Clinicopathological Features of Lipomas with Gene Fusions Involving HMGA2

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Abstract. Background: Despite accumulating knowledge of chimeric genes derived from fusion of the HMGA2 gene with multiple partners in lipomas, the different clinicopathological features of lipomas depending on different gene aberrations have not been well documented. The purpose of this study was to examine the clinical significance of the expression of fusion genes in lipomas. Patients and Methods: The expressions of three previously reported gene fusion transcripts, including HMGA2/LPP, HMGA2/RDC1 and HMGA2/NFIB, were analyzed in 102 tumors from patients with lipomas. Results: There were 23 cases (22.5%) expressing HMGA2/LPP, 2 cases (1.9%) expressing HMGA2/RDC1 and no cases of HMGA2/NFIB expression (0%). There were no significant intergroup differences in age, gender, body mass index, tumor size or location. The magnetic resonance images and pathological features were also not different in regard to the status of fusion gene expression. Conclusion: There were no significant differences of clinicopathological features in patients with lipoma with or without these fusion gene transcripts.

Lipoma, a benign tumor composed of mature adipocytes, is the most common soft tissue mesenchymal neoplasm in adults (1, 2). Cytogenetic analysis revealed that about half of all lipomas have aberrations in the chromosome 12q13-15 segment (3). The target gene for rearrangements involving 12q13-15 is HMGA2 (also known as HMGIC), which encodes a protein belonging to a high mobility family, members of which are important in terms of the regulation of chromatin structure and gene expression (4, 5). The creation of chimeric genes derived from fusion of the HMGA2 gene with multiple partners represents a common molecular alteration with a putative role in the development of benign mesenchymal tumors. HMGA2/LPP, which is the most frequent gene aberration in lipoma (4-6), is not specific for lipoma but can occur in other benign mesenchymal tumors, such as pulmonary chondroid hamartoma and soft tissue chondroma (7, 8). This suggests that gene fusion as such is not decisive for tumor cell differentiation but may activate the common regulatory pathway, leading to the development of benign tumors (8). The question arises whether the fusion genes present in lipoma cause any differences in their clinical features. To address this question, we evaluated clinical data (age, sex, body mass index (BMI), tumor size and location), magnetic resonance (MR) imaging findings, pathological features and the expression of three previously reported gene fusion transcripts, including HMGA2/LPP, HMGA2/RDC1 (9) and HMGA2/NFIB (10), in 102 lipomas.

Patients and Methods

Lipomas were obtained from 102 patients at the time of surgery at the Niigata Cancer Center Hospital or Niigata University Hospital between October 2001 and December 2006. The study was approved by the Ethics Committee of the institutions. The patients were 49 men and 53 women aged 22-83 years (mean, 52.5 years). Four patients presented with multiple lipomatosis. In the MR imaging study, to assess the non adipose tissue component, the existence of a thick fibrous septa (thicker than 2 mm) or nodular area (11, 12) in all 102 cases was assessed, and as was signal enhancement after gadolinium diethylene triamine penta-acetic acid (Gd-DTPA) injection in 39 cases for which enhanced images were available. All pathological specimens had been examined at our institutions; additionally, all tumors were diagnosed as lipoma. In histological specimens, the presence of non adipose tissue, such as bone and cartilage components, was evaluated.

Frozen specimens were processed immediately after surgical excision, cryopreserved and stored at −70°C. Total RNA was prepared using phenol-guanidine thiocyanate (ISOGEN-LS; Wako Pure Chemical Ltd., Osaka, Japan) from lipoma tissues according to the manufacturer’s recommendations. Synthesis of cDNA and
polymerase chain reaction (PCR) were performed mainly using a TaKaRa RNA PCR Kit (Takara Shuzo Co., Ltd., Tokyo, Japan). To confirm the synthesis of cDNA, β-actin Primer Pairs (Promega, cat. No. G57409) were used: forward primer 5'-TCA TGA AGT G TG AC G TTG ACA TCC G T-3' and reverse primer 5'-CTT AGA AGC ATT TG C GGT GC A CGA TG -3'. Table I shows the sequences of primers used to detect fusion gene transcripts in this study. PCR conditions were as follows: after the reaction mixture was heated for 3 min at 94˚C, 30 cycles of 30 s denaturation at 94˚C, 30 s annealing at 55˚C, and 30 s extension at 72˚C, followed by one cycle of 7-min extension at 72˚C using a Gene Amp PCR System 9700 (Perkin Elmer, Foster City, CA, USA). Six to 8 μl of PCR products were electrophoresed on 1.5% agarose gel and visualized by ethidium bromide staining. Nucleotide sequences of PCR products were confirmed using an ABI PRISM TM310 Genetic Analyzer (Perkin Elmer).

Association of the expression of fusion gene with clinical features was assessed by χ² test and unpaired Student’s t-test, when appropriate.

