

# Fusion of the *TBL1XR1* and *HMGA1* genes in splenic hemangioma with t(3;6)(q26;p21)

IOANNIS PANAGOPOULOS<sup>1,2</sup>, LUDMILA GORUNOVA<sup>1,2</sup>, BODIL BJERKEHAGEN<sup>3</sup>,  
INGVILD LOBMAIER<sup>3</sup> and SVERRE HEIM<sup>1,2,4</sup>

<sup>1</sup>Section for Cancer Cytogenetics, Institute for Cancer Genetics and Informatics, The Norwegian Radium Hospital, Oslo University Hospital; <sup>2</sup>Centre for Cancer Biomedicine, Faculty of Medicine, University of Oslo; <sup>3</sup>Department of Pathology, The Norwegian Radium Hospital, Oslo University Hospital; <sup>4</sup>Faculty of Medicine, University of Oslo, Oslo, Norway

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**Abstract.** RNA-sequencing of a splenic hemangioma with the karyotype 45~47,XX,t(3;6)(q26;p21) showed that this translocation generated a chimeric *TBL1XR1-HMGA1* gene. This is the first time that this tumor has been subjected to genetic analysis, but the finding of an acquired clonal chromosome abnormality in cells cultured from the lesion and the presence of the *TBL1XR1-HMGA1* fusion in them strongly favor the conclusion that splenic hemangiomas are of a neoplastic nature. Genomic PCR confirmed the presence of the *TBL1XR1-HMGA1* fusion gene, and RT-PCR together with Sanger sequencing verified the presence of the fusion transcripts. The molecular consequences of the t(3;6) would be substantial. The cells carrying the translocation would retain only one functional copy of the wild-type *TBL1XR1* gene while the other, rearranged allele could produce a putative truncated form of TBL1XR1 protein containing the LiSH and F-box-like domains. In the *TBL1XR1-HMGA1* fusion transcript, furthermore, untranslated exons of *HMGA1* are replaced by the first 5 exons of the *TBL1XR1* gene. The result is that the entire coding region of *HMGA1* comes under the control of the *TBL1XR1* promoter, bringing about dysregulation of *HMGA1*. This is reminiscent of similar pathogenetic mechanisms involving high mobility genes in benign connective tissue tumors such as lipomas and leiomyomas.

## Introduction

Splenic hemangiomas, although infrequent, represent the most common benign neoplasms of the spleen with an incidence of

0.03-14% found in large autopsy series (1,2). In the vast majority of cases, splenic hemangiomas are incidental surgical, radiologic, or autopsy findings, made during evaluation for other disorders (1-3). Arber *et al* (3) reported 7 localized splenic hemangiomas all of which were discovered incidentally in surgical patients. In a large series of 32 patients with splenic hemangioma, only 6 presented with abdominal symptoms and only 4 had a palpable spleen (1).

Because of the general absence of presenting symptoms and the typically incidental nature of splenic hemangiomas, the age at presentation varies considerably. Several non-autopsy, surgical series revealed an average age at detection/presentation of between 51 and 63 years (1,3). However, examination of autopsy data reveals a much younger average patient age (4) indicating that these benign lesions are likely to be present but remain undetected for long periods of time. No gender or race predilection has been reported (1).

Splenic hemangiomas are thought to be congenital in origin, arising from sinusoidal epithelium (5). Whether they are neoplastic or represent some other type of misgrowth, remains uncertain. They typically appear as circumscribed, non-encapsulated, honeycomb-like, red-purple masses that frequently blend imperceptibly into the surrounding splenic parenchyma (4). The spaces are composed of sponge-like tissue filled with blood and separated by fibrous septa. Occasional calcification may be seen, often in association with an organized infarct (6). Microscopically, the majority of hemangiomas are cavernous in nature, consisting of large interconnected, dilated, blood-filled spaces lined by a monolayer of cytologically bland endothelial cells separated by thin fibrous septa or splenic pulp tissue. Pure capillary architecture is less common. Instead, many lesions contain varying proportions of both cavernous and capillary components (4). Immunophenotypically, splenic hemangiomas show reactivity of endothelial lining cells for CD31, von Willebrand factor, Ulex europeaus, lectin I, and CD34. This pattern raises the possibility that splenic hemangioma may derive from a combination of splenic venous structures as well as from splenic sinusoidal cells (4).

