Expression of *HMGA2-LPP* and *LPP-HMGA2* Fusion Genes in Lipoma: Identification of a Novel Type of *LPP-HMGA2* Transcript in Four Cases

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Abstract. *Background: 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. Although the expression of HMGA2-LPP fusion gene has been reported in lipomas, the reciprocal LPP-HMGA2 fusion gene has rarely been described. Materials and Methods: Ninety-eight cases of lipoma were analyzed for the possible expression of HMGA2-LPP and LPP-HMGA2 fusion genes using a reverse-transcription polymerase chain reaction method. Results: Ten lipomas (10%) revealed both HMGA2-LPP and LPP-HMGA2 fusion transcripts, nine (9%) only HMGA2-LPP, and three (3%) only LPP-HMGA2. DNA sequencing analysis demonstrated that the HMGA2-LPP transcript in 19 lipomas consisted of exons 1-3 of HMGA2 and exons 9-11 of LPP, which was described previously. Out of 13 lipomas with LPP-HMGA2 transcript, 9 were associated with a previously reported LPP-HMGA2 fusion transcript, which fuses exon 8 of LPP to exon 4 of HMGA2, while 4 with a novel type of LPP-HMGA2 fusion transcript, which fuses exon 7 of LPP to exon 4 of HMGA2. Conclusion: In addition to the HMGA2-LPP fusion gene, the LPP-HMGA2 fusion gene could have some specific roles for lipomagenesis. The biological implications of the expression and the variation of LPP-HMGA2 fusion transcripts need to be elucidated.*

Lipoma, the most common tumor in adults, is a benign neoplasm composed of mature adipose tissue (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 (2-4). The *HMGA2-LPP* fusion transcript usually consists of exons 1-3 of *HMGA2* and exons 9-11 of *LPP*, and its expression has been reported in cases of lipoma (5-7). In contrast, the reciprocal *LPP-HMGA2* fusion transcript, which potentially consists of exons 1-8 of *LPP* and exons 4-5 of *HMGA2*, has rarely been described (8). Therefore, the *HMGA2-LPP* fusion is considered responsible for lipomagenesis associated with t(3;12).

To the best of our knowledge, the frequency of *HMGA2-LPP* and *LPP-HMGA2* fusion gene expression has not been established in lipoma. Here we report the fusion gene analysis of 98 cases of lipoma, in four of which we identified a novel type of *LPP-HMGA2* fusion transcript.

Materials and Methods

Tissue samples. Tissues from 98 lipomas, including three cases which have been described for expression of *HMGA2-LPP* fusion gene (5, 6), were obtained at the time of surgery with written informed consent and stored at –80˚C. 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.

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Table I. *Twenty-two cases of lipoma associated with HMGA2-LPP and/or LPP-HMGA2 fusion transcripts.*

Patient	Age (years)	Gender	Lipoma location	*HMGA2-LPP*	*LPP-HMGA2*	Reference
1	57	M	Knee	+	+ (type 1)	(5)
2	54	M	Thigh	+	+ (type 1)	(6)
3	50	F	Leg	+	+ (type 1)	(6)
4	55	F	Leg	+	+ (type 1)	
5	25	F	Back	+	+ (type 1)	
6	62	F	Arm	+	+ (type 1)	
7	57	M	Back	+	+ (type 1)	
8	69	F	Ankle	+	+ (type 2)	
9	49	M	Chest wall	+	+ (type 2)	
10	46	F	Back	+	+ (type 2)	
11	51	M	Arm	+	–	
12	59	F	Shoulder	+	–	
13	43	F	Elbow	+	–	
14	74	F	Elbow	+	–	
15	65	M	Back	+	–	
16	57	F	Foot	+	–	
17	59	F	Shoulder	+	–	
18	50	M	Arm	+	–	
19	61	M	Back	+	–	
20	60	F	Forearm	–	+ (type 1)	
21	79	M	Back	–	+ (type 1)	
22	68	F	Axilla	–	+ (type 2)	

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'. The *LPP-HMGA2* fusion transcript was amplified in a nested manner using the LPP-specific forward primers LPP exon 5 up: 5'-ctggacgctgagattgac-3' and LPP exon 6 up: 5'-acagcctctcctccagtt-3', and the HMGA2-specific reverse primers HMGA2 exon 5 down-1: 5'-ccaccccagatgaaagtg-3' and HMGA2 exon 5 down-2: 5'-ctacaggagaagccgtctgagaac-3' (9). A 124 bp fragment of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA was amplified using the forward primer 5'-cagcgacacccactcctccacctt-3' and the reverse primer 5'-catgaggtccaccaccctgttgct-3' as a control.

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, the first round reaction was performed as described above using the primers LPP exon 5 up and HMGA2 exon 5 down-1. In the second round reaction, 1 μl of the first PCR reaction mix was used as a template and the primers used were LPP exon 6 up and HMGA2 exon 5 down-2.