Results

The gene fusion transcript of HMGA2/LPP was detected in 23 cases (22.5%) and HMGA2/RDC1 in 2 cases (1.9%) (Figure 1 a, b). Sequencing of the fusion transcripts showed that the translocation breakpoints were located at identical sites as previously described (8, 9). No case expressed the fusion gene transcript of HMGA2/NFIB. The clinicopathological data was compared between the two subsets (positive for HMGA2/LPP and HMGA2/RDC1 vs. negative for tested fusion gene transcripts). As shown in Tables II and III there were no significant intergroup differences in age, sex, BMI, tumor size or location. In regard to MR imaging features, the prevalence of a thick fibrous or nodular septa in lipomas with fusion gene transcripts, of 4.0% (1/25) was no different from that in these without fusion gene transcripts of 6.5% (5/77). Similarly, the prevalence of signal enhancement after Gd-DTPA injection in lipomas with fusion gene transcripts (12.5%; 2/16) was no different from that in lipomas without fusion gene transcripts, (26.1%; 6/23; Table IV). The pathological features of lipomas expressing fusion gene transcripts were also no different from those lipomas lacking fusion gene transcripts. Bone and cartilage components were found in one of 25 cases (4.0%) of lipoma with fusion gene transcripts and in one of 77 cases (1.3%) without fusion gene transcripts (Table IV).

Table I. Primers used to detect fusion gene transcripts.

<table>
<thead>
<tr>
<th>Primers</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>HMGIC-LPP fusion</td>
<td></td>
</tr>
<tr>
<td>5'-ACT TCA GCC C AG GGA CAA-3'</td>
<td>(HMG848F out)</td>
</tr>
<tr>
<td>5'-GGG CTC AGA AGA GAG GAC-3'</td>
<td>(HMG878F in)</td>
</tr>
<tr>
<td>5'-CTA AAG GTC AGT GCT CGC CTT G-3'</td>
<td>(LPP1980R in)</td>
</tr>
<tr>
<td>5'-CCA TCT AGG GAC AAC C-3'</td>
<td>(HMG846F out)</td>
</tr>
<tr>
<td>5'-CAG CGC CTC AGA AGA GAG GAC G-3'</td>
<td>(HMG876F in)</td>
</tr>
<tr>
<td>5'-TGG GTG GAG GTG GTG GCC ATG-3'</td>
<td>(HMG2CHR2 in)</td>
</tr>
<tr>
<td>5'-TGG ACA TTG GCC GGT AAG ATG G-3'</td>
<td>(HMG2CHR2 out)</td>
</tr>
<tr>
<td>HMGIC-RDC1 fusion</td>
<td></td>
</tr>
<tr>
<td>5'-CCA TCT CAG CCC AGG GAC A-3'</td>
<td>(HMG846F out)</td>
</tr>
<tr>
<td>5'-CAG CGC CTC AGA AGA GAG GAC G-3'</td>
<td>(HMG876F in)</td>
</tr>
<tr>
<td>5'-TGG GTG GAG GTG GTG GCC ATG-3'</td>
<td>(HMG2CHR2 in)</td>
</tr>
<tr>
<td>5'-TGG ACA TTG GCC GGT AAG ATG G-3'</td>
<td>(HMG2CHR2 out)</td>
</tr>
<tr>
<td>HMGIC-NFIB fusion</td>
<td></td>
</tr>
<tr>
<td>5'-CCA TCT CAG CCC AGG CAC A-3'</td>
<td>(HMG846F out)</td>
</tr>
<tr>
<td>5'-CAG CGC CTC AGA AGA GAG GAC G-3'</td>
<td>(HMG876F in)</td>
</tr>
<tr>
<td>5'-TGG CCG GTA AGA TGG GTG GCC TCC T-3'</td>
<td>(NF1475-005 R in)</td>
</tr>
<tr>
<td>5'-TGG ACA TTG GCC GGT AAG ATG G-3'</td>
<td>(NF 1475-006 R out)</td>
</tr>
</tbody>
</table>

Figure 1. Expression of fusion gene transcripts as determined by gel electrophoresis of PCR products. a) Lane 1 50 bp ladder, lane 2 expression of the fusion gene transcript of HMGA2/LPP. b) Lane 1 50 bp ladder, lane 2 expression of the fusion gene transcript of HMGA2/RDC1.
Discussion

Although the number of identified gene fusions involving HMGA2 in lipomas has increased (6, 9, 10), the prevalence of such gene fusions, as well as the clinicopathological characteristics of lipomas depending on different gene aberrations, have not been well documented. The current genetic analysis of 102 lipomas revealed that 23 cases (22.5%) had the gene fusion transcript of HMGA2/LPP and 2 cases (1.9%) had that of HMGA2/RDC1. No case expressed the fusion gene transcript of HMGA2/NFIB. We did not find any significant differences in clinicopathological features, including, age, sex, BMI, tumor size or location, MR imaging features and histological appearance in two subsets of lipomas with or without known fusion gene transcripts. Several papers have indicated a relationship between the development of lipoma and obesity (1, 13), but there have been no definitive data supporting this correlation. In our study, there was no difference in BMI between the two subsets of patients with lipomas, and the BMI in both groups was within the normal range of Japanese people (14), suggesting that obesity is not an associated factor in lipomas.

