Array Comparative Genomic Hybridization Reveals Distinct DNA Copy Number Differences between Gastrointestinal Stromal Tumors and Leiomyosarcomas

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Abstract

Leiomyosarcomas are spindle cell tumors showing smooth muscle differentiation. Until recently, most gastrointestinal stromal tumors (GIST) were also classified as smooth muscle tumors, but now GISTs are recognized as a separate entity, defined as spindle cell and/or epithelioid tumors localized in the gastrointestinal tract. Using microarray-based comparative genomic hybridization (array CGH), we have created a detailed map of DNA copy number changes for 7 GISTs and 12 leiomyosarcomas. Considerable gains and losses of chromosomal segments were observed in both tumor types. The most frequent aberration observed in GISTs was loss of chromosomes 14 and 22, with minimal recurrent regions in 14q11.2-q32.33 (71% of the tumors) and 22q12.2-q13.31 (100%). In leiomyosarcomas, frequent loss of chromosome 10 and 13q was observed, with minimal recurrent regions in 10q21.3 (75%) and 13q14.2-q14.3 (75%). Recurrent high-level amplification of 17p13.1-p11.2 was detected in leiomyosarcomas. Expression profiling using cDNA microarrays revealed four candidate genes in this region with high expression (AURKB, SREBF1, MFAP4, and FLJ10847). Altered expression of AURKB and SREBF1 has been observed previously in other malignancies. Hierarchical clustering of all samples separated GISTs and leiomyosarcomas into two distinct clusters. Statistical analysis identified six chromosomal regions, 1p36.11-p13.1, 9q21.11-9q34.3, 14q11.2-q23.2, 14q31.3-q32.33, 15q24.3-q26.3, and 22q11.21-q13.31, which were significantly different in copy number between GISTs and leiomyosarcomas. Our results show the potential of using array comparative genomic hybridization to classify histologically similar tumors such as GISTs and leiomyosarcomas. (Cancer Res 2006; 66(18): 8984-93)

Introduction

Sarcomas, malignant tumors of mesenchymal origin, are histologically classified according to the normal tissue they resemble. Leiomyosarcomas, malignant tumors displaying features of smooth muscle differentiation, are characterized by spindle cells with elongated blunt-ended nuclei and eosinophilic cytoplasm (1). Leiomyosarcomas can occur at many anatomic locations and account for 5 to 10% of all soft tissue sarcomas (2). Leiomyosarcomas develop principally in adults (50-60 years of age) and are most common in women. According to their localization, they can be subdivided into different subgroups: deep soft tissue tumors (retroperitoneum, abdominal cavity, and intramuscular), uterine tumors, (sub)cutaneous tumors, and vascular leiomyosarcomas (i.e., venous cava). The subgroups also differ in their clinical characteristics, with cutaneous tumors showing a better prognosis (2).

Several studies have identified recurrent copy number changes at the cytogenetic level in leiomyosarcomas. Using chromosome-based comparative genomic hybridization (CGH), recurrent losses of 2p, 2q, 10q, 11q, and 13q have been observed, as well as frequent gains of 1q, 5p, 8q, and 17p (3–6). Some of these regions, e.g., loss of 2q, 10q, and 12p, as well as gain of 1q and 17p, have been associated with more aggressive tumor behavior (7).

Until recently, most gastrointestinal spindle cell sarcomas were classified as smooth muscle tumors. However, based on histological data and immunoreactivity, these tumors, which show almost no smooth muscle differentiation, have been reclassified as gastrointestinal stromal tumors (GIST), a separate entity from leiomyosarcomas. GISTs have relatively simple karyotypes compared with leiomyosarcomas, including recurrent losses of 1p, 9p, 11p, 14q, and 22q, as well as gains of 8q and 17q, among other regions (8–10). Some of these aberrations, e.g., alterations of the p16/INK4a locus in 9p21.3 and gain of 5p, 8q, 17q, and 20q, have been associated with more aggressive tumor behavior (8, 11).

In 95% of GISTs, a gene encoding a type II receptor kinase (KIT) is expressed and is a target of activating mutations (12). Approximately 70 to 80% of these mutations occur in exon 11, leading to ligand-independent phosphorylation of the KIT tyrosine kinase, which can induce malignant transformation (12, 13). A minority of GISTs show activating mutations of the platelet-derived growth factor receptor α (PDGFRα), another receptor tyrosine kinase (14). Recently, small-molecule inhibitors such as imatinib that specifically target tyrosine kinases have been developed (15). These inhibitors have proven highly effective in treating GISTs (16), a fact that highlights the importance of distinguishing GISTs from leiomyosarcomas.

