Molecular characterization of the t(4;12)(q27~28;q14~15) chromosomal rearrangement in lipoma

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Abstract. Lipomas are common benign soft tissue tumors whose genetic and cytogenetic features are well characterized. The karyotype is usually near- or pseudodiploid with characteristic structural chromosomal aberrations. The most common rearrangements target the *high mobility group AT-hook 2* (*HMGA2)* gene in 12q14.3, with breakpoints occurring within or outside of the gene locus leading to deregulation of *HMGA2*. The most common fusion partner for *HMGA2* in lipoma is *lipoma-preferred partner* (3q27), but also other genes frequently recombine with *HMGA2*. Furthermore, truncated *HMGA2* transcripts are recurrently observed in lipomas. The present study describes 5 lipomas carrying the translocation t(4;12)(q27~28;q14~15) as the sole chromosomal anomaly, as well as 1 lipoma in which the three-way translocation t(1;4;12)(q21;q27~28;q14~15) was identified. Molecular analyses performed on 4 of these cases detected 4 truncated forms of *HMGA2*. In 3 tumors, the *HMGA2* truncated transcripts included sequences originating from the chromosomal sub-band 4q28.1. Notably, in 2 of these cases, the fourth exon of *HMGA2* was fused to transposable elements located in 4q28.1.

Introduction

Lipomas are common fat cell tumors that most often occur in middle-aged or elderly patients. Lipomas may arise subcutaneously (superficial lipomas) or in deep soft tissues (deep-seated intra- or intermuscular lipomas). Superficial lipomas are usually smaller (<5 cm) than their deep-seated counterparts (>5 cm) (1). The genetic features of lipomas have been characterized in considerable detail. The karyotype of lipomas (2-4) is usually near- or pseudodiploid with structural chromosomal rearrangements, the most common of which involve the chromosomal segment 12q13~15 (5). According to the Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer (http://cgap.nci.nih.gov/Chromosomes/Mitelman; database updated on May 7, 2015), 476 lipomas have been reported with chromosomes aberrations, with 12q13~15 being targeted in >300 of them. The recombination with 12q13~15 occurs with multiple other chromosome bands, the most common of which is 3q27~28, corresponding to the translocation t(3;12)(q27~28;q14~15). Other chromosome segments that are frequently recombined with 12q13~15 are 1p36, 1p32~34, 2p22~24, 2q35~37, 5q33, 9p21~22, 12p11~13 and 13q12~14. The rearrangements often target the *high mobility group AT-hook 2* (*HMGA2)* gene in 12q14.3, regardless of whether the breakpoints occur within or outside the gene locus; the essential outcome is the deregulation of *HMGA2*. The most common gene fusing with *HMGA2* in lipoma is *lipoma-preferred partner (LPP)* (3q27) (6), but also other genes located in the common breakpoint regions are known to recombine with *HMGA2* recurrently. In addition, the chromosomal rearrangements may occasionally lead to truncated *HMGA2* transcripts (7).

According to the Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer, the translocation t(4;12)(q27;q15) and variants thereof have been reported in 6 lipomas: In 2 of them, as the sole chromosomal aberration; in other 2, as a three-way translocation; and in the last 2, as part of more complex karyotypes (http://cgap.nci.nih.gov/Chromosomes/Mitelman; database updated on May 7, 2015). The present study describes another 5 cases of lipoma carrying the t(4;12)(q27~28;q14~15) as a sole anomaly and 1 case with a three-way translocation. Molecular analyses were performed on 4 of the tumors in order to discover the putative gene partner from 4q27~28 recombining with *HMGA2*, but none was identified. Instead, the analysis revealed 4 truncated forms of *HMGA2* in the examined samples. The *HMGA2* truncated transcripts included sequences located in the chromosomal sub-band 4q28.1 in 3 of these cases.

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Materials and methods

Ethics statement. The present study was approved by the Regional Committee for Medical and Health Research of the University of Oslo (Oslo, Norway; http://helseforskning.etikkom.no), and written informed consent was obtained from the patients.

Patients. Patients were admitted to the Norwegian Radium Hospital (Oslo, Norway) between January 1, 1998 and November 30, 2014. Table I shows the patients' gender, age, diagnosis and tumor location. All tumors were surgically removed.

Chromosome banding analysis. Samples from the operation specimens were mechanically and enzymatically disaggregated and short-term cultured as described by Mandahl (8). The cultures were harvested, and the chromosomes were G-banded using Wright's stain (Sigma-Aldrich, St. Louis, MO, USA). The subsequent cytogenetic analysis and karyotype description followed the recommendations of the International System for Human Cytogenetic Nomenclature (9).

