Specificity of Fusion Genes in Adipocytic Tumors

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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 LPP-HMGA2, 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.*

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The highest prevalence of lipoma among human tumors makes adipocytic tumors the largest single group of mesenchymal tumors, in which liposarcoma represents the most common single type of soft tissue sarcoma (1). In a subset of lipoma, a specific t(3;12)(q27-28;q14-15) chromosomal translocation leads to the fusion of the high mobility group A2 (*HMGA2*) gene and the lipoma preferred partner (*LPP*) gene (2-8). Previously, we analyzed 98 lipomas and reported that 22 cases (22.4%) were associated with either *HMGA2-LPP* or the reciprocal *LPP-HMGA2* fusion gene (9). This number was compatible with a study by others where 23 out of 102 lipomas (22.5%) expressed *HMGA2-LPP* fusion gene (10).

Among principal histological subtypes of liposarcoma, myxoid liposarcoma is the second most common, followed by well-differentiated liposarcoma (1). A significant proportion of myxoid liposarcoma has a cytogenetic hallmark, t(12;16)(q13;p11), which leads to the fusion of the CCAAT/enhancer binding protein (*C/EBP*) homologous protein (*CHOP*) and human translocation liposarcoma (TLS) genes, generating *TLS-CHOP* fusion transcript (11). In a minor subset of myxoid liposarcoma, a variant chromosomal translocation, t(12;22)(q13;q12), has been described, resulting in Ewing sarcoma (*EWS*)-*CHOP* fusion gene (11). However, other histological subtypes of liposarcoma, such as well-differentiated, have not been implicated in such chromosomal translocations or fusion genes (12, 13).

As far as we know, the specificity of these fusion genes has not been established in a variety of adipocytic tumors. Here, we report the fusion gene analysis of 172 cases of adipocytic tumors, comprising 98 cases of lipoma and 74 cases of liposarcoma. The results clearly demonstrated that *HMGA2-LPP* and *LPP-HMGA2* were specific to lipoma, and that *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 RNeasy 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'-gatgagcgcacgcggtgagg-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'-ctggacgctgagattgac-3') and LPP exon 6 up (second round) (5'-acagcctctcctccagtt-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'-ctacaggagaagccgtctgagaac-3') (14). *TLS-CHOP* fusion transcripts were amplified in a nested manner using forward primers *TLS* exon 3 F-3 (first round) (5'-cagggctattcccagcagagcagt-3') and *TLS* exon 3 F-2 (second round) (5'-cagagcagctattcttcttatggc-3'), and reverse primers *CHOP* exon 4 R-1 (first round) (5'-cttcatgcgctgctttccag-3') and *CHOP* 176R (second round) (5'-gagaaaggcaatgactcagctgcc-3'). *EWS-CHOP* fusion transcripts were amplified using primers EWS 501F (5'-ccagcccagcctaggatatggaca-3') and *CHOP* 194R (5'-ctggacagtgtcccgaaggagaaa-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'-cagcgacacccactcctc cacctt-3' and the reverse primer 5'-catgaggtccaccaccctgttgct-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.*

Tumor type (cases)	*HMGA2-LPP*	*LPP-HMGA2*	*TLS-CHOP*	*EWS-CHOP*
Lipoma (98)	19*	13*	0	0
Liposarcoma (74)	0	0	22	3
Histological subtype				
Well-differentiated (12)	0	0	1	0
Myxoid (41)	0	0	19	2**
De-differentiated (4)	0	0	1	1
Unclassified (17)	0	0	1	0

*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 ascertains 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.

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