Microarray-based CGH (array CGH) enables genome-wide, high-resolution analysis of DNA copy number alterations. In this study, we sought to investigate the use of DNA copy number changes as a classification tool and to identify novel candidate areas important to the biology of GISTs and leiomyosarcomas. We used array CGH to create a detailed map of DNA copy number changes in 7 GISTs.
and 12 leiomyosarcomas. Statistical analysis identified six chromosomal regions that can distinguish the two tumor types based on changes in DNA copy number. Further analysis of recurrent high-level amplification of 17p13.1-p11.2 in leiomyosarcomas revealed four frequently overexpressed candidate genes.

Materials and Methods

Tumor samples. Eighteen human sarcomas initially classified as leiomyosarcomas were selected from a tumor collection at the Department of Tumor Biology, Norwegian Radium Hospital (Oslo, Norway). The collection and use of the tumor panel were approved by the ethical committee of Southern Norway. Sarcomas were collected immediately after surgery, cut into small pieces, frozen in liquid nitrogen, and stored at −70°C until use. In addition, one tumor sample tested was grown s.c. in immunodeficient mice as xenografts. All tumors were diagnosed at the time of collection according to the WHO International Histological Classification. At study completion, all samples were reviewed by the pathologist and classified according to the current standard. Seven leiomyosarcomas were then reclassified as GISTs. In addition, one sample initially diagnosed as malignant fibrous histiocytoma was reclassified as leiomyosarcoma and included in the study. Clinical data for all samples, as well as immunohistochemical data, are given in Table 1.

Genomic microarray construction. Genomic microarrays spanning the entire human genome at ~1 Mb resolution were made using bacterial artificial chromosomes (BAC) and P1 artificial chromosomes (PAC), based on the 1 Mb clone set kindly provided by Dr. Nigel Carter at the Wellcome Trust Sanger Institute, United Kingdom (17).

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Results

Hierarchical clustering of tumors. DNA copy number changes of 18 tumors historically diagnosed as leiomyosarcomas and GISTs were analyzed blindly using a 1 Mb resolution BAC/PAC genomic microarray supplemented with a tiling path between 1q12 and the beginning of 1q25 (~35 Mb). Hierarchical clustering of the 18 tumor samples using normalized ratios identified two well-defined clusters. Pathologic reviewing (without knowledge of the results) revealed that all samples within one of these primary clusters were classified as GIST by current standards (Fig. 1A). After being reviewed, all of the samples in the second primary cluster, including one additional sample (LMS28) previously classified as malignant fibrous histiocytoma, were confirmed as leiomyosarcoma (Fig. 1B).

List of genomic clones showing significant copy number differences between leiomyosarcomas and GISTs was generated. Chromosomal segments represented by multiple significant clones were considered to discriminate leiomyosarcomas and GISTs. Significant recurrent regions were separately identified in GISTs and leiomyosarcomas (Table 2A and B). GISTs showed considerable losses and gains of large chromosomal regions. Of the identified recurrent regions, seven represented losses of chromosomal segments and seven gains. The most frequently observed aberration was loss of the whole or parts of chromosome 22; this aberration was observed in all tumors with a minimal recurrent region in 22q12.2-q13.31 (17.8 Mb). In six of the seven samples, a loss of one copy of chromosome 22 was observed. The second most frequent alteration was loss of chromosome 14; in five of seven samples, one copy of the entire chromosome was absent. In addition, in four of seven samples, three chromosomal regions were lost; 1p36.32-p13.1 (114.1 Mb), 13q12.11-q33.2 (86.5 Mb), and 15q13.2-qtel (71.8 Mb); and in three of seven samples, 9q13-q34.2 (65.3 Mb) was lost.

