Abstract. Background: In subsets of adipocytic tumors, specific chromosomal translocations lead to the generation of fusion genes. The high mobility group A2 (HMGA2)-lipoma preferred partner (LPP) and the reciprocal LPP-HMGA2 represent such fusion genes in lipoma, while the human translocation liposarcoma (TLS)-CCAAT/enhancer binding protein (C/EBP) homologous protein (CHOP) and the Ewing sarcoma (EWS)-CHOP in liposarcoma. However, the specificity of these fusion genes has not been established in a variety of adipocytic tumors. Patients and Methods: One hundred and seventy-two cases of adipocytic tumors, comprising 98 cases of lipoma and 74 cases of liposarcoma, were analyzed for the possible expression of HMGA2-LPP, LPP-HMGA2, TLS-CHOP and EWS-CHOP fusion genes, using a reverse-transcription polymerase chain reaction method. Results: In lipoma, twenty-two cases (22.4%) were associated with either HMGA2-LPP or the reciprocal LPP-HMGA2 fusion gene, while neither TLS-CHOP nor EWS-CHOP transcript was detectable. On the contrary, in liposarcoma, neither HMGA2-LPP nor LPP-HMGA2 transcript was detectable, although twenty-five cases (33.8%) were related to either TLS-CHOP or EWS-CHOP. Conclusion: HMGA2-LPP and LPP-HMGA2 were specific to lipoma, and TLS-CHOP and EWS-CHOP were specific to liposarcoma.
Patients and Methods

Tissue samples. Tissues from 98 lipomas (9) and 74 liposarcomas were obtained at the time of surgery with written informed consent and stored at ~80°C. Histological subtypes of liposarcoma consisted of 12 well-differentiated, 41 myxoid, 4 dedifferentiated, and 17 unclassified. All the diagnoses were made by pathologists. Procurement of frozen tissues and retrospective data collection were approved by the Review Boards of Tokushima University Hospital, Osaka Medical Center for Cancer and Cardiovascular Diseases, and Osaka University Hospital.

Reverse transcription-polymerase chain reaction (RT-PCR) and DNA sequencing analysis. Total RNA was extracted with RNAeasy Lipid Tissue Mini Kit (QIAGEN, Hilden, Germany), according to the manufacturer’s instructions. After DNase (Invitrogen, Carlsbad, CA, USA) treatment, cDNA was synthesized using 5 μg of total RNA, 50 ng of random hexamers, and 50 U Superscript II Reverse Transcriptase (Invitrogen) in a total volume of 20 μl. The HMGA2-LPP fusion transcript was amplified using the forward primer HMGA2 exon 1 (5'-ggatgagccacagccggtgagg-3') and the reverse primer LPP exon 11 (5'-ctaaaggtcagtgctcgccttg-3') (14). The LPP-HMGA2 fusion transcript was amplified in a nested manner using the LPP-specific forward primers LPP exon 5 up (first round) (5'-ctggagctgagattgacgag-3') and LPP exon 6 up (second round) (5'-caacagcttctcctgattc-3'), and the HMGA2-specific reverse primers HMGA2 exon 5 down-1 (first round) (5'-ccaccccagatgaaagtg-3') and HMGA2 exon 5 down-2 (second round) (5'-ctcaggagagcgttgctagac-3') (14). TLS-CHOP fusion transcripts were amplified in a nested manner using forward primers TLS exon 3 F-3 (first round) (5'-ccagcagaggctattcttctatggc-3') and TLS exon 3 F-2 (second round) (5'-cagcagacgttctcctatgctg-3'), and reverse primers CHOP exon 4 R-1 (first round) (5'-cagcagacgttctcctatgctg-3') and CHOP 176R (second round) (5'-cagcagacgttctcctatgctg-3'). EWS-CHOP fusion transcripts were amplified using primers EWS 501F (5'-ccagcagacgagcagctattct-3') and CHOP 194R (5'-ctggagctgagattgacgag-3') (15). The primers above were carefully chosen or newly designed so that all of the known variant fusion transcripts could be detected (9, 16, 17, 18). As a control, a 124 bp fragment of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA was amplified using the forward primer 5'-cagcagacgagcagctattct-3' and the reverse primer 5'-cagcagacgagcagctattct-3'.

For the PCR, 1 μl of single-stranded cDNA (derived from 250 ng total RNA) was used as a template. The 20 μl reaction contained 18 μl PCR SuperMix (Invitrogen) and 10 pmol of each primer. Denaturation for 2 minutes at 95°C was followed by 35 cycles of 30 seconds at 95°C, 30 seconds at 60°C and 60 seconds at 72°C. For the nested PCR, detecting LPP-HMGA2 and TLS-CHOP transcripts, the first round reaction was performed as described above. The second round reaction was performed using 1 μl of the first round PCR reaction mix as a template.

The PCR products were separated by 1% agarose gel electrophoresis and were visualized by ethidium bromide. After purification, the PCR products were directly sequenced using the PRISM BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and one of each primer set used for the PCR amplification.

Table I. Expression of HMGA2-LPP, LPP-HMGA2, TLS-CHOP and EWS-CHOP fusion transcripts in adipocytic tumors.