Most splenic hemangiomas tend to be small in size (<4 cm) although lesions ≤36 cm have been reported (4). They need not be entirely without complications as larger lesions may

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*Correspondence to:* Dr Ioannis Panagopoulos, Section for Cancer Cytogenetics, Institute for Cancer Genetics and Informatics, The Norwegian Radium Hospital, Oslo University Hospital, P.O. Box 4953 Nydalen, NO-0424 Oslo, Norway  
E-mail: ioannis.panagopoulos@rr-research.no

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rupture with resulting intra-abdominal hemorrhage (7-11). In some patients, they cause the Kasabach-Meritt syndrome (12).

The etiology and pathogenesis of splenic hemangiomas are unclear and no cytogenetic or molecular genetic information about the disease has been published. We here describe the cytogenetic analysis of a splenic hemangioma and the fusion gene corresponding to the chromosomal translocation thus found.

## Materials and methods

**Ethical approval.** The study was approved by the Regional Committee for Medical and Health Research Ethics, South-East Norway (REK Sør) <http://helseforskning.etikk.no>. Written informed consent was obtained from the patient. The consent included acceptance that the clinical details be published. The ethics committee's approval included a review of the consent procedure. All patient information has been anonymized and de-identified.

**Patient.** A twenty-nine-year-old woman was incidentally diagnosed with a splenic hemangioma during an ultrasound examination for cholecystitis. She had been without symptoms attributable to the splenic lesion, possibly except some pressure in the upper left abdomen. Because of continuous growth of the hemangioma, it was decided to do a splenectomy. Histological examination (Fig. 1) showed that the lesion was composed of large, blood-filled vessels lined by flat endothelium and separated by thin fibrous septa or splenic pulpa. Immunohistochemical analysis showed positivity for CD31 and ERG.

**Control sample.** The control sample was FirstChoice human spleen total RNA (Life Technologies, Carlsbad, CA, USA).

**G-banding and karyotyping.** Fresh tissue from a representative area of the tumor was received and analyzed cytogenetically as part of our diagnostic routine. The sample was disaggregated mechanically and enzymatically with collagenase II (Worthington, Freehold, NJ, USA). The resulting cells were cultured and harvested using standard techniques. Chromosome preparations were G-banded with Wright stain and examined. Peripheral blood lymphocytes stimulated with phytohemagglutinin (PHA) for 72 h were also karyotyped. The karyotypes were written according to the International System for Human Cytogenetic Nomenclature (ISCN) 2009 guidelines (13).

**RNA and DNA extraction.** Tumor tissue adjacent to that used for cytogenetic analysis and histologic examination had been frozen and stored at -80°C. Total RNA was extracted using miRNeasy Mini kit according to the manufacturer's instructions (Qiagen Nordic, Oslo, Norway). Tumor tissue was disrupted and homogenized in Qiazol Lysis Reagent (Qiagen) using a 5-mm stainless steel bead and TissueLyser II (Qiagen). Subsequently, total RNA was purified using QIAcube (Qiagen). The RNA quality was evaluated using the Experion Automated Electrophoresis system (Bio-Rad Laboratories, Oslo, Norway). The RNA quality indicator (RQI) was 9.0. Genomic DNA was extracted using the Maxwell 16 Instrument System

and the Maxwell 16 Tissue DNA Purification kit (Promega, Madison, WI, USA), and the concentration and purity of DNA were measured using NanoVue Plus Spectrophotometer (GE Healthcare Life Sciences, Oslo, Norway).

**High-throughput paired-end RNA-sequencing.** Three micrograms of total RNA were sent for high-throughput paired-end RNA-sequencing at the Norwegian Sequencing Centre, Ullevål Hospital (<http://www.sequencing.uio.no/>). The RNA was sequenced using an Illumina HiSeq 2000 instrument and the Illumina software pipeline was used to process image data into raw sequencing data. The regular TruSeq library preparation protocol ([http://support.illumina.com/downloads/truseq\\_rna\\_sample\\_preparation\\_guide\\_15008136.ilmn](http://support.illumina.com/downloads/truseq_rna_sample_preparation_guide_15008136.ilmn)) was used. The reads obtained had a length of 100 base pairs (bp). A total of 74 million reads were obtained. The quality of the raw sequence data was assessed using FastQC software (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). The software TopHat-Fusion was used for the discovery of fusion transcripts (14,15). To verify further the fusion gene which was found by TopHat-Fusion, the 'grep' command (<http://en.wikipedia.org/wiki/Grep>) was used to search the fastq files of sequence data ([http://en.wikipedia.org/wiki/FASTQ\\_format](http://en.wikipedia.org/wiki/FASTQ_format)). Our 'specific expression' was a sequence of 20 nucleotides at the fusion point, 10 bases upstream (5'-end gene), and 10 bases downstream from the junction (3'-end gene). The expression was 'CGACCAATAGGTCCCCAAGT'. The sequences obtained by 'grep' were blasted against the human genomic plus transcript database (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) as well as the sequences with accession numbers NM\_024665.4 (*TBL1XR1*) and NM\_002131.3 (*HMGAI*) and DB051170.1 (TEST12 *Homo sapiens* cDNA clone TEST12040990, 5', mRNA sequence).