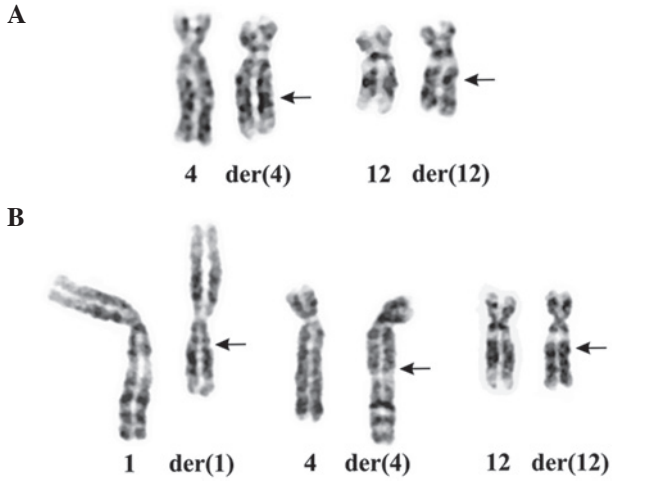

Figure 1. Partial karyotypes of cases 1 and 2. (A) In case 1, the der(4) t(4;12)(q27~28; q14~15) and der(12) t(4;12), together with the corresponding normal chromosome homologs, are shown. (B) In case 2, the der(1), der(4) and der(12) are shown, together with the corresponding normal chromosome homologs from the t(1;4;12)(q21;q27~28;q14~15). Breakpoint positions are indicated by arrows. der, derivative chromosome.

RNA extraction. Tumor tissue adjacent to that used for cytogenetic analysis and histologic examination had been frozen and stored at -80˚C from 4 tumors (cases 1-4). Total RNA was extracted using miRNeasy kit, TissueLyser II and QIAcube according to the manufacturer's protocol (Qiagen GmbH, Hilden, Germany). The concentration and purity of the RNA was measured with the NanoVue Spectrophotometer (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA).

3'-Rapid amplification of complementary DNA (cDNA) ends (3'-RACE). 3'-RACE was performed using a protocol described previously (10). Total RNA (100 ng) was reverse-transcribed in a 20-μl reaction volume using A3RNV-RACE (5'-ATCGTTGAGACTCGTACCAGCAGA GTCACGAGAGAGACTACACGGTACTGGTTTTTTTTT TTTTT-3') as a primer and iScript Select cDNA Synthesis kit (Bio-Rad Laboratories AB, Oslo, Norway) according to the manufacturer's protocol. cDNA (1 μl) was used as template and amplified in a polymerase chain reaction (PCR) using the outer primer combination HMGA2-846F1 (5'-CCACTT CAGCCCAGGGACAACCT-3') and A3R-1New (5'-TCGTTG AGACTCGTACCAGCAGAGTCAC-3'). PCR cycling conditions consisted of an initial step of denaturation at 94˚C for 30 sec, followed by 35 cycles of 7 sec at 98˚C, 2 min at 68˚C and a final extension for 5 min at 72˚C. In total, 1 μl of the amplified products was used as template in nested PCR with the primers HMGA2-982F1 (5'-CAAGAGTCCCTCTAA AGCAGCTCA-3') and A3R3 (5'-CGAGAGAGACTACAC GGTACTGGT-3'). The nested PCR was performed using the Touchdown-PCR conditions described by Korbie and Mattick (11) in order to increase the specificity of the PCR and improve the quality of the products. For both PCRs, the 25-μl reaction volume contained 12.5 μl Premix Ex Taq (Takara Bio Europe SAS, Saint-Germain-en-Laye, France), template, and 0.4 μM of each of the forward and reverse primers. PCR products (3 μl) were stained with GelRed™ (Biotium, Inc., Hayward, CA, USA) and analyzed by electrophoresis using 1.0% agarose gels. The gel was scanned with G:BOX (Syngene, Frederick, MD, USA), and the images were acquired using GeneSnap (Syngene). The remaining 22 μl of the amplified fragments were purified using the QIAquick PCR purification kit (Qiagen AB). Direct sequencing was performed using the LIGHTRUN™ Sequencing Service of GATC Biotech (Konstanz, Germany; www.gatc-biotech.com/en/products/sanger-services/lightrun-sequencing.html). The Basic Local Alignment Search Tool (BLAST; http://blast.ncbi.nlm.nih.gov/Blast.cgi) and BLAST-like alignment tool (http://genome.ucsc.edu/cgi-bin/hgBlat) programs were used for computer analysis of the sequencing data.

