

Gene copy number profiling of soft-tissue leiomyosarcomas by array-comparative genomic hybridization

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Received 18 October 2005; received in revised form 16 January 2006; accepted 25 January 2006

Abstract

Leiomyosarcoma (LMS) is a rare malignant mesenchymal tumor of smooth muscle cells. Chromosomal aberrations in LMS have been studied, but the cytogenetic data that have been published so far are complex, limited, and incomplete. Here, we performed for the first time a high-resolution genome-wide array comparative genomic hybridization (CGH) analysis (aCGH) on a pool of 14 low- and high-grade LMS cases to obtain gene-level information about the amplified and deleted regions that may play a role in the development and progression of LMS. Our aCGH results indicated that 2,218 genes were involved in 25 altered chromosomal regions; 9 regions in low-grade LMS, 12 regions in high-grade LMS, and 4 minimal common regions shared by low- and high-grade LMS. The frequency of DNA copy number gains in high-grade LMS was threefold compared to low-grade LMS, whereas losses in low-grade LMS were almost twice as frequent as in high-grade LMS. Both low- and high-grade tumors shared two minimal common regions of gain (15q26~qter and 17p13.1~q11) and loss (6p12~p21.3 and 13q14.3~qter). Moreover, our findings indicated that low- and high-grade LMS and osteosarcoma share 12 genes located in the 17p amplicon. In conclusion, by using aCGH, we were able to define the precise location of the altered chromosomal areas and to identify putative tumor suppressor genes and oncogenes therein. The list of altered genes in the minimal common regions is available as Appendix 1 at our web site (http://www.helsinki.fi/cmg/microarray_data). © 2006 Elsevier Inc. All rights reserved.

1. Introduction

Leiomyosarcoma (LMS) is a malignant mesenchymal tumor composed of cells that show distinct smooth muscle differentiation [1]. The tumors are histologically composed of spindle cells with blunt-ended nuclei and eosinophilic cytoplasm, most of which are positive for α -smooth muscle-actin (SMA), desmin, and h-caldesmon.

LMS occurs primarily in the middle-aged or elderly but it may also develop in young adults and even in children in a wide variety of anatomic body sites. Although all soft-tissue LMS are histologically similar regardless of anatomic site, they are generally divided into site-related groups on the basis of clinical and biologic differences [1]. Soft-tissue

LMS is the third most common type of sarcoma after malignant fibrous histiocytoma and liposarcoma.

Conventional cytogenetic analyses using standard karyotyping and fluorescence in situ hybridization have been reported of about 100 LMS cases. They demonstrate that genetic changes in LMS show a diverse pattern with no single change common to all or most. Nonrandom structural aberrations have been reported (e.g., rearrangements involving 1p36, 1p32, 1p13, 1q32, 7p11.1~p21, 7q32, 10q22, 13q14, and 14p11). Numerical changes, predominantly loss of chromosomes 4, 9, 14, 15, 16, 18, 21, and 22, have been found. Cytogenetic signs of gene amplification (i.e., homogeneously staining regions and double-minute chromosomes) have also been seen in LMS. Most reported karyotypes, however, are complex and incomplete [2–7]. So far, comparative genomic hybridization (CGH) has been used to identify gains and losses of DNA copy number changes in about 200 primary LMS tumors [8–15].

In the present study we performed a high-resolution analysis of gene copy number gains, losses, and amplifications of

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small chromosomal regions by applying both conventional and array CGH (cCGH and aCGH, respectively) using a cDNA microarray with clones for 12,922 genes throughout the genome to obtain a genome-wide pattern of chromosomal imbalances in 14 cases of primary soft tissue LMS.

2. Material and methods

2.1. Tumor specimens

The material consisted of 14 primary soft-tissue LMS samples obtained from 14 patients treated at the Helsinki University Central Hospital, Helsinki. The diagnosis was confirmed by histopathologic review and appropriate immunohistochemical analysis. Furthermore, the histologic grading of the tumors was re-evaluated. A four-grade system used by the Scandinavian Sarcoma Group was applied, in which grades I–II are low-grade and grades III–IV are high-grade tumors. The main criteria for grading were mitotic activity, tumor necrosis, and cellular atypia. None of the patients had received chemo- and/or radiotherapy before surgery. All patients were surgically treated with local excision of the tumor. Clinical characteristics are shown in Table 1.