Recurred altered regions in leiomyosarcomas and GISTs. Significant regions of DNA copy number changes in each sample were identified using the ACE algorithm in CGH-Explorer. Minimal recurrent regions were identified in GISTs and leiomyosarcomas (Table 2A and B). GISTs showed considerable losses and gains of large chromosomal regions. Of the identified recurrent regions, seven represented losses of chromosomal segments and seven gains.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Patient age (y)</th>
<th>Initial Diagnosis</th>
<th>Revised Diagnosis</th>
<th>Grade/</th>
<th>Location</th>
<th>Size (cm)</th>
<th>Metastasis (mo)</th>
<th>Status</th>
<th>Follow-up (mo)</th>
<th>Desmin</th>
<th>SM</th>
<th>CD34</th>
<th>KIT</th>
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<tr>
<td>LMS1</td>
<td>59/M</td>
<td>LMS</td>
<td>LMS</td>
<td>High</td>
<td>Abdomen</td>
<td>15</td>
<td>11</td>
<td>DD</td>
<td>16</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>LMS2</td>
<td>67/M</td>
<td>LMS</td>
<td>LMS</td>
<td>Low</td>
<td>Small bowel</td>
<td>8</td>
<td>11</td>
<td>DD</td>
<td>23</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>LMS3</td>
<td>61/F</td>
<td>LMS</td>
<td>LMS</td>
<td>High</td>
<td>Small bowel</td>
<td>11</td>
<td>18</td>
<td>AD</td>
<td>135</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td></td>
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<tr>
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<td>LMS</td>
<td>LMS</td>
<td>Low</td>
<td>Rectum</td>
<td>1.5</td>
<td>93</td>
<td>DD</td>
<td>121</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td></td>
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<tr>
<td>LMS5</td>
<td>53/M</td>
<td>LMS</td>
<td>LMS</td>
<td>High</td>
<td>Liver</td>
<td>6</td>
<td>73</td>
<td>DD</td>
<td>101</td>
<td>f.p.</td>
<td></td>
<td>+</td>
<td></td>
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<tr>
<td>LMS6</td>
<td>74/M</td>
<td>LMS</td>
<td>GIST</td>
<td>High</td>
<td>Abdomen</td>
<td>20</td>
<td>m.d.</td>
<td>DD</td>
<td>13</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
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<td>LMS7</td>
<td>70/M</td>
<td>LMS</td>
<td>GIST</td>
<td>Low</td>
<td>Stomach</td>
<td>5.5</td>
<td>n.m.</td>
<td>NED</td>
<td>95</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td></td>
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<td>LMS8</td>
<td>59/F</td>
<td>LMS</td>
<td>LMS</td>
<td>4</td>
<td>Retroperitoneum</td>
<td>9</td>
<td>15</td>
<td>DD</td>
<td>176</td>
<td>f.p.</td>
<td>+</td>
<td>f.p.</td>
<td>–</td>
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<tr>
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<td>72/F</td>
<td>LMS</td>
<td>LMS</td>
<td>4</td>
<td>Retroperitoneum</td>
<td>20</td>
<td>54</td>
<td>DD</td>
<td>91</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td></td>
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<tr>
<td>LMS10</td>
<td>46/F</td>
<td>LMS</td>
<td>LMS</td>
<td>4</td>
<td>Uterus</td>
<td>13</td>
<td>m.d.</td>
<td>DD</td>
<td>11</td>
<td>f.p.</td>
<td>+</td>
<td>–</td>
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<tr>
<td>LMS11</td>
<td>71/F</td>
<td>LMS</td>
<td>LMS</td>
<td>4</td>
<td>thigh (i.m.)</td>
<td>8</td>
<td>n.m.</td>
<td>DOC</td>
<td>67</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>LMS12</td>
<td>67/F</td>
<td>LMS</td>
<td>LMS</td>
<td>4</td>
<td>Retroperitoneum</td>
<td>8</td>
<td>n.m.</td>
<td>NED</td>
<td>123</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>LMS13</td>
<td>72/M</td>
<td>LMS</td>
<td>LMS</td>
<td>3</td>
<td>Retroperitoneum</td>
<td>20</td>
<td>28</td>
<td>DD</td>
<td>63</td>
<td>–</td>
<td>+</td>
<td>f.p.</td>
<td>–</td>
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<td>LMS14</td>
<td>59/M</td>
<td>LMS</td>
<td>LMS</td>
<td>Uterus</td>
<td>8.5</td>
<td>12</td>
<td>DD</td>
<td>31</td>
<td>f.p.</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
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<tr>
<td>LMS15</td>
<td>46/F</td>
<td>LMS</td>
<td>LMS</td>
<td>Uterus</td>
<td>20</td>
<td>DD</td>
<td>62</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LMS16</td>
<td>31/F</td>
<td>LMS</td>
<td>LMS</td>
<td>4</td>
<td>Retroperitoneum</td>
<td>14</td>
<td>1</td>
<td>DD</td>
<td>18</td>
<td>+</td>
<td>–</td>
<td>w.f.p.</td>
<td>–</td>
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<tr>
<td>LMS17</td>
<td>72/F</td>
<td>LMS</td>
<td>LMS</td>
<td>4</td>
<td>thigh (femoralis)</td>
<td>10</td>
<td>m.d.</td>
<td>DD</td>
<td>41</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
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<tr>
<td>LMS18</td>
<td>66/F</td>
<td>LMS</td>
<td>LMS</td>
<td>4</td>
<td>Perineum</td>
<td>3</td>
<td>n.m.</td>
<td>DOC</td>
<td>51</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>LMS19</td>
<td>82/M</td>
<td>MFH</td>
<td>LMS</td>
<td>4</td>
<td>Knee (s.c.)</td>
<td>7</td>
<td>n.m.</td>
<td>DOC</td>
<td>79</td>
<td>f.p.</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: SM, smooth muscle; x, xenograft; Prim, primary tumor; Rec, recurrence; Met, metastasis; F, female; M, male; MFH, malignant fibrous histiocytoma; LMS, leiomyosarcoma; n.a., not available; m.d., metastasis at diagnosis; n.m., no metastasis; DD, dead of disease; AD, alive with disease; DOC, dead of other cause; NED, no evidence of disease; f.p., focal positive; w.f.p., weak focal positive.