Computed tomography (CT) and MR images of lipomas reveal a mass of homogenous adipose tissue, which is sufficiently characteristic to permit a correct diagnosis (11, 15). Lipomas, however, may contain non adipose tissue, such as muscle fibers, blood vessels, fibrous septa, areas of necrosis or inflammation, and metaplastic cartilage and bone formation, which produce inhomogenous features on MRI (12, 16, 17). Ten to 30% of lipomas exhibit marked non adipose areas; these features can confound the correct imaging diagnosis as they may mimic findings associated with well-differentiated liposarcomas, including thickened or nodular septa, associated non adipose masses, prominent foci of high T2 signal, and prominent areas of enhancement (11, 12). Recent publications have reported that several cases involving lipoma expressing HMGA2/LPP fusion gene transcripts showed heterogeneous signal enhancement along the thickened fibrous septa on MR images (18, 19). The question arises whether fusion genes are responsible for the formation of non adipose tissues in lipomas. Our comparison of two subsets of lipoma with or without fusion genes involving HMGA2 revealed no difference on MR imaging features concerning the prevalence of a thick fibrous septa and signal enhancement. Kubo et al. (20) suggested that the HMGA2/LPP fusion gene may promote chondrogenesis by up regulating cartilage specific collagen gene expression through N-terminal DNA binding domains of HMGA2; however, based on our data, 96% cases of lipoma with fusion gene transcripts did not contain bone or cartilage components, suggesting that these fusion genes are not essential factors for the differentiation of adipocytes towards bone or cartilage, but rather are regulators which stimulate the common signal pathway leading to the growth of benign mesenchymal tumors. Definitive pathogenesis for the development of non adipose tissue components in lipomas is unknown, but it is probably due to as yet unknown genetic alterations in addition to secondary effects, i.e. intratumoral necrosis, infarction, or bleeding (1)

The detection of fusion gene transcripts has led to enhanced diagnostic accuracy and a deeper understanding of a subset of bone and soft tissue sarcomas. Clearly, the molecular testing of fusion gene transcripts in lipomas will

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Table II. Clinical data vs. status of fusion gene transcripts.

<table>
<thead>
<tr>
<th>FG+ (N=25)</th>
<th>FG- (N=77)</th>
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</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>51.2±13.2</td>
</tr>
<tr>
<td><strong>BMI (kg/m^2)</strong></td>
<td>24.0±4.0</td>
</tr>
<tr>
<td><strong>Tumor size (cm)</strong></td>
<td>7.9±4.3</td>
</tr>
</tbody>
</table>

FG: fusion genes; BMI was calculated by the following formula: BMI=body weight/body weight^2.

Table III. Gender and location of lipoma vs. status of fusion gene transcripts.

<table>
<thead>
<tr>
<th>FG+</th>
<th>FG-</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td><strong>No. of cases (prevalence %)</strong></td>
</tr>
<tr>
<td>Male</td>
<td>11 (44.0)</td>
</tr>
<tr>
<td>Female</td>
<td>14 (56.0)</td>
</tr>
<tr>
<td><strong>Location</strong></td>
<td><strong>No. of cases (prevalence %)</strong></td>
</tr>
<tr>
<td>Neck</td>
<td>2 (8.0)</td>
</tr>
<tr>
<td>Upper extremity</td>
<td>12 (40.8)</td>
</tr>
<tr>
<td>Lower extremity</td>
<td>6 (24.0)</td>
</tr>
<tr>
<td>Trunk</td>
<td>3 (12.0)</td>
</tr>
<tr>
<td>Multiple</td>
<td>2 (8.0)</td>
</tr>
</tbody>
</table>

Table IV. MR image and pathological findings vs. status of fusion gene transcripts.

<table>
<thead>
<tr>
<th>FG+</th>
<th>FG-</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fibrous septa</strong></td>
<td>1 (4.0)</td>
</tr>
<tr>
<td><strong>Enhancement</strong></td>
<td>2 (12.5)</td>
</tr>
<tr>
<td><strong>Bone/cartilage</strong></td>
<td>1 (4.0)</td>
</tr>
</tbody>
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also contribute to the diagnosis of lipomatous tumors; however, only 24.4% of lipomas were found to express known fusion gene transcripts in the present study. Chromosomal analysis by the CHAMP study group reported that 22% of patients with lipoma had a normal karyotype (3). The remaining lipomas are known to be karyotypically heterogenous, making it difficult to diagnose lipoma genetically using simple techniques such as PCR; therefore, conventional imaging techniques such as CT and MRI as well as immunohistological analysis (21) are still valuable for differentiating lipoma from well differentiated liposarcoma. Further investigation is needed to identify additional genetic markers characteristic of lipoma in order to increase diagnostic accuracy, as well as to improve our understanding of the etiology of lipomatous tumors.

References


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