Leiomyosarcomas and Most Malignant Fibrous Histiocytomas Share Very Similar Comparative Genomic Hybridization Imbalances: An Analysis of a Series of 27 Leiomyosarcomas

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SUMMARY: Twenty-seven tumor samples with a diagnosis of leiomyosarcomas (LMS) were characterized by comparative genomic hybridization. The results were compared with immunohistochemical analysis of the smooth muscle profile of the tumors and expression of the RB1 gene protein. The comparative genomic hybridization profiles suggested that 7 of the 27 tumors might have been misclassified. High levels of DNA amplification were detected in 20 different small regions and recurrently involved bands 1p34, 1q21, 12q13–15, 17p, and 22q. Most recurrent simple gains were noted at sites such as 1p3, 1q21, 15q12–15, 16p, 17p and 17q, 19, 20q, 22q, and Xp. Significant losses of chromosome 13 were detected in 19 of the 27 tumors with a putative common region of loss in bands 13q14–21. Losses of chromosomes 1q, 2p and 2q, 4q, 9p, 10p and 10q, 11p and 11q23, and 16q were also highly recurrent. A comparative analysis between the most frequent genomic imbalances observed in this study of LMS and the genomic imbalances observed in a large proportion of malignant fibrous histiocytomas (MFH) from a previous study demonstrated that both types of tumors had similar recurrent imbalances. Although MFH were once thought to be a separate member of the soft tissue sarcoma family, our observations support the hypothesis that MFH are a morphologic modulation in the tumoral progression of other sarcomas, particularly LMS. (*Lab Invest 2001, 81:211–215*).

Leiomyosarcomas (LMS) are relatively frequent sarcomas of adult life. They are principally tumors of uterine or gastrointestinal origin, but also account for 5% to 10% of soft tissue sarcomas (STS) (Enzinger and Weiss, 1995). Within the past few years, with better identification of dedifferentiated liposarcomas and the recognition of malignant fibrous histiocytomas (MFH) as a separate entity, the relative incidence of LMS among STS has changed. The recent characterization of gastrointestinal stromal tumors (GIST) as a distinct classification also influences the incidence of gastrointestinal and retroperitoneal LMS. Contrary to MFH, osteosarcomas, or fibrosarcomas, LMS rarely occur after radiation, but may develop in patients with a constitutional inactivation of one allele of the RB1 gene and with a previous diagnosis of bilateral retinoblastoma. LMS are much more common in women than men, even for nonuterine locations. Until now, few cytogenetic or molecular data were available for LMS (Mandahl et al, 2000), and only a few series were analyzed by comparative genomic hybridization (CGH) (El-Rifai et al, 1998; Otano-Joos et al, 1998; Packenham et al, 1997; Parente et al, 1999). In this study, we analyzed a series of 27 LMS by immunohistochemistry and CGH. We found that the most frequent genomic alterations in LMS were losses in the 13q14–21 region. LMS and a large proportion of MFH were also found to share very similar CGH imbalance profiles, which suggests that these two tumors may correspond to different differentiation states of a single tumor type.

Results

Imbalances Detected by CGH

One of the 27 samples (LMS 23) did not exhibit any imbalance. A considerable contamination of this tumor sample by nontumoral cells might account for these findings. Another tumor (LMS 28) had a single aberration, which was not recurrently observed in the other samples. The regions of DNA copy number

imbalances observed in the 27 tumors are shown in Figure 1.

Gains of DNA. High levels of DNA amplification (green to red ratio greater than 1.5) were observed in 20 different small regions. Among them, bands 1p34, 1q21, 12q13–15, 17p, and 22q were recurrently observed as amplified regions in different tumors. The most recurrent simple gains involved 1p3, 1q21, 15q12–15, 16p, 17p and 17q, 19, 20q, 22q, and Xp.

Losses of DNA. As shown in Figure 1, significant losses in chromosome 13 were observed in 19 of the 27 tumors, with a putative common region of loss in bands 13q14–21. In one other tumor (LMS 7), probably contaminated by a large number of normal cells, chromosome 13 loss was suggested by a green to red ratio of 0.85. Losses of chromosome 1q, 2p and 2q, 4q, 9p, 10p and 10q, 11p and 11q23, and 16q were also recurrent.