2.2. cCGH and digital image analysis

DNA from paraffin-embedded tissue sections from all tumor samples were extracted according to the method of Isola et al. [16]. The proportion of tumor cells was evaluated by standard light microscopy from sections adjacent to the section, from which the samples for molecular analyses were taken. Reference DNA were extracted from peripheral blood cells of healthy individuals. CGH was performed on all samples according to the protocol described elsewhere [17,18]. Briefly, tumor DNA and reference DNA were labeled by nick translation. The hybridization mixture consisted of 400 ng tumor DNA, 400 ng reference DNA, and 10 μ g unlabeled human Cot-1 DNA (Gibco/BRL, Life Technologies, Gaithersburg, MD) dissolved in 10 μ L hybridization buffer. The denatured hybridization mixture was hybridized to a slide with normal metaphase spreads. After hybridization, the slides were washed and counterstained with 4',6-diamidino-2-phenyl-indole-dihydrochloride (Sigma Chemical Co., St. Louis, MO). CGH was performed on individual and pooled DNA from the LMS samples, divided according to both grade and the presence of DNA copy number changes in 17p, following the procedure reported elsewhere [19]. Finally, the hybridized slides were analyzed using an Olympus fluorescence microscope and the ISIS digital image analysis system (MetaSystems GmbH, Altlussheim, Germany). Thresholds for gains (1.17), high-level amplifications (1.5), and losses (0.85) were calculated as described elsewhere [18,20].

2.3. aCGH

aCGH was performed on the Agilent cDNA microarray containing about 13,000 cDNA clones (Agilent Technologies

Inc., Palo Alto, CA), using the DNA pooled for cCGH. Briefly, Alu 1 and Rsa 1 restriction enzymes were used to digest 20 μ g of tumor and reference DNA. Labeling was done as described previously [21]. Briefly, 6 μ g of genomic digested tumor and reference DNA were labeled with Cy3-dUTP and Cy5-dUTP (Amersham Biosciences Corp, Piscataway, NJ), respectively, by random priming using the RadPrime DNA labeling system kit (Gibco/BRL). After hybridization and post hybridization washes, the slides were scanned using the Agilent laser confocal scanner and the results were analyzed using the Agilent G2565AA Feature Extraction Software. Genomic basepair localizations of the clones were retrieved from the University of California Santa Cruz Genome Browser database (<http://www.genome.ucsc.edu/>) as described previously [22]. Genomic imbalances and their associated breakpoints were identified using genetic local search algorithms from the aCGHsmooth software package developed by Jong et al. [23]. Breakpoints were detected using lambda 8 for all the samples, and the amplification threshold for each sample was estimated according to the distribution of data points. Briefly, the aCGHsmooth software determines breakpoints within chromosomes by performing maximum likelihood estimation for each clone by calculating the probability that the studied clone lies within the set of previous clones. The putative breakpoints are then shifted randomly in both directions and an overall fitness is determined. This procedure is repeated either until no improvement in fitness can be achieved or once the maximum numbers of iterations have been completed. The mean values of breakpoint segments are then calculated, and closely smoothed levels are joined together. Changes were considered reliable only when five or more sequential clones were deleted or amplified. Mean ratios were calculated for duplicate clones or clones with identical genomic alignments.

3. Results

3.1. cCGH

Regardless of the tumor grade, all 14 LMS samples showed changes with a mean value of 9.71 ± 1.61 aberrations per sample (range, 2–20). Gains of DNA copy number changes were less frequent than losses (gains/losses = 1.0:1.3), with mean values of 3.86 ± 0.57 (range, 0–7) and 5.00 ± 1.17 (range, 0–13) aberrations per sample, respectively. High-level amplifications of small chromosomal regions were found in 8 of the 14 tumors analyzed (57%), with a mean value of 0.86 ± 0.25 aberrations per sample (range, 0–3; Table 2). Other less frequent gains, high-level amplifications, and losses are depicted in Figs. 1 and 2, for low- and high-grade LMS samples, respectively.

Gains of DNA sequence copy number were most commonly observed in chromosomes 1 (43%), 5 (29%), 8 (29%), 17 (43%), and 20 (29%). Losses most frequently affected chromosomes 2 (43%), 6 (50%), 10 (57%), 13 (71%), 16 (43%), and X (50%).