**RT-PCR and genomic PCR analyses.** The primers used for PCR amplification and Sanger sequencing are listed in Table I. For RT-PCR, 1 µg of total RNA was reverse-transcribed in a 20-µl reaction volume using iScript Advanced cDNA Synthesis kit for RT-qPCR according to the manufacturer's instructions (Bio-Rad Laboratories). The 25 µl PCR volume contained 12.5 µl Premix Ex Taq™ DNA Polymerase Hot Start Version (Takara Bio Europe/SAS, Saint-Germain-en-Laye, France), 1 µl of cDNA, and 0.4 µM of each of the forward and reverse primer. The primer sets TBL1XR1-229F1/DB051170-Intr2R1 and TBL1XR1-229F1/HMGAI-324R1 were used to detect possible *TBL1XR1-HMGAI* fusion transcripts. The quality of the cDNA synthesis was examined by amplification of a cDNA fragment of the *ABL1* gene using the primers ABL1-91F1 and ABL1-404R1 (16).

For genomic PCR, the 25 µl PCR volume contained 12.5 µl Premix Ex Taq™ DNA Polymerase Hot Start Version, 100 ng DNA, and 0.4 µM of each of the forward and reverse primers TBL1XR1-intron4-F1 and DB051170-intr2R1.

The PCR amplifications were run on a C-1000 Thermal cycler (Bio-Rad Laboratories) with an initial denaturation at 94°C for 30 sec, followed by 35 cycles of 7 sec at 98°C, 30 sec at 55°C (58°C for genomic PCR), 1 min at 72°C, and a final extension for 5 min at 72°C. For amplification of the *ABL1* cDNA fragment, the PCR cycling was an initial denaturation at 94°C for 30 sec followed by 35 cycles of 7 sec at 98°C and 2 min at