*Grading is based on a four-tiered system used in the Scandinavian Sarcoma Group. Uterine leiomyosarcomas are not graded. GISTs are graded according to size and mitotic count.

1 Largest diameter of the tumor.

2 Time to first metastasis from diagnosis.

3 Time to last follow-up from diagnosis.

4 Additional stainings have been done. Sample is estrogen- and progesterone receptor–negative.

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high-level amplifications and homozygous deletions were observed in some of the samples. No recurrent region of aberration showed frequent high-level amplification (log<sub>2</sub> ratio > 1) or homozygous deletion (log<sub>2</sub> ratio < -1). Figure 2A shows a genome-wide frequency plot of copy number aberrations for the GIST group, whereas Fig. 2B shows a representative ratio plot for one GIST sample (GIST1). Genome-wide ratio plots for all GISTs are shown in Supplementary Fig. S1A.

Leiomyosarcomas showed more recurrent losses than gains. Eighteen of 32 recurrent regions were losses, compared with 14 regions of increased DNA copy number. The most frequent minimal regions of loss were in 10q21.3 and 13q14.2-q14.3, both detected in 9 of 12 samples. Chromosome 2 was a frequent target for deletion; multiple recurrent regions were identified at 2p25.1-p21 (35.9 Mb), 2p14-p13.1 (8.8 Mb), 2q24.1-q31.2 (41.8 Mb), and 2q37.1-q37.2 (9.4 Mb), with frequencies ranging from 6 of 12 samples to 7 of 12.

Figure 1. A, hierarchical clustering dendrogram and heat map showing DNA copy number ratios in tumor samples compared with a pool of normal diploid DNA. A total of 3,329 unique genomic clones are shown in chromosomal order from 1p<sub>tel</sub> to 22q<sub>tel</sub>. Red, increases in DNA copy number; green, decreases in DNA copy number. Chromosomes are indicated with black and gray bars. B, hierarchical clustering dendrogram indicating anatomic location of the tumor samples.
samples (Fig. 2C). Additional regions with decreased copy number were observed at 1p36.32-p36.21 (7.9 Mb), 4q31.3-qtel (36.2 Mb), 6p25.2-p22.3 (21.2 Mb), 6q14.1-q23.3 (58.6 Mb), 7p22.3-p13 (43.4 Mb), 11p15.5-p15.4 (2.4 Mb), 11q22.1-q24.1 (21.9 Mb), 16q21.2-q22.1 (20.2 Mb), 18q11.2-qtel (57.9 Mb), 21q21.1-q22.11 (18.4 Mb), and 22q13.1-q13.33 (11.4 Mb); all occurring in at least 4 of 12 samples (see Fig. 2C).

A considerable number of gains were also observed in leiomyosarcomas. The most frequently affected region was 17p13.1-p11.2, in which high-level amplification was found in four of six samples with increased DNA copy number (see Fig. 3A). The minimal recurrent region was limited to 1.9 Mb in 17p11.2, in which three samples showed high-level amplification. The long arm