<table>
<thead>
<tr>
<th>Tumor type (cases)</th>
<th>HMGA2-LPP</th>
<th>LPP-HMGA2</th>
<th>TLS-CHOP</th>
<th>EWS-CHOP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipoma (98)</td>
<td>19*</td>
<td>13*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Liposarcoma (74)</td>
<td>0</td>
<td>0</td>
<td>22</td>
<td>3</td>
</tr>
<tr>
<td>Histological subtype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well-differentiated (12)</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Myxoid (41)</td>
<td>0</td>
<td>0</td>
<td>19</td>
<td>2**</td>
</tr>
<tr>
<td>De-differentiated (4)</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Unclassified (17)</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

*Previously published data (9), each including 10 cases with both HMGA2-LPP and LPP-HMGA2. **Including 1 case previously published (17).

Results

Expression of fusion genes in lipoma. Out of 98 lipomas, 19 (19%) were associated with HMGA2-LPP fusion transcript, while 13 (13%) with LPP-HMGA2 fusion transcript, each including 10 with both HMGA2-LPP and LPP-HMGA2 transcripts, as we reported previously (9) (Table I). On the other hand, neither TLS-CHOP nor EWS-CHOP fusion transcript was detectable in lipoma.

Expression of fusion genes in liposarcoma. Neither HMGA2-LPP nor LPP-HMGA2 fusion transcript was detectable in liposarcoma (Table I). Conversely, out of 74 liposarcomas, 22 (30%) were associated with TLS-CHOP fusion transcript, while 3 (4%) with EWS-CHOP fusion transcript (Table I). Histological subtypes of TLS-CHOP detection in liposarcoma consisted of 1 well-differentiated (8% of the subtype), 19 myxoid (46% of the subtype), 1 de-differentiated (25% of the subtype) and 1 unclassified (6% of the subtype). In turn, histological subtypes of EWS-CHOP detection in liposarcoma included 2 myxoid (2% of the subtype) and 1 de-differentiated (25% of the subtype).

Discussion

In this retrospective analysis of 172 adipocytic tumors, the results clearly established the specificity of fusion gene expression. In particular, HMGA2-LPP and LPP-HMGA2 were specific to lipoma, and TLS-CHOP and EWS-CHOP were specific to liposarcoma.

HMGA2-LPP or LPP-HMGA2 fusion transcripts have been linked to benign mesenchymal tumors, such as lipoma (2-10), pulmonary chondroid hamartoma (14), and soft tissue chondroma (19). However, the specificity of these fusion genes in adipocytic tumors was not determined. Recently, Ida
et al. described that HMGA2-LPP transcripts were not detectable in 20 cases of well-differentiated liposarcoma (7). Here, for the first time to the best of our knowledge, we report that neither HMGA2-LPP nor LPP-HMGA2 fusion transcript was detectable in 74 cases of liposarcoma, comprising a variety of histological subtypes. Together, the specificity of HMGA2-LPP and LPP-HMGA2 to lipoma among adipocytic tumors might aid in distinguishing lipoma from candidate liposarcoma, once HMGA2-LPP or LPP-HMGA2 transcript is detectable.

Expression of TLS-CHOP or EWS-CHOP fusion gene was implicated in liposarcoma (11, 18). Then again, the specificity of these fusion genes in adipocytic tumors was not revealed. The present study provides the first evidence, as far as we know, showing that neither TLS-CHOP nor EWS-CHOP fusion transcript was detectable in lipoma. The consequent significance should be that the specificity of TLS-CHOP and EWS-CHOP to liposarcoma among adipocytic tumors helps distinguish liposarcoma from candidate lipoma, once TLS-CHOP or EWS-CHOP transcript is detectable.

Among distinct histological subtypes of liposarcoma, TLS-CHOP and EWS-CHOP fusion transcripts have exclusively been related to myxoid liposarcoma. Antonescu et al. reported that TLS-CHOP rearrangement was absent from 12 cases of predominantly myxoid well-differentiated liposarcoma, and 10 cases of other liposarcoma (12). Domoto et al. described that TLS-CHOP fusion transcripts were not detectable in 5 cases of well-differentiated liposarcoma (13). In this study, on the contrary, we showed that subtypes of liposarcoma other than myxoid liposarcoma gave detectable TLS-CHOP or EWS-CHOP fusion transcripts. TLS-CHOP fusion transcripts were detectable in 1 case of well-differentiated liposarcoma, 1 case of de-differentiated liposarcoma, and 1 case of unclassified liposarcoma, while 1 case of de-differentiated liposarcoma revealed EWS-CHOP expression. At this moment in time, it is unclear whether these four cases should be re-diagnosed as myxoid liposarcoma, or whether TLS-CHOP or EWS-CHOP fusion transcripts may be detectable in other histological subtypes.

In summary, this report illustrates the specificity of the representative fusion genes in a variety of adipocytic tumors. Taking advantage of the fusion genes as molecular markers, the distinction of lipoma from liposarcoma could be facilitated in some circumstances. Further studies on the correlation between fusion genes and clinicopathological features of adipocytic tumors are encouraged to establish the specific identity of each tumor.

Acknowledgements

This work was supported, in part, by grants from the Japan Society for Promotion of Science (grant no. 18591664) and the Osaka Medical Research Foundation for Incurable Diseases.

References