Table 1

Clinical data from 14 primary soft-tissue leiomyosarcoma samples analyzed by cCGH and aCGH

Sample no.	Sex/age at diagnosis (yr)	Tumor site	Tumor size (cm)	Tumor grade	Local recurrence (mo)	Metastasis (mo)	Follow-up (mo)	Last follow-up status	Cause of death
Low-grade LMS with changes in chromosome 17p									
1	F/44	Lower leg, subcutaneous ^d	6.0	II			138.40	NED	
2	M/36	Head and neck, cutaneous	1.2	II	9.93		182.50	NED	
3	F/64	Foot, subcutaneous ^{a,b,d}	3.0	II			77.07	NED	
Low-grade LMS with changes in chromosome 17p									
4	M/57	Upper trunk, cutaneous	2.0	I			97.10	NED	
5	M/78	Upper trunk, cutaneous ^d	0.7	II			29.93	DEA	WHT
6	F/25	Upper arm, cutaneous ^d	1.0	II			182.77	NED	
7	M/65	Upper trunk, cutaneous	0.9	II			30.77	NED	
High-Grade LMS with changes in chromosome 17p									
8	F/80	Hand, subcutaneous ^{a,b,d}	2.0	III			72.50	DEA	WHT
9	M/61	Thigh, deep ^d	8.0	III	40.43		211.87	NED	
10	M/46	Thigh, cutaneous ^d	2.3	III			49.30	NED	
High-grade LMS without changes in chromosome 17p									
11	F/91	Thigh, subcutaneous	13.0	IV		4.10	8.00	DEA	FT
12	F/55	Thigh, subcutaneous ^{a,d}	2.0	IV		78.93	135.23	DEA	FT
13	F/77	Upper trunk, deep ^{a,b,d}	6.0	IV	6.87	6.87	7.57	DEA	FT
14	M/35	Lower leg, deep ^c	10.0	IV		0.00	11.33	DEA	FT

Abbreviations: NED, no evidence of disease; DEA, death; FT, from tumor; WHT, without tumor.

^a Vascular connection.

^b Postoperative radiotherapy.

^c Postoperative chemotherapy.

^d Adequate local treatment.

Regardless of the tumor grade, the minimal common regions of gain in DNA pooled from all 14 LMS samples were narrowed down to 1cen~q21 (cases 8–10 in the high-grade LMS pool with changes in chromosome 17p, and cases 1–3 in the low-grade LMS pool with changes in chromosome 17p) as well as 19p (cases 1–3 in the low-grade LMS pool with changes in chromosome 17p). 1cen~q21 was gained in 4 of the 14 single cases (28.6%), whereas 19p was amplified in three of the 14 single cases analyzed (21.4%). The minimal common regions of loss were 4p15.1~p15.2 (cases 1–3 in the low-grade LMS pool with changes in 17p); 10q23~q26.2 (cases 8–10 in the high-grade LMS pool with changes in 17p); 13q12.2~q12.3 (cases 1–3 in the low-grade LMS pool with changes in 17p); 13q14~q32 (cases 8–10 in the high-grade LMS pool with changes in 17p); and 13q (cases 4–7 in the low-grade LMS pool without changes in 17p). In single tumors, these changes were present at frequencies of 7.1, 57.1, 57.1, 42.8, and 35.7%, respectively. High-level amplification in 17p was present in 28.6% of the tumors analyzed case-by-case by cCGH (Figs. 1 and 2).

3.2. aCGH

Regardless of the tumor grade, the minimal common regions of gene amplifications in the DNA pools were narrowed down to 15q26~qter (50.0%; cases 1–3 in the low-grade LMS pool with changes in 17p, and cases 8–10 in the high-grade LMS pool with changes in 17p) as well

as 17p13.1~q11 (50.0%; cases 1–3 in the low-grade LMS pool with changes in 17p, and cases 8–10 in the high-grade LMS pool with changes in 17p). 15q26~qter was amplified in 2 of the 14 single cases (cases 3 and 10; 14.3%) and 17p13.1~q11 in 3 of the 14 single cases analyzed (cases 2, 9, and 10; 21.4%). The minimal common regions of gene deletions were 6p12~p21.3 (50.0%; cases 1–3 in the low-grade LMS pool with changes in 17p); 13q14.3~qter (75.0%; cases 8–10 in the high-grade LMS pool with changes in 17p; cases 1, 2, and 3 in the low-grade LMS pool with changes in 17p; and cases 4–7 in the low-grade LMS pool without changes in 17p). In single tumors, these changes were present at frequencies of 28.6 and 42.9%, respectively (Table 3; Figs. 1 and 2). Figs. 1 and 2 show the other recurrent areas of gene amplification and deletion that are summarized in Table 3. The minimal common regions of DNA copy number gains and losses are presented in Appendix 1 (http://www.helsinki.fi/cm/microarray_data). The original microarray data are posted at <http://www.cangem.org/>.