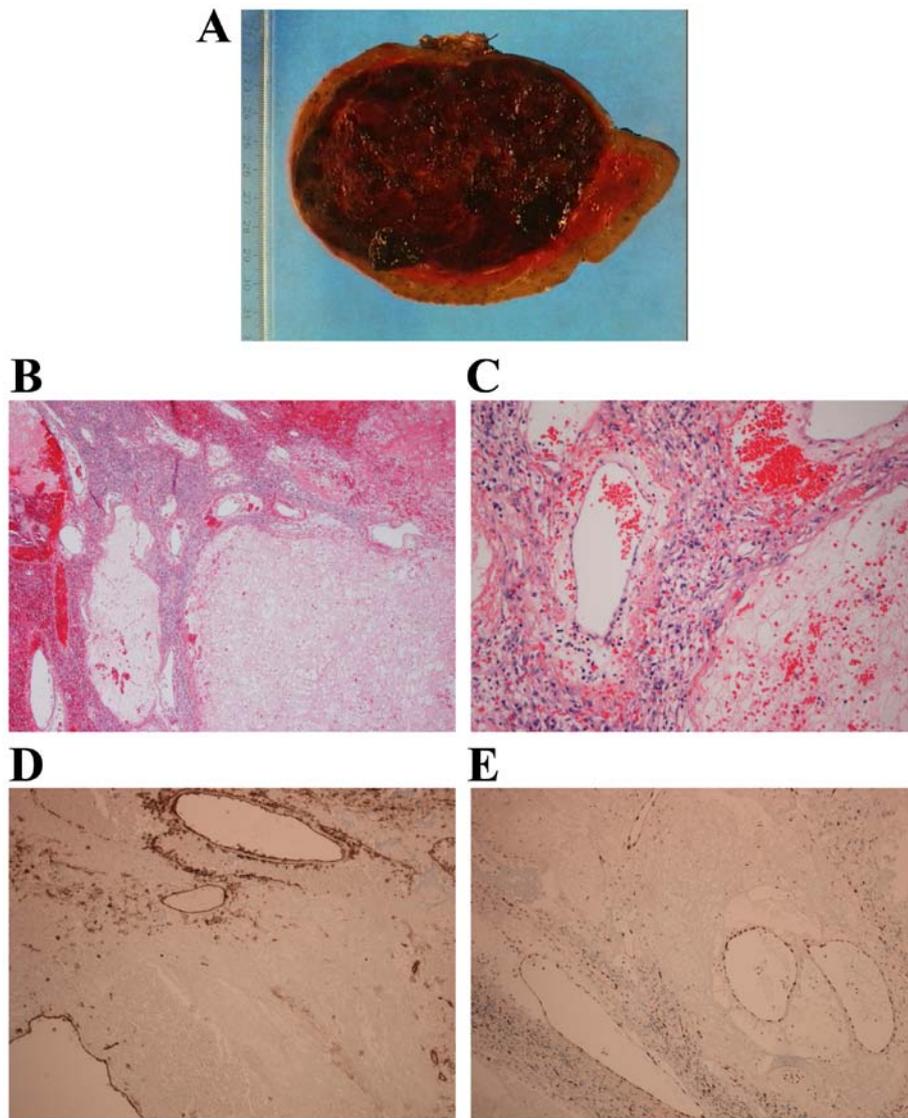


Figure 1. Pathologic examination of the splenic hemangioma. (A) Resected spleen with a well circumscribed hemangioma. (B and C) Microscopic image of splenic hemangioma with blood filled caverns with endothelial cell lining (H&E staining). (D) The vessels are highlighted with CD31 staining. (E) The endothelial cell nuclei are highlighted with ERG staining.

Table I. Primers used for PCR amplification and Sanger sequencing analyses.

Name	Sequence (5'-3')	Direction in PCR	Position on Human Feb. 2009 (GRCh37/hg19) Assembly
TBL1XR1-229F1	TGTTGTGACCTCATGGTTTAAGTGG	Forward	chr3:176,782,774-176,782,798
TBL1XR1-intron4-F1	AAGCAGTCATTTCCAGTTGCTGC	Forward	chr3:176,769,680-176,769,702
HMGA1-324R1	GGACTTCGAGCTCGACTCACTCA	Reverse	chr6:34,208,559-34,208,581
DB051170-Intr2R1	GTACACCCAAGGGAGGCTTCATAC	Reverse	chr6:34,203,951-34,203,974
DB051170-Intr2R2	TGATCAAGGTGGAGCCTTCCAGC	Reverse	chr6:34,204,001-34,204,023
ABL1-91F1	CAGCGGCCAGTAGCATCTGACTTTG	Forward	chr9:133,729,459-133,729,483
ABL1-404R1	CTCAGCAGATACTCAGCGGCATTGC	Reverse	chr9:133,730,335-133,730,359

68°C, and a final extension for 5 min at 68°C. Three microliters of the PCR products were stained with GelRed (Biotium, Hayward, CA, USA), analysed by electrophoresis through

1.0% agarose gel, and photographed. The remaining 22 µl PCR products were purified using the MinElute PCR purification kit (Qiagen Nordic) and sequenced at GATC Biotech

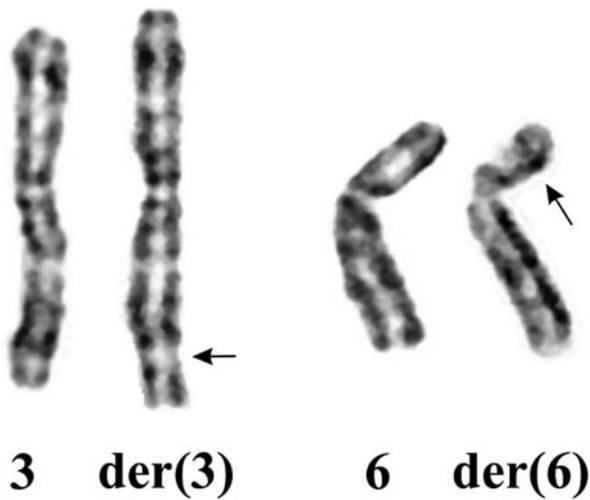


Figure 2. Partial karyotype showing the der(3)t(3;6)(q26;p21) and der(6)t(3;6)(q26;p21) together with the corresponding normal chromosome homologs; breakpoint positions are indicated by arrows.