Table 2. Minimal recurrent regions altered in GISTs and leiomyosarcomas

<table>
<thead>
<tr>
<th>Cytoband</th>
<th>Aberration</th>
<th>Start clone</th>
<th>End clone</th>
<th>Size (Mb)</th>
<th>Frequency</th>
<th>Observation</th>
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<tbody>
<tr>
<td>(A) Minimal recurrent regions altered in GISTs (n = 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1p36.32-p13.1</td>
<td>Loss</td>
<td>RP1-785P20</td>
<td>RP11-27K15</td>
<td>114.1</td>
<td>4/7</td>
<td>Four samples with −1p</td>
</tr>
<tr>
<td>4qtel-q13.2</td>
<td>Gain</td>
<td>CTC-35P21</td>
<td>RP11-211G17</td>
<td>67.2</td>
<td>3/7</td>
<td></td>
</tr>
<tr>
<td>5p15.33-q35.3</td>
<td>Gain</td>
<td>CTD-2265D9</td>
<td>RP11-451H32</td>
<td>177.5</td>
<td>3/7</td>
<td>Three samples with +5</td>
</tr>
<tr>
<td>8p23.3-5cen</td>
<td>Gain</td>
<td>RP11-338B2</td>
<td>RP11-215H11</td>
<td>43.0</td>
<td>4/7</td>
<td>Three samples with +8</td>
</tr>
<tr>
<td>9p21.3</td>
<td>Loss*</td>
<td>RP11-149F2</td>
<td>RP11-468C2</td>
<td>3.2</td>
<td>3/7</td>
<td>Two samples with −9</td>
</tr>
<tr>
<td>9q13-q42.4</td>
<td>Loss</td>
<td>RP11-274P21</td>
<td>RP11-153P4</td>
<td>65.3</td>
<td>3/7</td>
<td>Two samples with −9</td>
</tr>
<tr>
<td>13q12.1-q3.2</td>
<td>Loss</td>
<td>RP11-86C10</td>
<td>RP11-417P24</td>
<td>85.1</td>
<td>5/7</td>
<td>All with −14</td>
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<tr>
<td>15q2.2-q13.1</td>
<td>Loss</td>
<td>RP11-506G7</td>
<td>RP11-600E6</td>
<td>1.1</td>
<td>3/7</td>
<td>Two samples with +15q</td>
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<tr>
<td>17q21.2-q21.31</td>
<td>Gain</td>
<td>RP11-429O1</td>
<td>GS1-50C4</td>
<td>30.0</td>
<td>3/7</td>
<td>Two samples with +17q</td>
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<tr>
<td>18q11.2-qtel</td>
<td>Gain</td>
<td>RP11-296E23</td>
<td>CTC-964M9</td>
<td>58.8</td>
<td>3/7</td>
<td>Two samples with +18</td>
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<td>20q12.1-q13.12</td>
<td>Gain</td>
<td>RP11-338B2</td>
<td>RP11-138B7</td>
<td>4.1</td>
<td>3/7</td>
<td>Two samples with +20</td>
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<td>22q12.1-q13.31</td>
<td>Loss</td>
<td>RP1-76B20</td>
<td>LL22NC03-75H12</td>
<td>17.8</td>
<td>7/7</td>
<td>Six samples with −22</td>
</tr>
</tbody>
</table>

| (B) Minimal recurrent regions altered in leiomyosarcomas (n = 12) |
| 1p36.32-p36.21 | Loss | RP1-37J18 | RP1-436F13 | 7.9 | 4/12 | Three samples with +1q |
| 1q21.1-q23.2 | Gain | RP11-277L2 | RP11-406G20 | 86.5 | 5/12 | Three samples with +1q |
| 1q23.2-q23.3 | Gain | RP11-259G7 | RP11-406G20 | 86.5 | 5/12 | Three samples with +1q |
| 2p14.1-p13.1 | Loss | RP11-524F11 | RP11-162E17 | 1.4 | 6/12 | Two samples with −2p |
| 4q13.3-qtel | Gain | RP11-11L10 | RP11-447J13 | 12.0 | 5/12 | Two samples with +3 |
| 5p13.2-qcen | Gain | RP11-11L10 | RP11-447J13 | 12.0 | 5/12 | Two samples with +3 |
| 6q14.1-q33.3 | Loss | RP11-274P21 | RP11-153P4 | 65.3 | 3/7 | Two samples with −9 |
| 6q22.1-q22.3 | Loss | RP11-138B7 | RP11-166D19 | 21.9 | 5/12 | Two samples with −11 |
| 7p22.1-p13 | Loss | RP11-429O1 | GS1-50C4 | 30.0 | 3/7 | Two samples with +17q |
| 8p23.3-5cen | Gain | RP11-296E23 | CTC-964M9 | 58.8 | 3/7 | Two samples with +18 |
| 9q13-q42.4 | Loss | RP11-338B2 | RP11-138B7 | 4.1 | 3/7 | Two samples with +20 |
| 13q12.1-q13.12 | Gain | RP11-338B2 | RP11-138B7 | 4.1 | 3/7 | Two samples with +20 |
| 17p21.2-q21.31 | Gain | RP11-429O1 | GS1-50C4 | 30.0 | 3/7 | Two samples with +17q |
| 18q11.2-qtel | Gain | RP11-296E23 | CTC-964M9 | 58.8 | 3/7 | Two samples with +18 |
| 20q12.1-q13.12 | Gain | RP11-338B2 | RP11-138B7 | 4.1 | 3/7 | Two samples with +20 |
| 22q12.1-q13.31 | Loss | RP1-76B20 | LL22NC03-75H12 | 17.8 | 7/7 | Six samples with −22 |