Immunohistochemistry

Results are summarized in Table 1 along with the main clinical data. In general, expression of the smooth muscle markers, common muscle actin (HHF 35), smooth muscle actin, and desmin, did not strictly correlate with tumor grade. Some low-grade LMS were negative for desmin expression, whereas some high-grade LMS were strongly immunoreactive for the three smooth muscle markers. There was no correlation between grade, intensity of immunoreactivity of the smooth muscle markers, and expression of Rb1 gene protein. With one exception, all tumors were

Figure 1.
Ideogram of regions of DNA copy number imbalances. *Vertical lines* on the right and left sides of chromosomes indicate gains and losses, respectively.

negative for CD 34 and CD 117. The one exception (LMS 14) originated in the retroperitoneum, was of a large volume (15 cm in diameter), and was immunoreactive for common muscle actin (HHF 35) and smooth muscle actin.

Discussion

The CGH analysis of a series of 27 LMS showed the occurrence of genomic imbalances in all tumors but one. In this tumor, LMS 23, we did not find either chromosome imbalance by CGH or allelic imbalance by microsatellite allelotyping (data not shown). The presence of a prominent inflammatory component in this tumor could account for the normal CGH profiles. In LMS 28, the only detected imbalance corresponded to an intercalary deletion of the 9q21–31 bands. At the time of diagnosis, the patient had two synchronous tumors; one tumor within the transverse colon (4 cm in diameter) and a second tumor within the pelvis (12 cm in diameter). This patient is alive and free of disease 5 years later, which raises the possibility that both synchronous low grade LMS were in fact multiple leiomyomas. Three unique tumors from the abdominal cavity, LMS 43, LMS 45, and LMS 20, expressed the three muscular markers and did not express CD34 or CD117. All three tumors had losses of whole 1p; LMS 43 also had a gain of whole 1q and LMS 45 also had a loss of chromosome 14. These profiles are suggestive of GIST (Breiner et al, 2000; Derré et al, 2000; El-Rifai et al, 1996, 2000). Moreover, a number of GIST can express muscular markers (Miettinen et al, 1999; Sircar et al, 1999). Three other tumors (LMS 40, LMS 21, and LMS 47) were characterized by simple CGH profiles associated with multiple small amplifications (such as bands 12q14–15). This is reminiscent of the profiles found in well-differentiated liposarcomas (Suijkerbuijk et al, 1994). The morphologic characterization of LMS 14 was difficult to assess because two muscular markers (HHF35 and smooth muscle actin) were expressed along with moderate CD 117 expression, which could be suggestive of GIST, but the CGH profile of LMS 14 was incompatible with a GIST diagnosis. Thus, the CGH analysis of this series of 27 tumors suggests that 7 of them might have been misclassified by histopathologic and immunohistochemistry criteria alone.

We did not find clear correlations between the sizes of the tumors, grades, and any of the aberrations observed. In particular, gains of 16p, 17p, 1q, and 8q, previously reported as correlating with size (El-Rifai et al, 1998), seemed relatively widespread in the tumors analyzed in our study. Our immunohistochemical findings did not support this correlation.