(Germany, <http://www.gatc-biotech.com/en/home.html>). The BLAST software (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) was used for computer analysis of sequence data.

**Results**

*G-banding.* The G-banding analysis of the splenic hemangioma yielded a karyotype with a single clonal chromosome abnormality: 45~47,XX,t(3;6)(q26;p21)[cp22] (Fig. 2). The G-banding analysis of PHA-stimulated peripheral blood cells yielded a normal 46,XX karyotype. Thus, the translocation t(3;6)(q26;p21) was found in cells of the splenic hemangioma only.

*High-throughput paired-end RNA-sequencing analysis.* Using the TopHat-Fusion on the raw sequencing data obtained by Norwegian Sequencing Centre, four fusions were found between chromosome bands 3q26 and 6p21 (Table II). Among them was a fusion between *TBLIXR1* (from 3q26) and the sequence with accession number DB051170 which is an alternative splicing transcript of *HMGAI* found in testis (17).

In order to verify this fusion, we used the ‘grep’ command utility to search for expressions composed of 10 nt of *TBLIXR1* and 10 nt of *HMGAI* upstream and downstream of the fusion point (Table III). Using the expression ‘CGACCAATAGGTCCCAAGT’, which is composed of 10 nt, ‘CGACCAATAG’, from *TBLIXR1* and 10 nt, ‘GTCCCAAGT’, from *HMGAI*, 16 sequences were retrieved. BLAT of these sequences on the human genome browser-hg19 assembly (<http://genome-euro.ucsc.edu/cgi-bin/hgGateway>) showed that they were chimeric cDNA fragments composed of nucleotides which mapped on 3q26 in the coding region of *TBLIXR1* and nucleotides mapped on chromosome band 6p21, circa 700 bp upstream of the *HMGAI* sequence with accession number NM\_002131 and within the sequence with accession number DB051170 which is an alternative splicing transcript of *HMGAI* found in testis (17). The fusion had occurred between nt 496 of *TBLIXR1* mRNA reference sequence NM\_024665.4

Table II. Fusions between chromosome bands 3q26 and 6p21 which were found with the TopHat-Fusion program.<sup>a</sup>

3q26-fusion point	6p21-fusion point	Fifty bases on the left side of the fusion (3p21)	Fifty bases on the right side of the fusion (6p21)
166506603	30593594	GGGATTACA AACTCCTCCTGGCATT CAGGAGTGAGCCACCACCATGCCTGGCC	TCTCTTCCCAGTCTACAGCTCCTACAGACACTCTGAGGGCCCTGTCTGCC
<b>176769483</b>	<b>34203899</b>	<b>CACAATTTTCCAAATCCAGGATGG</b> <b>TACCTTGTTTGATGGTCGACCAATAG</b>	<b>GTCCCAAGTGGGCCCTGCCGTATCTCCAGAACACCAATCTAAGTCACCTCAA</b>
176799652	35441544	TCTTTAGTCAGTATAATTACAGT ATTGTATATAATAATAACAATAATAAAA	CCTAGTGGGGTTTGGAGGTGGCCCTGAGCGATATGCAAACAGTGAGGACC
161838267	39157355	TTGGGTTTGAGACTATAGCTAAA ATATAATAAAACAAAAAATAACAAATA	CATTCAAGACACAGTGGGCCAGCCCCAGGGTGAACATCATGGAGAACCCA

<sup>a</sup>The sequences corresponding to *TBLIXR1*-*HMGAI* fusion are in bold.

Table III. Sequences retrieved with the 'grep' command using the expression 'CGACCAATAGGTCCCAAGT'.<sup>a</sup>