*Aberration detected in only two samples by ACE. Additional sample annotated manually, small homozygous deletion.
of chromosome 1 was also a target of frequent gains; five samples showed increased copy number of a large region of 1q, and three recurrent regions at 1q21.1-q23.2 (11.3 Mb), 1q23.2-q23.3 (0.5 Mb), and 1q23.3-q25.1 (9.9 Mb) could be delimited. Increased copy number of chromosome 14 was observed in 6 of 12 samples, 4 of them involving almost the complete chromosome. In five samples, smaller regions of gain at 14q12-q21.1 (9.1 Mb), 14q21.2-q21.3 (3.6 Mb), and 14q31.3-q32.2 (9.7 Mb) were observed. A number of other recurrent amplified regions were identified in leiomyosarcomas at 3p12.3-p12.1 (12 Mb), 5p13.2-pcen (14.7 Mb), 9q21.13-q31.3 (39.8 Mb), 12p11.22-p11.21 (2.3 Mb), 15q11.1-q12 (3.5 Mb), 15q25.1-q26.3 (21.2 Mb), and 20q11.21-q13.33 (32.1 Mb); all were altered in >30% of the samples (see Table 2B). A frequency plot of gains and losses for leiomyosarcomas is shown in Fig. 2C, as well as a representative ratio plot for this type of tumor (LMS23; Fig. 2D). Genome-wide ratio plots for all leiomyosarcomas are shown in Supplementary Fig. S1B.

A list of all recurrent aberrations for GISTs and leiomyosarcomas is shown in Table 2A and B. A detailed description of all defined regions of gain and loss from the ACE analysis is presented in Supplementary Table S1A and B.

Characterization of the 17p13-p11 amplification in leiomyosarcomas. A region in 17p13.1-p11.2 showed the highest level of amplification in the leiomyosarcoma samples. The region encompassed 12.7 Mb and was represented by 23 BACs and PACs, starting with RP11-404G1 and ending with RP11-121A13. Six of the 12 leiomyosarcomas showed increased copy number of all or parts of this region, with LMS1, -7, -21, and -23 showing particularly high levels of amplification. Figure 3A shows the copy number of chromosome 17 for the six leiomyosarcoma samples with increased copy number.

As part of an ongoing study, gene expression was analyzed in a panel of soft tissue sarcomas using cDNA microarrays. In the current study, in order to narrow the list of candidate target genes for this amplification, expression levels in six of the leiomyosarcomas analyzed by array CGH and one additional sample (LMS29) were investigated. According to Ensembl, 172 genes are located within the amplified region. Probes for 70 of these genes were present on the cDNA microarray. The expression levels of genes determined to have a value in at least four leiomyosarcomas (43 of 70 genes) are shown in Fig. 3B.

Four genes located within the amplified region showed increased expression relative to the median for soft tissue sarcoma (log2 ratio >1) in three or more of the seven leiomyosarcomas analyzed. The expression levels of these genes in the seven leiomyosarcomas are shown in Fig. 3C. Microfibrillar-associated protein 4 (MFAP4) was overexpressed in four leiomyosarcomas, whereas aurora kinase B (AURKB) and sterol regulatory element binding transcription factor 1 (SREBF1) were overexpressed in three leiomyosarcomas. In addition, one gene with unknown function, FLJ10847, showed increased expression in three leiomyosarcomas.

SREBF1 showed the highest level of expression; its expression was >16-fold higher in LMS1 and LMS3 compared with the median for soft tissue sarcoma. All genes, except AURKB, were located within the minimal recurrent region of amplification in 17p11.2 identified after ACE analysis.

Copy number changes distinguishing GISTs from leiomyosarcomas. SAM was used to identify genomic regions that can differentiate leiomyosarcomas and GISTs by means of DNA copy number changes. SAM uses a modified t test to identify genes or genomic clones in a microarray data set whose alteration significantly differs between groups. Using a two-class unpaired design and a false discovery rate of 1%, 238 genomic clones that were significantly different between leiomyosarcomas and GISTs were identified. The 238 clones identified six primary chromosomal regions in 1p, 9q, 14q (two segments), 15q, and 22q, all more frequently deleted in GISTs.

Eighty-nine of 100 genomic clones (89%) between 1p36.11 and p13.1 were identified by SAM to be frequently deleted in GISTs compared with leiomyosarcomas. On chromosome 9, 51 of 94 clones (54%) between 9q21.11 and 9q34.3 were identified. Two chromosomal segments were identified in 14q; 14q11.2-q23.2 and 14q31.3-q32.33 were deleted in GISTs, 29 of 42 (69%) and 19 of 20 (95%) clones, respectively. Five of 20 clones (25%) in 15q24.3-q26.3 were identified by SAM, in addition to 40 of 44 clones (91%) in 22q11.32-13.31. Only 5 of the 238 significant clones did not map to any of the six regions mentioned.