4. Discussion

We analyzed 14 primary soft-tissue LMS using aCGH. The study represents the first application of cDNA-based CGH microarray methodology to genome-wide analysis of copy number changes in LMS.

Table 2
cCGH and aCGH results from 14 primary soft-tissue leiomyosarcoma samples

Sample no.	DNA sequence copy number changes
Low-grade LMS with changes in chromosome 17p	
cCGH	
1	rev ish enh(1p13~q21, 3p14~pter, 16p, 19, 22) dim(1q31~qter, 8q13~qter, 9p21~pter, 10q21~qter, 13q12~q31, 16q, Xq21~qter) amp(17p)
2	rev ish enh(6q, 17cen~q21, 19pter~q13.2) dim(6p, 13cen~q21) amp(17p)
3	rev ish enh(1, 12q, 15q15~qter, 16cen~p11.2) dim(2q35~qter, 6p21.3~pter, 7q33~qter, 8pter~q22, 10q, 12p, 13, 16q, 18, 21, 22, Xq) amp(17p)
Pooled DNAs (1–3)	rev ish enh(1p13.2~q21.2, 19p) dim(4p15.1~p15.2, 13q12.2~q12.3) amp(17p) cDNA-based aCGH
Pooled DNAs (1–3)	–2p25 (5 genes), –6p12~pter (328 genes), +6p12~qter (221 genes), –10q26.3~qter (14 genes), –12p13.3 (36 genes), –13q14~q34 (154 genes), +15q26 (5 genes), +17p13.1~q11 (65 genes), –19q13.33~q13.43 (130 genes), –22q11.21 (13 genes)
Low-grade LMS without changes in chromosome 17p	
cCGH	
4	rev ish enh(5p) dim(6, 10, 16, 17)
5	rev ish enh(2p12~p14, 14) dim(2p21~pter, 10, 13, 16)
6	rev ish dim(1p21~p31, 6, 9p, 13, 14q11.2~q24, Xq22~qter)
7	rev ish dim(13, 16)
Pooled DNAs (4–7)	rev ish dim(13q) cDNA-based aCGH
Pooled DNAs (4–7)	–13cen~qter (189 genes)
High-grade LMS with changes in chromosome 17p	
cCGH	
8	rev ish enh(1q21~q22, 8q, 9q, 17p, 20q, Xp) dim(1q24~q32, 2q33~qter, 6q, 8p, 9p, 10q, 11p, 11q14~qter, 13q14~qter, 18, Xq) amp(17p12, Xp21~pter)
9	rev ish enh(1pter~q31, 6p, 9, 10p12~pter, 17, 20, Xp) dim(2q22~qter, 3p, 4p, 12p, Xq21~qter) amp(1q21~q24, 5p, 17p12~pter)
10	rev ish enh(9q11~q21, 14, 15, 17cen~q21) dim(6p, 7, 10q21~qter, 13, 16q, X) amp(17p)
Pooled DNAs (8–10)	rev ish enh(1cen~q21) dim(10q23~q26.2, 13q14~q32, 16q22) amp(17p) cDNA-based aCGH
Pooled DNAs (8–10)	+10pter~q11.22 (96 genes), –10q11.22~q24.3 (282 genes), –13q14.3~q34 (65 genes), +15q11~q26.3 (285 genes), +17p13.1~q11 (49 genes), –17q25.3 (36 genes), +19p13.2~p13.3 (59 genes), +20p11 (9 genes), –Xq13.2~q28 (189 genes)
High-grade LMS without changes in chromosome 17p	
cCGH	
11	rev ish enh(1q21~q23, 3q26.3~qter, 5p, 16p, 19, 20q) dim(1q32~qter, 2p12~pter, 2q32.3~qter, 5q32~qter, 6q, 8p, 10, 11, 12, 13q12~q31, 17q, 22, Xpter~q25) amp(20p)
12	rev ish enh(1q12~q23, 8) dim(2, 4q, 10, 13q14~q22)
13	rev ish enh(2, 3q, 7p, 8q)
14	rev ish enh(5, 8q21.3~qter, 20, 22, X) amp(5p13~pter, 8q24.2~qter)
Pooled DNAs (11–14)	rev ish normal cDNA-based aCGH
Pooled DNAs (11–14)	–6p21~q26 (211 genes), –10p12.32~pter (43 genes), +20pter~q11.2 (118 genes), +20q13~qter (6 genes)