1	<b>CTTGT</b> TTGATGTCGACCAATAGGTCCCAAGTGGGCTCGCTAATCTCCAGAACACCAATCTAAGTCACTCAAGGTATGAAGCCTCCCTTGGGTGTACCTG
2	<b>ATTTT</b> CCAAATCCAGGATGGTACCTTGTGATGGTCGACCAATAGTCCCAAGTGGGCTCGCTAATCTCCAGAACACCAATCTAAGTCACTCAAGGTAT
3	<b>CTTGT</b> TTGATGTCGACCAATAGGTCCCAAGTGGGCTCGCTAATCTCCAGAACACCAATCTAAGTCACTCAAGGTATGAAGCCTCCCTTGGGTGTACCTG
4	<b>GGTAC</b> CTTGTGATGTCGACCAATAGGTCCCAAGTGGGCTCGCTAATCTCCAGAACACCAATCTAAGTCACTCAAGGTATGAAGCCTCCCTTGGGTGT
5	<b>AGAAG</b> CAGAAAGTTAGTATTAAATGAGGATGGTACCTTGTGATGGTCGACCAATAGTCCCAAGTGGGCTCGCTAATCTCCAGAACACCAATCTAAGTCACT
6	<b>ATTTT</b> CCAAATCCAGGATGGTACCTTGTGATGGTCGACCAATAGTCCCAAGTGGGCTCGCTAATCTCCAGAACACCAATCTAAGTCACTCAAGGTAT
7	<b>GATGT</b> AGAAGCAGAAAGTTAGTATTAAATGAGGATGGTACCTTGTGATGGTCGACCAATAGTCCCAAGTGGGCTCGCTAATCTCCAGAACACCAATCTA
8	<b>CAGTAT</b> GAGAACGCAAGTTAGTATTAAATGAGGATGGTACCTTGTGATGGTCGACCAATAGTCCCAAGTGGGCTCGCTAATCTCCAGAACACCAATC
9	<b>CTATC</b> ATCCAGAAAGTCTACAGTATGAGAACGCAAGTTAGTATTAAATGAGGATGGTACCTTGTGATGGTCGACCAATAGTCCCAAGTGGGCTCGCTG
10	<b>CTATC</b> ATCCAGAAAGTCTACAGTATGAGAACGCAAGTTAGTATTAAATGAGGATGGTACCTTGTGATGGTCGACCAATAGTCCCAAGTGGGCTCG
11	<b>CTATC</b> ATCCAGAAAGTCTACAGTATGAGAACGCAAGTTAGTATTAAATGAGGATGGTACCTTGTGATGGTCGACCAATAGTCCCAAGTGGGCTCG
12	<b>TTCTAT</b> CATCCAGAAAGTCTACAGTATGAGAACGCAAGTTAGTATTAAATGAGGATGGTACCTTGTGATGGTCGACCAATAGTCCCAAGTGGGCTCG
13	<b>TTTATTT</b> CATCCAGAAAGTCTACAGTATGAGAACGCAAGTTAGTATTAAATGAGGATGGTACCTTGTGATGGTCGACCAATAGTCCCAAGTGGGCTCG
14	<b>AGAAG</b> CAGAAAGTTAGTATTAAATGAGGATGGTACCTTGTGATGGTCGACCAATAGTCCCAAGTGGGCTCGCTAATCTCCAGAACACCAATCTAAGTCACT
15	<b>AGAAG</b> CAGAAAGTTAGTATTAAATGAGGATGGTACCTTGTGATGGTCGACCAATAGTCCCAAGTGGGCTCGCTAATCTCCAGAACACCAATCTAAGTCACT
16	<b>GGTAC</b> CTTGTGATGTCGACCAATAGTCCCAAGTGGGCTCGCTAATCTCCAGAACACCAATCTAAGTCACTCAAGGTATGAAGCCTCCCTTGGGTGT

<sup>a</sup>The sequences of *TBL1XR1* are in bold.

and nt 215 of the sequence with accession number DB051170.1 (Table III). We therefore decided to investigate the tumor further for the presence of the *TBL1XR1-HMGA1* fusion transcript using molecular techniques. No other fusions were examined.

*Molecular genetic confirmation of the TBL1XR1-HMGA1 fusion.* A 338-bp *ABL1* cDNA fragment was amplified indicating the good quality of the synthesized cDNA (Fig. 3A).

To verify the data obtained with the RNA-sequencing/TopHat-Fusion software and 'grep' command, PCR amplifications were performed using forward *TBL1XR1* and reverse *HMGA1* primers corresponding to sequences located upstream and downstream of the putative breakpoint, respectively. PCR with the two primer combinations, TBL1XR1-229F1/DB01170-intr2R1 and TBL1XR1-229F1 and HMGA1-324R1, amplified fragments from the cDNA of splenic hemangioma but not from cDNA of the normal spleen. The results strongly suggested the presence of *TBL1XR1-HMGA1* chimeric transcript in splenic hemangioma (Fig. 3A). Direct Sanger sequencing of the amplified fragments showed that both were *TBL1XR1-HMGA1* chimeric cDNA fragments with the fusion point identical to that found with TopHat-Fusion and the 'grep' command, i.e., the fusion had occurred between nt 496 of *TBL1XR1* mRNA reference sequence NM\_024665.4 and nt 215 of the sequence with accession number DB051170.1 (Fig. 3B).