As previously noted in a small series of LMS (Otano-Joos et al, 1998), chromosome 13 loss, observed in 19 of the tumors in our series, was the most frequent genomic aberration. In the current study, CGH demonstrated that the smallest region of overlap of these losses was localized to the 13q14–21 region. As recently demonstrated for MFH (Chibon et al, 2000), these observations could suggest that the RB1 gene is also the target of these deletions. Our immunohistochemical analysis, which showed that most tumors
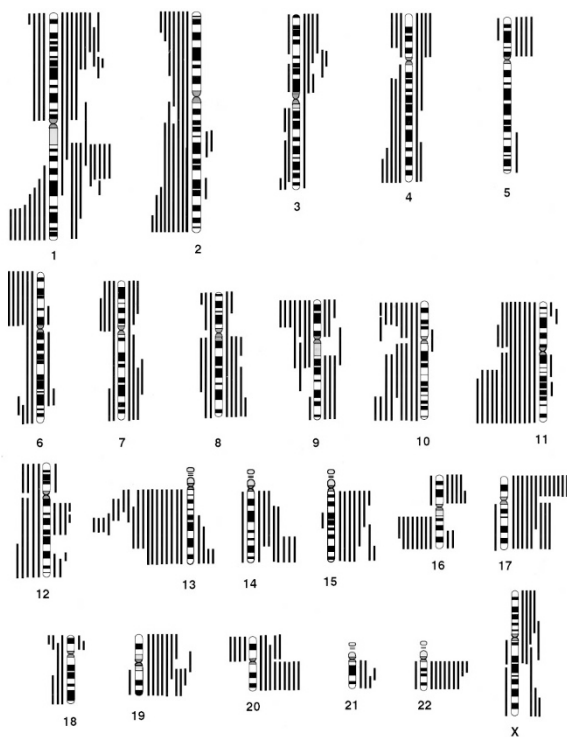
Table 1. Results of Immunohistochemistry

Cases	Sex	Age	Grade	PT/R	Tt	Site	Size	HHF35	Sarc	Smooth	Desm	Vim	CD34	CD117	RB1
LMS 05	M	75	II	R	RT	Back	3	0	0	0	+	+++	0	0	0
LMS 07	F	88	III	PT		Breast	8	+	0	0 to +	0	+++	0	0	+
LMS 10	F	60	III	PT	CT	Breast	9	++	+	+	++	++	0	0	+ to ++
LMS 11	F	64	III	PT	CT	Thigh	13	0	0	+	0	+++	0	0	0
LMS 12	M	59	I	PT		Arm	3	+++	0	+++	0	0 to +	0	0	0 to +
LMS 14	M	49	III	PT		Retrop	15	++	0	++	0	0 to +	+++	+ to ++	0
LMS 17	F	41	II	PT		Breast	3	0	0	0	++	+++	0	0	+
LMS 19	M	64	III	PT	CT	Neck	10	++	0	+	0	+++	0	0	0 to +
LMS 20	F	64	III	PT		Small int	10	+++	0	+++	+++	+++	0	0	0 to +
LMS 21	M	57	III	PT		Retrop	10	+	0	++	0	+++	0	0	++
LMS 22	F	42	III	PT	RT	Pelvis	11	+	++	0	+	+++	0	0	0 to +
LMS 23	F	52	III	PT		Trunk	11	+	0	++	0	+++	0	0	++
LMS 24	F	49	III	PT		Retrop	17	+++	0	+++	+++	+++	0	0	0
LMS 25	F	70	I	PT		Leg	10	+++	0	+++	+++	++	0	0	0 to +
LMS 26	F	50	III	PT		Trunk	15	+++	0	+++	++	++	0	0	0 to +
LMS 28	M	24	I	PT		Colon	11	+++	0	+++	+++	++	0	0	0 to +
LMS 29	F	44	III	PT		Pelvis	10	+++	0	+++	+++	+++	0	0	0 to +
LMS 30	F	67	III	PT		Cervix	11	0	0	+	0	+++	0	0	0 to +
LMS 31	F	36	II	PT		Uterus	10	++	0	++	0 to +	+++	0	0	0 to ++
LMS 32	F	75	II	PT		Groin	3	+++	0	+++	0 to +	++	0	0	0
LMS 36	M	26	II	PT	CT	Femur	5	+	0	+	+++	+++	0	0	+
LMS 38	F	73	I	PT		V. cava	5	+++	0	+++	+++	0 to +	0	0	+
LMS 40	M	64	III	PT		Thigh	8	++	0	++	++	+++	0	0	+ to ++
LMS 41	F	52	II	PT		Iliac	2	+++	+	+++	+++	+++	0	0	+ to ++
LMS 43	F	65	I	R		Pelvis	13	+++	0	+++	+++	++	0	0	0 to ++
LMS 45	F	56	I	PT		Retrop	10	++	0	++	++	+	0	0	0 to +
LMS 47	M	61	III	PT		Stom	9	++	0	++	++	++	0	0	0 to +

LMS, leiomyosarcoma; PT, primary tumor; R, relapse; Tt, treatment; RT, radiotherapy; CT, chemotherapy; Sarc, sarcomeric; Desm, desmin; Vim, vimentin; F, female; M, male.

with chromosome 13 deletions did not express the RB1 protein, supports this hypothesis. Our data is also in agreement with the known occurrence of LMS in patients with bilateral retinoblastoma, who have constitutional inactivation of one allele of RB1 (Dunkel et al, 1998).