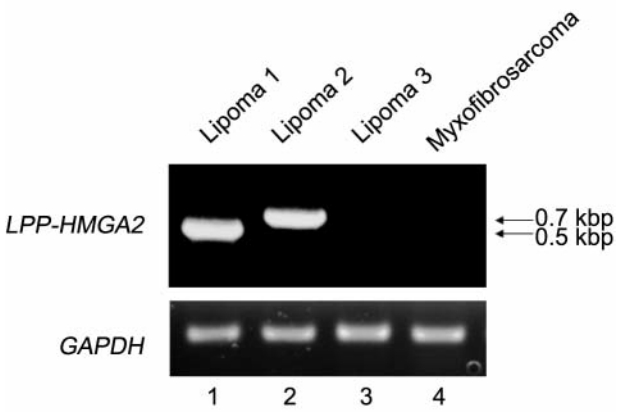

Figure 1. *Detection of LPP-HMGA2 fusion transcripts by RT-PCR. Lane 1, lipoma with LPP-HMGA2 fusion transcript (type 2 variant); Lane 2, lipoma with LPP-HMGA2 fusion transcript (type 1 variant); Lane 3, lipoma without LPP-HMGA2 fusion transcript; Lane 4, myxofibrosarcoma (as a negative control).*

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.

Results

Frequency of HMGA2-LPP and LPP-HMGA2 fusion gene expression. Out of 98 lipomas, ten (10%) revealed both *HMGA2-LPP* and *LPP-HMGA2* fusion transcripts, nine (9%) only *HMGA2-LPP* fusion transcript, and three (3%) only *LPP-HMGA2* fusion transcript (Table I).

Identification of each HMGA2-LPP or LPP-HMGA2 fusion transcript. RT-PCR analysis for *HMGA2-LPP* amplified a 0.7-kbp fragment from cDNAs of 19 lipomas (data not shown). DNA sequencing analysis demonstrated that the *HMGA2-LPP* transcript in the 19 lipomas consisted of exons 1-3 of *HMGA2* and exons 9-11 of *LPP*, which was described previously (5-7). RT-PCR analysis for *LPP-HMGA2* amplified a 0.5-kbp DNA fragment from cDNAs of 4 lipomas, and a 0.7-kbp fragment from cDNAs of 9 lipomas (Figure 1). DNA sequencing analysis of the 0.7-kbp fragment from the 9 lipomas showed that it corresponded to a previously reported *LPP-HMGA2* fusion transcript (type 1 variant), in which exon 8 of *LPP* was in-frame fused to exon 4 of *HMGA2* (Figure 2). The 0.5-kbp DNA fragment from 4 lipomas was identified to be a novel *LPP-HMGA2* fusion transcript (type 2 variant), in which exon 7 of *LPP* was fused to exon 4 of *HMGA2* (Figure 2). This fusion caused frame-shift at the junction and the LPP sequence was followed by 14 amino acids derived from exon 4-5 of *HMGA2* (Figure 3).

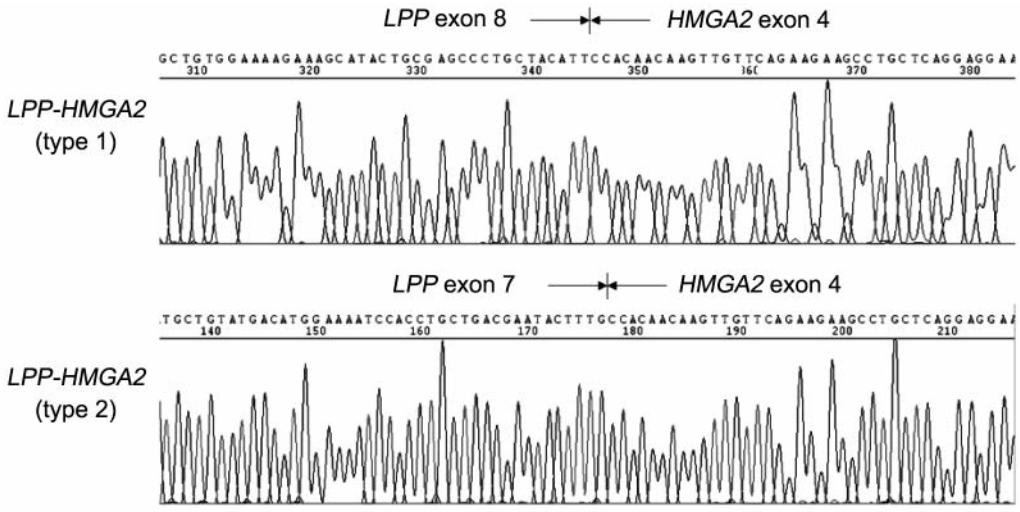

Figure 2. *The LPP-HMGA2 fusion transcripts. Partial nucleotide sequence and sequence chromatogram of the 0.7-kbp (top, type 1 variant) and the 0.5-kbp (bottom, type 2 variant) fragment cDNAs showing the junction of the LPP and HMGA2 genes.*