Results

Pathology and cytogenetics. Table I contains the patients' gender, age and diagnosis, and the location, karyotype, *HMGA2* expression and *HMGA2* fusions of the examined lipomatous tumors. In all 6 cases (3 males and 3 females), there was recombination between the chromosome bands 12q14~15 and 4q27~28. In total, 5 cases carried t(4;12)(q14~15;q27~28) as the sole karyotypic aberration, whereas 1 lipoma (case 2) had a three-way translocation t(1;4;12)(q21;q27~28;q14~15) (Fig. 1).

Molecular genetics. 3'-RACE on the lipomas of cases 1-4 (Table I) amplified fragments which were revealed to be chimeric *HMGA2*-cDNA fragments by Sanger sequencing analysis (Fig. 2). In lipomas 1, 2 and 4, *HMGA2* was fused with sequences from intergenic regions of 4q28.1. In lipoma 1, the exon 4 of *HMGA2* was fused with the transposable elements long interspersed element L2a and mammalian interspersed repetitive located in 4q28.1 (Fig. 3), whereas lipoma 2 exhibited *HMGA2* exon 3 fused with a circa 750-bp fragment from band 4q28.1. In lipoma 4, *HMGA2* exon 4 was fused with two Alu sequences from chromosome band 4q28.1

Table I. Clinical, cytogenetics and molecular data on the 6 lipomas.

Case no.	Gender/ age, years	Diagnosis	Location	Diameter (cm)	Karyotype [no. cells carrying karyotype]	Genome coordinates of the fusion[a]
1	M/47	Lipoma	Subcutaneous, posterior axillary fold	8.0	46,XY,t(4;12) (q27~28;q14~15)[cp15]	Chr12:65951415 Chr4:1234122125
2	F/73	Lipoma	Intramuscular, throat	7.0	46,XX,t(1;4;12) (q21;q27~28;q14~15) [12]/45,idem,-20[3]	Chr12:65838592 Chr4:126766222
3	F/69	Lipoma	Intramuscular, flank	10.0	46,XX,t(4;12)(q27~28; q14~15)[15]/46,XX[2]	Chr12:65838592 Chr12:65915527
4	F/33	Lipoma (relapse)	Supraclavicular fossa	5.5	46,XX,t(4;12)(q27~28; q14~15)[cp6]	Chr12:65951415 Chr4:123450402
5	M/61	Lipoma	Intramuscular, right thigh	20.0	46,XY,t(4;12)(q27~28; q14~15)[27]/46,XY[3]	Chr12: Not available Chr4: Not available
6	M/14	Lipoma	Intramuscular, left forearm	11.0	46,XY,t(4;12)(q27~28; q14~15)[15]/46,XY[5]	Chr12: Not available Chr4: Not available

[a]Genome coordinates obained from the genome browser of University of California, Santa Cruz (Santa Cruz, CA,USA), assembly December 2013 (GRCh387hg38). M, male; F, female.