Apart from the chromosome 13 deletion, which is common to most tumors, our CGH results are similar to those observed in approximately 80% of MFH (Mairal et al, 1999), particularly the results of tumors that seem correctly diagnosed. A comparative analysis between the most frequent genomic imbalances observed in the present series of LMS and those of our previous findings on MFH (Mairal et al, 1999; and unpublished data) are presented in Figure 2. Almost all recurrent imbalances are observed in both series, especially the most frequently recurring imbalances, which are thought to correspond to precocious oncogenic events. The complexity of the CGH profiles and the fact that similar profiles have not been described in other malignancies (for review, see Knuutila et al: CGH Data of DNA Sequence

Number Changes in Human Neoplasms, http://www.helsinki.fi/lgl_www/CMG.html), strongly suggest that the profiles are not similar by chance. The morphologic studies to characterize MFH were not able to delineate specific markers or to describe the phenotype for this sarcoma of supposed "fibrohistiocytic" lineage. There is growing evidence that MFH is a second component in another sarcoma and represents a morphologic modulation resulting from tumor progression (Dehner, 1988; Fletcher, 1987; Hollowood and Fletcher, 1995). The five previously described subtypes of MFH seem to have few links, if any, to tumors of fibrohistiocytic differentiation, and the angiomatoid variant is no longer thought to be a fully sarcomatous member of STS (Costa and Weiss, 1990; Fanburg-Smith and Miettinen, 1999). Rather, the giant cell variant shares characteristics common to giant-cell–rich osteosarcoma of soft tissues (Guccion and Enzinger, 1972) and a number of inflammatory MFH overlap with inflammatory LMS (Merchant et al, 1995). Additionally, poorly differentiated myxoid MFH merge with pleomorphic MFH (Weiss and

Enzinger, 1977), which are no longer thought to be tumors of true histiocytic differentiation. Our results suggest that MFH do not represent a specific sarcomatous entity, but may instead represent a common oncogenic pathway in the progression of LMS and possibly other STS. If this hypothesis is correct, most MFH could represent an ultimate state of tumor progression for LMS. Nevertheless, among the 26 imbalances listed in Figure 2, 6 (+14, +15q, +18p, −6p, +6p, and −3p) showed different occurrences in LMS versus MFH. If our hypothesis of a common oncogenesis for LMS and MFH is verified, genes important for smooth muscle cell differentiation may be localized in some of these six chromosomal regions.

Materials and Methods

Tumor Samples

The 27 frozen samples were obtained from three different pathology laboratories. The diagnosis and grading was established according to the recommendations of the French "Sarcoma Group" of the FN-CLCC (Fédération Nationale des Centres de Lutte Contre le Cancer). The 27 tumors were from 27 patients. Two of the tumors were from relapses, and 25 from primary tumors. Six tumors were of grade I, 6 were of grade II, and 15 were of grade III. Six of the samples were obtained after a pretreatment of radiotherapy or chemotherapy. Patients consisted of 18 women and 9 men, and ranged in age from 24 to 88 years, mean age of 56.5 years. The main clinical and pathology data are listed in Table 1.