Figure 4 shows the genomic areas that were significantly different in copy number between GISTs and leiomyosarcomas. The complete list of selected clones is presented in Supplementary Table S2.
Discussion

We have used array CGH to compare DNA copy number changes in two groups of mesenchymal malignancies, GISTs and leiomyosarcomas. The initial aim of the study was to determine recurrent copy number aberrations in leiomyosarcomas and identify novel candidate proto-oncogenes and/or tumor suppressor genes. Pathologic review of the leiomyosarcomas revealed two distinct tumor entities based on the current classification standard. Seven of the initial 18 samples were reclassified as GISTs, which until recently, were difficult to distinguish from smooth muscle malignancies like leiomyosarcomas. Samples belonging to the biobank used in this study have been collected during the past 20 years and classified at the time of diagnosis.

Loss of DNA material was as frequent as gain in the GIST samples, seven minimal recurrent regions of loss were determined, as well as seven regions of gain. All chromosomes showed different levels of aberration, and chromosomes 3 and 22 were the least and most altered, respectively. GISTs tend to have simple karyotypes, losses of 14q and 22q are the most common cytogenetic findings, followed by loss of 1p, 9p, or 11p (10, 22–27). In our study, the two most frequent aberrations observed in GISTs were losses of 22q and chromosome 14. These alterations, together with loss of 1p, are believed to be early events in tumor development because they are found in benign as well as malignant and metastatic GISTs (8, 26). With our limited number of samples, we were able to identify a minimal recurrent region of loss between 14q11.2 and q32.33. This segment includes the two segments previously identified by conventional CGH and microsatellite analysis (9).

Gain of 5p and 20q was observed in three of seven GISTs, and previous reports have indicated that these regions are associated with aggressive and metastatic behavior (8). The same study also showed that gain of 8q and 17q and loss of 13q were frequently observed in aggressive and metastatic GISTs. In our panel, recurrent gain of 17q and loss of 13q were observed in three and four of seven GISTs, respectively. Three samples showed gain of 8q, but the only minimal recurrent aberration in chromosome 8 was situated in 8p23.3-cen (gained in four of seven samples). All tumor samples showing alterations in chromosomes 8, 13, and/or 17 belonged to patients that developed metastasis, and all these patients, except one, died of the disease.

Loss of heterozygosity of 9p and loss of the p16\(^{ink4A}\) locus have also been associated with aggressive GISTs (11, 28). Our analysis revealed that three of seven samples showed loss in 9p21.3, in which the p16\(^{ink4A}\) locus resides. Two tumor samples were classified as high risk and one as low risk, and all three patients developed metastasis and died of cancer.

Eighteen recurrent regions of loss and 14 of gain were identified in our leiomyosarcoma panel. The presence of a large number of alterations per tumor has been correlated with high-grade tumors (7), which also characterizes our tumor panel. The most frequent aberrations observed were losses of 10q and 13q in 9 of 12 samples. Deletion of 10q was recently associated with aggressive tumor behavior and is frequently found in large tumors and tumors that metastasize (29). The minimal recurrent deleted area in 10q21.3 contains only the gene CTNN\(\alpha\)3, a cadherin-associated protein possibly involved in the organization of the actin cytoskeleton and in cell adhesion (30, 31). CTNN\(\alpha\)3, like other \(\alpha\)-catenins, inhibits Wnt signaling and expression of T cell transcription factor target genes (30). The CTNN\(\alpha\)3 locus has also recently been identified as a common fragile site (32), which might play a role in its frequent deletion. Deletion of CTNN\(\alpha\)3, a gene associated with cell adhesion, would be a likely target during early steps of the metastatic process.

A 2.7 Mb minimal recurrent region of loss between 13q14.2 and q14.3 was also identified; this chromosomal segment contains the known tumor suppressor gene RB1. Deletion of chromosome 13 in leiomyosarcomas has been previously identified in a number of conventional CGH studies (3, 4, 7). Loss of 13q has been found in all stages and grades of leiomyosarcomas (7), indicating that inactivation of a tumor suppressor gene on this chromosome arm may be an early event in the development of leiomyosarcoma. Additional evidence supporting RB1 as a target for inactivation comes from negative RB1 immunostaining of tumors showing deletion of this region (3), as well as generally frequent alteration of RB1 in soft tissue sarcomas (33).

Deletion of segments of 10q, 2p, and 12p, as well as gains in 1q and 17p, have been shown to be more frequent in aggressive, high-grade, and recurrent leiomyosarcomas (7). Our analysis found deletions of chromosome 2 to be among the most frequent alterations in leiomyosarcomas, multiple recurrent regions were identified on the short and long arm of this chromosome. No highly recurrent deletion in 12p was observed in our study. Multiple areas of gain were identified in 1q, among them 1q21.1-q23.2 and 1q23.3-q25. Alteration of 1q21 was initially identified and characterized in sarcomas by our group (34, 35). Further analysis identified three novel candidate genes for the 1q21 amplicon (36). One of these, PPIL4, previously termed COAS2, is located within the minimal region of aberration in 1q21 observed in leiomyosarcomas.