The reasons why aCGH was performed on pooled DNA were economical, practical, and biologic. The present cDNA-based CGH microarrays require a rather large amount of DNA (20 µg) that sometimes cannot be obtained from clinical material. Furthermore, the costs associated with array technology are still relatively high. The general concept is that biologically significant “driver” genes co-exist in losses and amplifications, whereas many other genes in the amplicon or loss area are secondary and biologically nonessential. Thus, secondary changes that vary from case to case are concealed in pooled DNA while driver genes become visible in a cumulative manner. Craig et al. [24] have shown that pooled DNA samples on single-nucleotide polymorphism microarrays allow accurate

identification of relevant genomic regions. Sample pooling reduced the effects of biological variation on gene expression arrays and yielded comparable expression measurements from pools and individuals [25]. Zhang and Gant [26] have recently quantitatively characterized the effect of sample pooling on identification of differentially expressed genes using microarrays.

To test the correspondence between the DNA copy number changes detected by pooled and nonpooled approaches, we performed cCGH on pooled cases and individual cases. The comparison was restricted to cCGH results because the amount of DNA was too limited for individual aCGH. Importantly, pooled aCGH showed all the changes that were frequent in the nonpooled approach. Thus, the array results

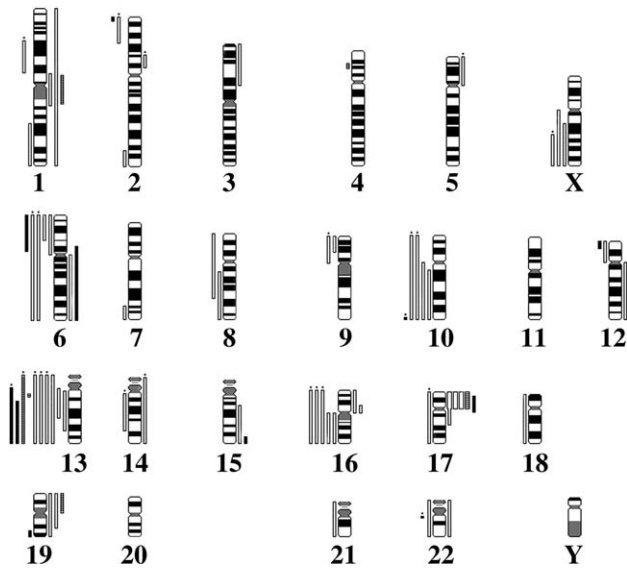


Fig. 1. Summary of DNA copy number gains, losses, and high-level amplifications of small chromosomal areas detected by cCGH in seven low-grade soft-tissue leiomyosarcomas. Each hollow bar represents aberrations found in one sample. Losses are shown on the left, gains on the right of each chromosome, and wide bars depict high-level amplifications. Striped bars show the cCGH results obtained using pooled DNA, and solid bars represent aCGH results. A dot over a bar indicates that the aberration was present in a sample with at least one DNA copy number change in chromosome 17p.

from the pooled approach can be interpreted as characteristic biologically significant changes in LMS, even when some less frequent changes may have remained undetectable in our pool.

Since the 17p amplicon has been reported to be a characteristic change in LMS, we selected the samples for pooling according to this chromosomal feature, in addition to tumor grading. As expected, the pooled approach showed amplified genes only in the 17p amplicon pool but not in the pool without changes in 17p.

Our aCGH results revealed 25 altered chromosomal regions (at least five consecutive genes gained or lost) involving a total of 2218 genes. Previous cCGH reports demonstrate that among the most prominent DNA copy number changes in LMS are losses in 10q and 13q, as well as gains in 16p and gains and/or amplifications in 17p [27,28]. Our cCGH results agree with previous observations. Our study did not reveal any novel amplicons or losses, but all previously mentioned changes were found in all samples, regardless of the tumor grade. Therefore we suggest that these changes may harbor genes involved in the tumorigenesis of LMS.