CGH and Image Analysis

CGH was performed as previously described (Kallionemi et al, 1992). Tumor DNA was directly labeled by nick-translation using an FITC-dUTP nucleotide (DuPont NEN, Boston, Massachusetts). The nick-translation conditions were adjusted to obtain DNA fragments ranging from 500 to 2000 bp. SpectrumRed normal male reference DNA (Vysis, Downers Grove, Illinois) was used as control DNA. Four hundred nanograms of tumor DNA, 400 ng of control DNA, and 80 μg of Cot-1 DNA (GibcoBRL, Life Technologies, Gaithersburg, Maryland) were mixed in 15 μl of hybridization buffer (50% formamide, 40 mm NaH_2PO_4, 0.1% SDS, 10% dextran sulfate, 2x SSC). CGH pictures from a mean of 10 metaphases were captured using a Leica DMRB fluorescence microscope and a Photometrics Nu200 CCD camera, and analyzed with the Quips XL software (Vysis).

Green to red ratios greater than 1.2 and less than 0.8 were regarded as gains and losses, respectively. Ratios greater than 1.5 were regarded as high level amplifications. Negative controls corresponding to normal DNA hybridized to the same batches of metaphase spreads were included in each series of experiments. Only experiments fulfilling previously defined quality criteria were included (Kallionemi et al, 1994). The analysis of the profiles observed in some GC-rich chromosome regions, such as 1p, 19, and 22, known to give some false-positive results, were interpreted with caution, particularly for the low level of imbalances.

Immunohistochemistry

Tissue sections were obtained from paraffin blocks. They were deparaffinized in xylene, and rehydrated in a series of ethanol baths. For antigen retrieval, slides were incubated in 10 mM citrate buffer, pH 6.1, for 20 minutes in a microwave oven. Antibodies used to characterize the tumors were purchased from Dako Corporation (Carpinteria, California). The antibodies used were common-muscle actin HHF-35 (Dako M 0635), sarcomeric actin (Dako M 0874), smooth muscle actin (Dako M 0851), desmin (Dako M 0760), vimentin (Dako M 0725), CD 34 (Dako M 7165), and
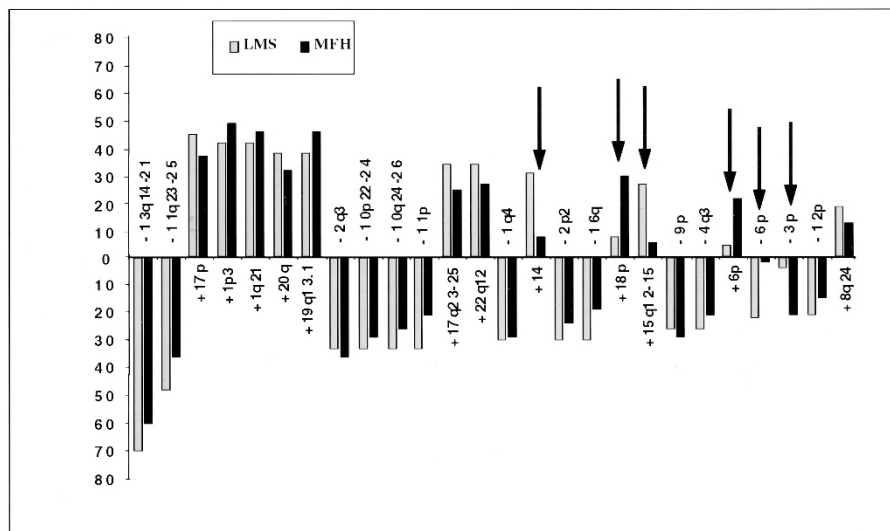

Figure 2.

Comparative frequencies of recurrent imbalances between leiomyosarcoma and malignant fibrous histiocytoma. The six main differences are indicated by *arrows*.

CD 117 (c-kit oncoprotein, Dako A 4502). Rb1 1F8, a mouse monoclonal antibody against RB1 protein was purchased from Zymed (San Francisco, California) and used at a dilution of 1:50. Immunohistochemical reactions were performed with an Avidin-Biotin peroxidase complex (Vectastain Elite ABC, Vector, Burlingame, California), using a biotinylated goat anti-mouse secondary antibody and diaminobenzidine as a chromogen. Normal tissue known to express the Rb1-encoded protein was used as a positive control. The original histologic and immunohistochemical slides were not used for the current study and all tissue samples were reprocessed in the same laboratory at the Institut Curie, Paris, France.

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