Alterations on the short arm of chromosome 17 were also seen in more than half of the leiomyosarcoma samples, and a 12.7 Mb region in 17p13.1-p11.2 showed the highest level of amplification. Six of the 12 leiomyosarcomas showed increased copy number of all or part of this region (see Fig. 3A), with four samples showing particularly high levels of amplification. The ACE analysis identified a minimal recurrent region within 17p11.2, in addition to a region of loss in 17p13.2-p13.1. In previous CGH studies, frequent high-level amplification of 17p has been observed in leiomyosarcomas (3–5, 7).

In order to identify candidate genes for this amplification, we investigated the expression level of genes present in this region.
In an ongoing expression profiling study, 70 of the 172 genes located within 17p13.1-p11.2 have been analyzed in a panel of soft tissue sarcomas. The expression profiling of seven leiomyosarcomas, six of them analyzed here by array CGH, revealed four genes with >2-fold greater expression in at least three of the seven leiomyosarcomas compared with the median for soft tissue sarcoma (see Fig. 3B and C). Three of the genes encode proteins with known function, whereas the function of the fourth protein, encoded by FLJ10847, is undescribed. The MFP4 gene was overexpressed in four tumors, whereas SREBF1, FLJ10847, and AURKB were overexpressed in three tumors. All genes except AURKB were located within the minimal recurrent region of amplification in 17p11.2 that was identified after ACE analysis.

MFP4 is an extracellular matrix protein possibly involved in calcium-dependent cell adhesion or intercellular interactions, and it has been shown to stimulate ex vivo expansion of hematopoietic stem cells (37). Based on these findings, MFP4 could play a role in tumor growth.

SREBF1 is a transcriptional activator that regulates the transcription of genes for sterol biosynthesis and the low-density lipoprotein receptor gene. SREBF1 has been shown to be up-regulated in prostate cancer during progression to androgen independence (38), and participates in the transcriptional regulation of proliferation-associated fatty acid synthesis in colorectal cancer (39), making SREBF1 a possible growth-promoting gene. SREBF1, as well as FLJ10847, was also shown to be highly expressed in leiomyosarcomas in another microarray study (40). AURKB is a member of the aurora subfamily of serine/threonine protein kinases. It plays an essential role in chromosome segregation and cytokinesis (41). Overexpression of AURKB is observed in a variety of tumors and has been correlated with malignancy and cell proliferation in prostate cancer (42), level of genetic instability in primary non–small cell lung carcinomas (43), and histological malignancy and clinical outcome in high-grade gliomas (44). AURKB has also been shown to be involved in Ras-mediated cell transformation (45).

High-level amplification of 17p12-p11.2 is also common in osteosarcomas, malignant bone tumors (46, 47). Several studies have reported recurrent amplification of the genes PMP22, COPS3, TOP3A, and MAP7 in osteosarcomas (48, 49). COPS3 and PMP22 have also been shown to be frequently overexpressed (48, 50), with COPS3 consistently overexpressed in osteosarcomas with amplification of the gene. In our panel of leiomyosarcomas, MAP7 was overexpressed (log2 ratio >1) in two tumor samples, whereas COPS3 and PMP22 were overexpressed in only one sample each (see Fig. 3B). Expression of TOP3A was undetectable in all the leiomyosarcomas.

Based on their functions and association with cancer, several of the genes found to be overexpressed may be interesting candidate targets for the 17p13.1-p11.2 amplification in leiomyosarcomas. Altered expression of these genes may play a role in the development and/or progression of leiomyosarcoma. Judging from the frequency of overexpression in our data, MFP4 seems to be the most likely candidate, although it is possible that genes not represented on our expression array are also of importance in leiomyosarcoma biology.

In order to investigate whether there are regions that can differentiate leiomyosarcomas and GISTs by means of DNA copy number, we used statistical analysis in SAM. Six regions in 1p, 9q, 14q (two segments), 15q, and 22q were identified to have a significantly lower copy number in GISTs compared with leiomyosarcomas. Although some of the leiomyosarcoma samples also showed loss of these regions, it was to a much lower extent than what is observed within GISTs.

In summary, our study shows that array CGH can be used to differentiate histologically similar tumors such as GISTs and leiomyosarcomas, although further validation is required on a larger tumor set. The implementation of whole-genome amplification protocols is bringing array CGH closer to clinical use, enabling the analysis of small numbers of cells such as those obtained from thin-needle biopsies. Thus, array CGH has the potential to play an important role in differentiating and classifying tumors.

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