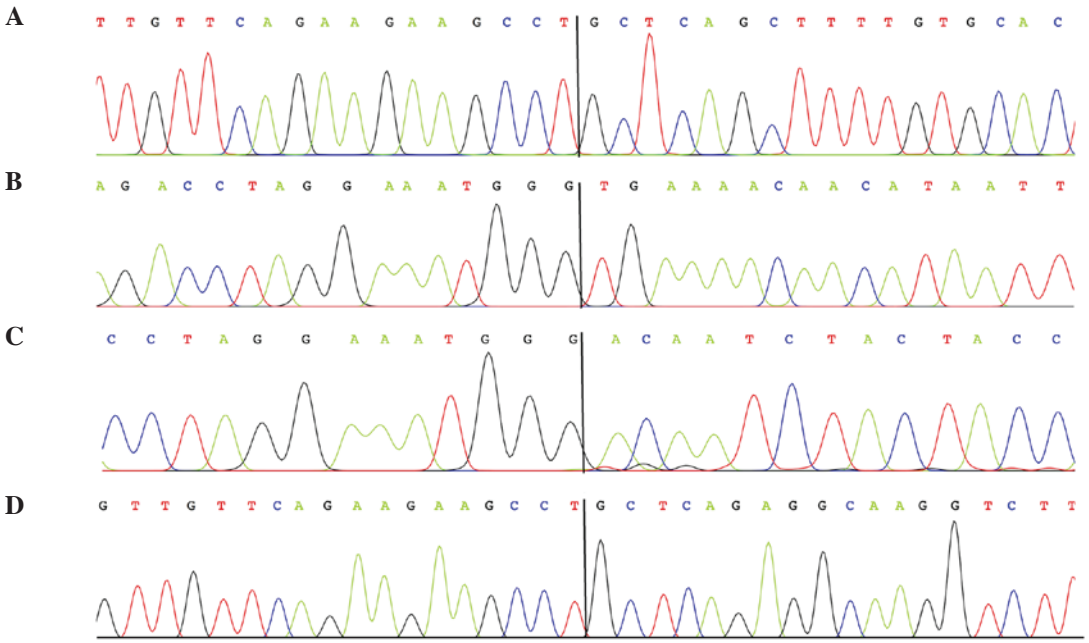

Figure 2. Truncated *HMGA2* transcripts identified in lipomas with t(4;12)(q27~28;q14~15). Chromatograms of the 3'-rapid amplification of complementary DNA ends products. (A) Fusion between *HMGA2* exon 3 and a chromosome 4 long interspersed element sequence was noticed in case 1. (B) Transcript observed in case 2; junction between *HMGA2* exon 3 and chromosome 4. (C) Truncated transcript observed in case 3; junction between the third exon and the first intronic fragment of *HMGA2*. (D) Case 4. Junction between *HMGA2* exon 4 and the first chromosome 4 Alu sequence. *HMGA2, high mobility group AT-hook 2*.

Figure 3. Chromosome 4 transposable elements identified in *high mobility group AT-hook 2*-fusion transcripts. Basic Local Alignment Search Tool-like alignment results for the chromosome 4 sequences observed in (A) case 1 and (B) case 4. BLAT, Basic Local Alignment Search Tool-like alignment; LINE, long interspersed element; MIR, mammalian interspersed repetitive; SINE, short interspersed element.

(Fig. 3). In lipoma 3, the third exon of *HMGA2* was fused with two *HMGA2* intron 3 sequences of 469 and 305 bp, respectively, with a distance between them of ~75 kbp.

Discussion

HMGA1 and HMGA2 are non-histone proteins involved in a wide variety of nuclear processes, from chromatin dynamics to gene regulation (12). *HMGA* family genes are expressed during embryonic development (13), but are largely unexpressed in adult normal tissues (14). High expression levels of *HMGA2* in tissues are usually associated with neoplastic transformation (15). Indeed, recent studies highlight a pivotal role of *HMGA2* in cancer pathogenesis and progression (16). The *HMGA2* gene has been previously observed to be disrupted, due to rearrangement of chromosomal bands 12q13~15, in different connective tissue tumors, including lipomas (7), pleiomorphic adenomas of the salivary gland (17), uterine leiomyomas (18) and lung hamartomas (19). These alterations usually involve exon 3 and cause deletion of downstream regions, leading to a truncated transcript that can evade microRNA-dependent gene silencing (20). Alternatively, chromosomal rearrangements of 12q13~15 may lead to the formation of a fusion gene (15). In lipoma tumorigenesis, the most common fusion partner for *HMGA2* is *LPP* (3q27). Other frequent fusion partners of *HMGA2* are *C-X-C chemokine receptor type 7* (2q37), *early B-cell factor 1* (5q33) and *neurofibromin 1B* (9p23) (5).

The present analyses revealed that the translocation t(4;12)(q27~28;q14~15) is recurrent in lipomas and leads to the truncation of *HMGA2*. Notably, 4 of the 6 lipomas analyzed were intramuscular and large; whether this represents a general feature of t(4;12)-positive tumors remains to be investigated. In 3 lipomas (cases 1, 2 and 4), sequences located in 4q28.1 were fused with *HMGA2*. The ensuing *HMGA2*-fusion transcripts coded for putative proteins which contain amino acid residues 1-83 of HMGA2 protein (accession number NP_003474.1), corresponding to exons 1-3 of the *HMGA2* gene, and amino acid residues from the fused sequences. This pattern is similar to that observed as a result of other rearrangements of *HMGA2* in lipomas, where disruption of the *HMGA2* locus leaves intact exons 1-3 of the gene (which encode the AT-hooks domains) and separates them from the 3'-terminal part of the gene (12). Notably, in cases 1 and 4, the *HMGA2* exon 4 was fused with transposable elements. The Human Genome Project findings predicted that approximately half of the genome consists of such elements, which are capable of integrating at new genomic sites within the cell of origin (21), thus leading to transposable element-driven transcript diversification mediated by alternative splicing (22).

In conclusion, the present study characterized the translocation t(4;12)(q27~28;q14~15) in four lipomas and showed that it disrupts the *HMGA2* locus separating exons 1-3 of the gene from the 3'-untranslated region. This finding contributes to the understanding of *HMGA2* genetics in lipomas.

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