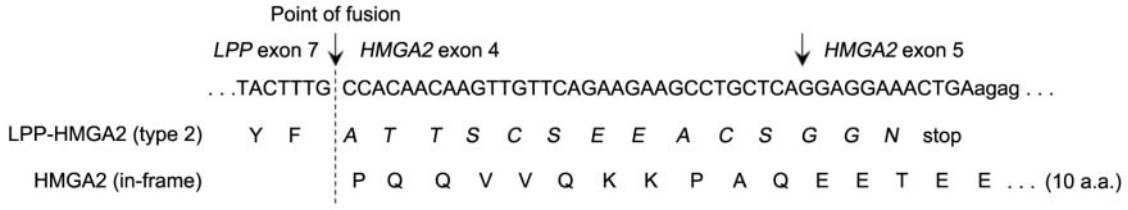

Figure 3. *C-terminal amino acid sequence of the LPP-HMGA2 type 2 variant. The fusion causes frame-shift at the junction. Fourteen predicted amino acids (a.a.) are shown in italic letters below the nucleotide sequence of LPP-HMGA2 gene. The in-frame HMGA2 amino acid sequence is shown for comparison (bottom).*

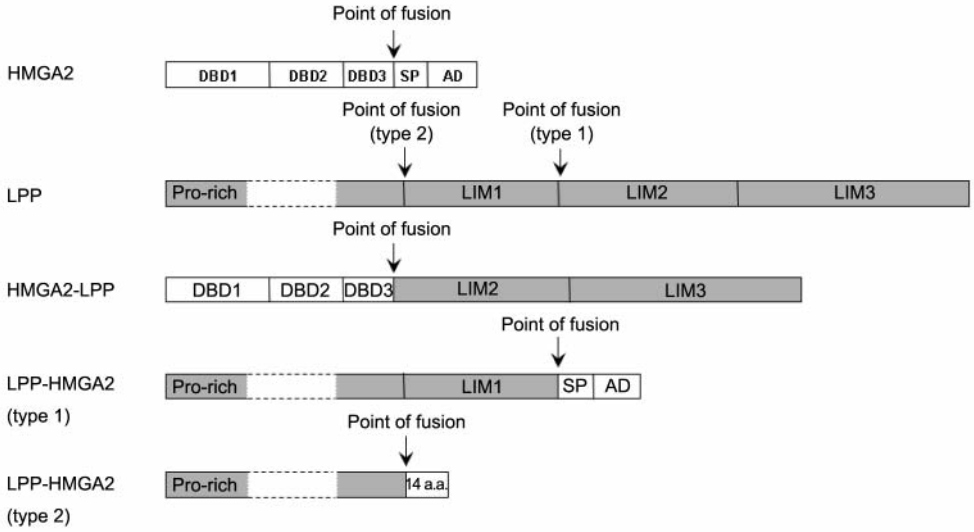

Figure 4. *Diagram showing the structural variation of LPP-HMGA2 fusion proteins. DBD, DNA-binding domain; LIM, LIM domain; SP, short spacer peptide; AD, acidic domain; a.a., amino acids.*

Discussion

The results established the frequency of *HMGA2-LPP* and *LPP-HMGA2* fusion gene expression in lipoma. A total of twenty-two cases (22%) were associated with either *HMGA2-LPP* or *LPP-HMGA2*, indicating the presence of t(3;12). Nineteen cases (19%) were associated with the *HMGA2-LPP* fusion, and thirteen cases (13%) with the *LPP-HMGA2* fusion. This observation was compatible with the recent report where 23 out of 102 lipomas (22.5%) expressed *HMGA2-LPP* (10). Nine cases with *HMGA2-LPP* and three cases with *LPP-HMGA2* did not carry the reciprocal fusion transcript, suggesting the involvement of a large deletion at the junction of the reciprocal locus (11). Several lines of evidence suggest that *HMGA2* truncation is implicated in lipomagenesis (12). On the other hand, the implication of *LPP* truncation has not been clarified. As far as we know, no previous report described a case of lipoma with *LPP-HMGA2* fusion transcript without the reciprocal *HMGA2-LPP*. From the current finding that three lipomas exclusively revealed the *LPP-HMGA2* fusion transcript, we speculated that *LPP* truncation could have some specific roles in lipomagenesis.

In this study, we identified a novel type of *LPP-HMGA2* fusion transcript (type 2 variant), in which exon 7 of *LPP* was fused to exon 4 of *HMGA2*. Out of four cases with the novel transcript, three were associated with the reciprocal *HMGA2-LPP* fusion transcript, which consisted of exons 1-3 of *HMGA2* and exons 9-11 of *LPP*, indicating a genomic deletion spanning exon 8 of *LPP*. Since the novel type of *LPP-HMGA2* was exclusively found in one case, it is also possible to speculate that the type 2 variant is related to lipomagenesis.

Structural comparison between the LPP-HMGA2 variants demonstrates that the type 2 variant replaces LIM1 domain, short spacer peptide, and acidic domain with a novel 14 amino acids (Figure 4). If both of the LPP-HMGA2 variants are involved in lipomagenesis, the common part, *i.e.* the *N*-terminal proline-rich domain, could be of functional interest.

In summary, a potential role for lipomagenesis was implicated not only with *HMGA2-LPP*, but also with *LPP-HMGA2*. The biological implications of the expression and the variation of *LPP-HMGA2* fusion transcripts need to be elucidated.

Acknowledgements

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