Most chromosomal regions with consecutively amplified or deleted genes (<http://www.cangem.org/>) were complex and contained amplified, nonamplified, and even deleted genes, supporting previous observations from microsatellite marker analyses [29]. Furthermore, the aCGH results (Table 3) revealed that the number of areas affected by gene copy number losses in the low-grade LMS pool

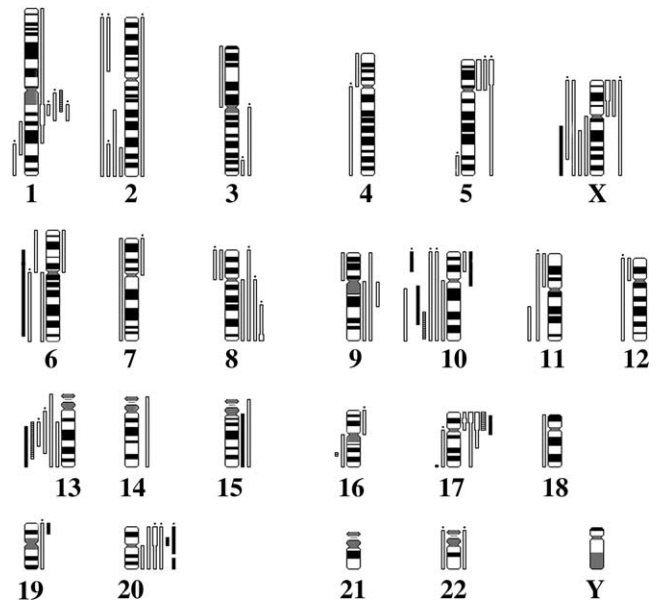


Fig. 2. Summary of DNA copy number gains, losses, and high-level amplifications of small chromosomal areas detected by cCGH in seven high-grade soft-tissue leiomyosarcomas. Each hollow bar represents aberrations found in one sample. Losses are shown on the left, gains on the right of each chromosome, and wide bars depict high-level amplifications. Striped bars show the cCGH results obtained using pooled DNA, and solid bars represent aCGH results. A dot over a bar indicates that the aberration was present in a sample with at least one DNA copy number change in chromosome 17p.

(10 areas) was higher in comparison to the high-grade LMS pool (7 areas). The number of chromosomal areas with gene copy number gains in high-grade tumors (9 areas) was threefold in comparison to low-grade tumors (3 areas). This suggests an increasing trend in the number of gene DNA sequences during the progression of LMS.

The altered areas varied in size from 118 Mbp (+6p12~qter) to 1 Mbp (+15q26~qter), both present in the low-grade LMS pools detected by aCGH. The low-grade and high-grade LMS pools shared two minimal common regions of gains (15q26~qter and 17p13.1~q11) and two regions of losses (6p12~p21.3 and 13q14.3~qter). Besides, we observed nine different altered areas only in the low-grade LMS pool (-2p25, -6p22~pter, +6p12~qter, -10q26.3, -12p13.3, -13cen~q13, -13q14.1~q14.2, -19q13.33~q13.43, and -22q11.21) and 12 areas only in the high-grade LMS pool (-6p11.2~q26, +10pter~q11.22, -10p12.32~pter, -10q11.22~q24.2, +15cen~q25.3, -17q25.3, +19p13.2~p13.3, +20p11, +20pter~p12, +20q11.1~q11.2, +20q13~qter, and -Xq13.2~qter). In the minimal common regions, the number of genes involved in each region varied between the low- and high-grade pools. Accordingly, the affected chromosomal areas may contain not only genes necessary for the tumorigenesis of LMS in general, but also genes specific to its initiation and progression (present most probably in low-grade but not in high-grade LMS), and to its aggressiveness (present most probably in high-grade but not in low-grade LMS) (detailed

Table 3
Amplified and deleted chromosomal areas detected by aCGH in
14 primary soft-tissue leiomyosarcomas^a

