genetic profiles emerging from both MFH and LMS are difficult to evaluate in the absence of specific diagnostic chromosomal rearrangements. Both the present results and our previous reports revealed that MFH and LMS show similar CGH imbalances [45,46]. Although some studies have demonstrated that both the expression profiles of some specific genes (e.g., *VEGF* [51−53]) and allelic imbalances in some chromosomal areas in LMS and MFH share similar patterns (i.e., 9 p at D9S230 [37,43]), no comprehensive and comparative genome-wide studies of DNA copy number changes and gene expression profiles have been reported to date.

Using fine-resolution oligonucleotide array CGH, we have recently demonstrated equally complex structural patterns of the 17 p amplicon in LMS and MFH [25]. Most MFHs appear to represent the final tumor progression stage of LMS. Furthermore, the multivariate analysis in the present study showed that most CGH imbalances are common to MFH and LMS. Additional molecular studies at the gene level are required to elucidate this aspect and to understand the gene expression involved in the initiation and progression pathways and to determine whether these neoplasms really exist as two different entities. If the hypothesis concerning a common oncogenic pattern shared by LMS and MFH is valid, some or all of the common chromosomal imbalances are likely to harbor the genes relevant to smooth muscle cell differentiation. Nonetheless, the possibility that

the imbalances commonly observed in both MFH and LMS may harbor genes involved in the neoplasia of this cell lineage keeping these cells in an undifferentiated stage cannot be ruled out.

Acknowledgments

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Appendix A

Clinical, histopathological, and comparative genomic hybridization data from 82 primary leiomyosarcomas and 102 malignant fibrous histiocytomas. Available online at http://www.sciencedirect.com/science/journal/01654608.

Appendix B

Distribution of DNA copy number changes per chromosomal band among all tumors with DNA copy number changes (79 primary leiomyosarcomas and 85 malignant fibrous histiocytomas). Available online at http://www.sciencedirect.com/science/journal/01654608.

Appendix C

Specific similarities between leiomyosarcoma and malignant fibrous histiocytoma cases at the level of single chromosome bands. Available online at http://www.sciencedirect.com/science/journal/01654608.

Appendix D

Cluster analysis of overall differences between leiomyosarcomas and malignant fibrous histiocytomas. Available online at http://www.sciencedirect.com/science/journal/01654608.

Supplementary material

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.cancergencyto.2008.06.005

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