Chromosome	Low-grade leiomyosarcomas ^{b,c}	High-grade leiomyosarcomas ^{b,c}
2	–2p25 (5/1.2)	
6	–6p12~p21.3 (218/17) +6p12~qter (221/118) –6p22~pter (54/25)	–6p11.2~q26 (183/104) –6p12~p21.3 (22/6)
10	–10q26.3~qter (14/3.4)	+10pter~q11.22 (98/43) –10q11.22~q24.2 (158/55) –10p12.32~pter (41/5)
12	–12p13.3 (36/6)	
13	– 13cen~q13 (72/22.3) – 13q14.1~q14.2 (42/10.1) – 13q14.3~qter (72/30)	–13q14.3~qter (65/27)
15	+15q26~qter (5/1)	+15cen~q25.3 (252/64) +15q26~qter (14/11)
17	+17p13.1~q11 (62/7)	+17p13.1~q11 (49/13) +17q25.3 (36/3)
19	–19q13.33~q13.43 (130/9)	+19p13.2~p13.3 (59/5)
20		+ 20p11 (32/8) +20p12~pter (51/9) +20q11.1~q11.2 (19/3) +20q13~qter (6/2)
22	– 22q11.21 (13/1.9)	
X	–Xq13.2~qter (189/78)	

^a Only regions containing at least five consecutive deleted or gained genes are included.

^b Chromosomal areas seen both in samples with and without changes in chromosome 17p are in boldface.

^c The number of genes involved/region size in Mbp are in parentheses.

lists of the genes at <http://www.cangem.org/>). Some of the affected regions detected by aCGH were so small that cCGH would not have been likely to show them [e.g., –2p25 (1.2 Mbp), –12p13.3 (6 Mbp), +15q26~qter (1 Mbp), and –22q11.21 (1.9 Mbp) in the low-grade pools (pool of cases 1–3 and pool of cases 4–7) and –6p12~p21.3 (6 Mbp), –17q25.3 (3 Mbp), +19p13.2~p13.3 (5 Mbp), and +20q13~qter (2 Mbp) in high-grade pools (pools of cases 8–10 and pool of cases 11–14)]. Only 32 of the 507 commonly altered genes, which are found both in the low- and high-grade LMS, have been reported to be associated with other human neoplasms. Nineteen of them (59%) are located in 6p, one of the most commonly deleted chromosomal arms in LMS, and seven (22%) are located in 17p, one of the most commonly amplified chromosomal arms in LMS, while the remaining six genes are present in 13q14.3~qter (five genes, 16%) and 15q26~qter (one gene). All these genes have been observed altered in a wide variety of human neoplasias affecting both solid and hematologic tissues (detailed information of each gene with bibliographic references in Appendix 1).

Increased DNA sequence copy number at 1q21~q23 has been detected in several malignancies, but especially in sarcomas including LMS, liposarcoma, osteosarcoma, chondrosarcomas, and malignant fibrous histiocytoma (see

Knuutila et al. [19] and references therein). It has been demonstrated that the expression of cellular retinoic acid-binding protein 2 (*CRABP2*) located at 1q21.3 is 5- to 10-fold in LMS as compared with normal myometrium [30]. This region has been reported to harbor several other genes of potential significance [i.e., the octamer-binding transcription factor (*OTFI*; 1q22~q25) and several members of the *S-100* gene family cluster, including *CACY* and *CAPL* (1q21~q25), as well as *MUC1* (1q21)] [31–33]. The 1q amplicon in human sarcomas has been characterized recently using molecular analysis. Although *FLG* (1q21), *NTRK1* (1q21~q22), and *SPRR3* (1q21~q22) were the most frequently amplified genes, none of them were amplified in all of the samples with increased copy number at 1q21~q22 [10,31]. In our material, while cCGH revealed this amplicon at 1q in 6 of the 14 samples (43%), aCGH was unable to detect it although a high-level amplification in one of the high-grade LMS samples was observed. So far, we do not have any plausible explanation for this peculiar finding, but further molecular cytogenetic analysis by in situ hybridization with locus-specific probes for 1q21~q23 could give some clues.

Gains as well as high-level amplifications at 17p11~p12 have been reported as one of the most commonly observed alterations in sarcomas, including LMS, osteosarcoma, chondrosarcoma, and malignant fibrous histiocytoma [27]. Using aCGH, we were able to define 17p13.1~q11 as a common recurrently amplified area both in the low- and high-grade pools. The size of the amplified area appeared to vary, as did the number of genes therein. The 17p13.1~q11 region spanned from 13 to 7 Mbp in the high- and low-grade tumors, respectively. Notwithstanding, several common genes were equally overrepresented irrespective of the tumor grade and the size of the 17p amplicon. The original microarray data are posted at <http://www.cangem.org/>. Our present LMS findings show that several genes within the amplicon are common both in LMS and osteosarcoma [21]. Among them are *COX10* (cytochrome c oxidase assembly protein, heme A), *PMP22* (peripheral myelin protein), *ADORA2B* (adenosine A2b receptor), *ZNF287* (zinc finger protein 287), *M-RIP* (myosin phosphatase-Rho interacting protein), *COPS3* (constitutive photomorphogenic homolog subunit 3), *PMT* (phosphatidylethanolamine N-methyltransferase), *SREBF1* (sterol regulatory element binding transcription factor 1), *TOM1L2* (target of myb1-like 2 (chicken)), *TOP3A* [topoisomerase (DNA) III α], and *MAPK7* (mitogen-activated protein kinase 7). Further analyses by quantitative real-time polymerase chain reaction are required to confirm our results.

Losses in 13q, 16q, and 10q have been recently reported to be recurrent changes in LMS [9,12]. By aCGH, we observed DNA copy number losses in 10q, specifically at 10q26.3~qter and 10q11.22~q24.2 in the low- and high-grade LMS pools, respectively. These findings are consistent with previous studies on LMS. The tumor suppressor gene *PTEN* (10q23) has been mapped to this overlapping

region [34], which has been reported to be lost in uterine LMS but not in leiomyomas [35]. Loss of heterozygosity and mutation of the remaining allele of the *PTEN* gene have been identified frequently in other neoplasms (e.g., glioblastoma and prostate, breast, and endometrial carcinoma) [36–39]. Moreover, *MXI1*, a negative regulator of the MYC oncoprotein with tumor suppressing function, has been mapped to the nearby region at 10q25. Altered *MXI1* function as such may directly contribute to tumorigenesis, as has been observed in astrocytomas, prostate cancer, and melanoma [40–42].

A complete list of the genes in the minimal common region is shown in Appendix 1 (http://www.helsinki.fi/cm/microarray_data). Among the most intriguing genes, whose copy number sequence was revealed to be altered by aCGH (Appendix 1), are *VEGF* (6p21), *RUNX2* (6p21), *CDKN1A* (6p21), *BAK1* (6p21), and *COPS3* (17p11.2). *COPS3* (COP9 constitutive photomorphogenic homolog subunit 3) is also known to be involved in other sarcoma types (e.g., osteosarcoma) [43]. *COPS3* was found amplified both in the LMS samples included in this study and in the osteosarcoma samples reported in our previous study [21]. The tumor suppressor gene *RUNX2* (runt-related transcription factor 2) was deleted in low-grade LMS but not in high-grade LMS, suggesting loss or reduction in activity of the gene in the early stages of the tumorigenesis. *CDKN1A* (cyclin-dependent kinase inhibitor 1A) and *BAK1* (BCL2-antagonist/killer 1) genes, which are involved in cell cycle arrest and regulation of apoptosis, were deleted only in low-grade LMS, suggesting that they may act as tumor suppressors in LMS. *VEGF* (vascular endothelial growth factor) was deleted in our LMS, although the gene is expressed in several primary brain tumors as an angiogenic growth factor that induces the formation of cysts in brain tumors.

In summary, chromosomal CGH performed on single cases and pooled DNA from soft-tissue LMS samples combined with cDNA-based array-CGH analysis of global gene copy number changes accurately revealed the chromosomal areas that harbor putative tumor suppressor genes and oncogenes that could play a role in LMS tumorigenesis. Details of the amplified and deleted genes in each amplified and deleted chromosomal area (http://www.helsinki.fi/cm/microarray_data; <http://www.cangem.org/>) provide valuable raw data for further gene expression studies. Additional functional studies are required to determine not only the biologic but also the clinical significance of the genetic imbalances we observed.

Acknowledgments

We thank Tiina Wirtanen and Aki Väyrynen for technical assistance in CGH analysis. This work was supported by the Finska Läkaresällskapet, the K. Albin Johansson Foundation, the Kurt and Doris Palander Foundation (C.S.), Helsinki University Central Hospital research funds

in Finland, and the European Commission grant (Prothets contract no. LSHC-CT-2004-503036), as well as the National Council of Scientific and Technological Research (CONICET) and National University of La Plata (Grant Number 11/N325, M.L.L.) in Argentina.

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