Genomic PCR with the primer combination TBL1XR1-intron4-F1/DB051170-intr2R1 amplified a fragment from the DNA of the splenic hemangioma. Direct Sanger sequencing showed that it was a genomic hybrid DNA fragment with sequences from the *TBL1XR1* and *HMGA1* genes (Fig. 4B). The junction point was identical to the fusion point found with TopHat-Fusion and the 'grep' command and with RT-PCR.

**Discussion**

We describe the first cytogenetic and molecular genetic analysis of a splenic hemangioma. The tumor had an acquired chromosomal translocation, t(3;6)(q26;p21), which resulted in fusion of the *TBL1XR1* (from 3q26) and *HMGA1* (from 6p21) genes. The fact that an acquired genetic aberration was found in the splenic hemangioma cells argues strongly that the disease is neoplastic.

The protein encoded by the *TBL1XR1* gene has sequence similarity with members of the WD40 repeat-containing protein family (18). The WD40 group is a large family of proteins which appear to have a regulatory function (19-21). WD40 repeats mediate protein-protein interactions and members of the family are involved in signal transduction, RNA processing, gene regulation, vesicular trafficking, cytoskeletal assembly, and they may also play a role in the control of cytotypic differentiation (19-21). *TBL1XR1* is a core component of NCoR (nuclear receptor corepressor) and SMRT (silencing mediator of retinoic acid and thyroid hormone receptors) repressor complexes and is essential in targeting SMRT/NCoR corepressor complexes to the promoter of target genes. It is also required for transcriptional activation by nuclear receptors and other regulated transcription factors (18). *TBL1XR1* is further essential in the activation of Wnt-β-catenin and NF-κB signaling pathways (18). *De novo* deletions

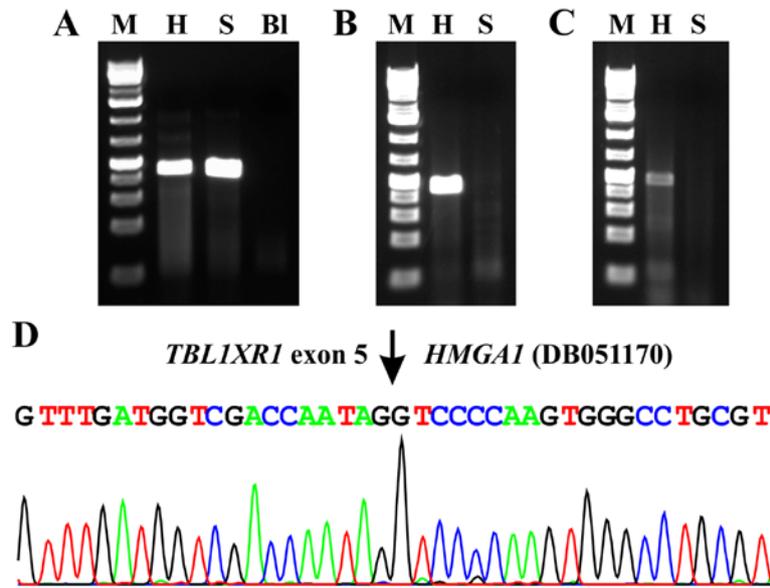


Figure 3. RT-PCR analyses of splenic hemangioma (H) together with normal spleen (S). (A) Amplification of an *ABL1* cDNA fragment using the primers *ABL1*-91F1 and *ABL1*-404R1. (B) Amplification of a cDNA fragment using the primers *TBLIXR1*-229F1 and *DB051170*-Intr2R1. (C) Amplification of a cDNA fragment using the primers *TBLIXR1*-229F1 and *HMGA1*-324R1. M, 1 kb DNA ladder (GeneRuler, Fermentas); BI, blank, water in cDNA synthesis. (D) Partial sequence chromatogram of the cDNA fragment showing the fusion (arrow) of *TBLIXR1* with *HMGA1* (sequence with accession number *DB051170*).

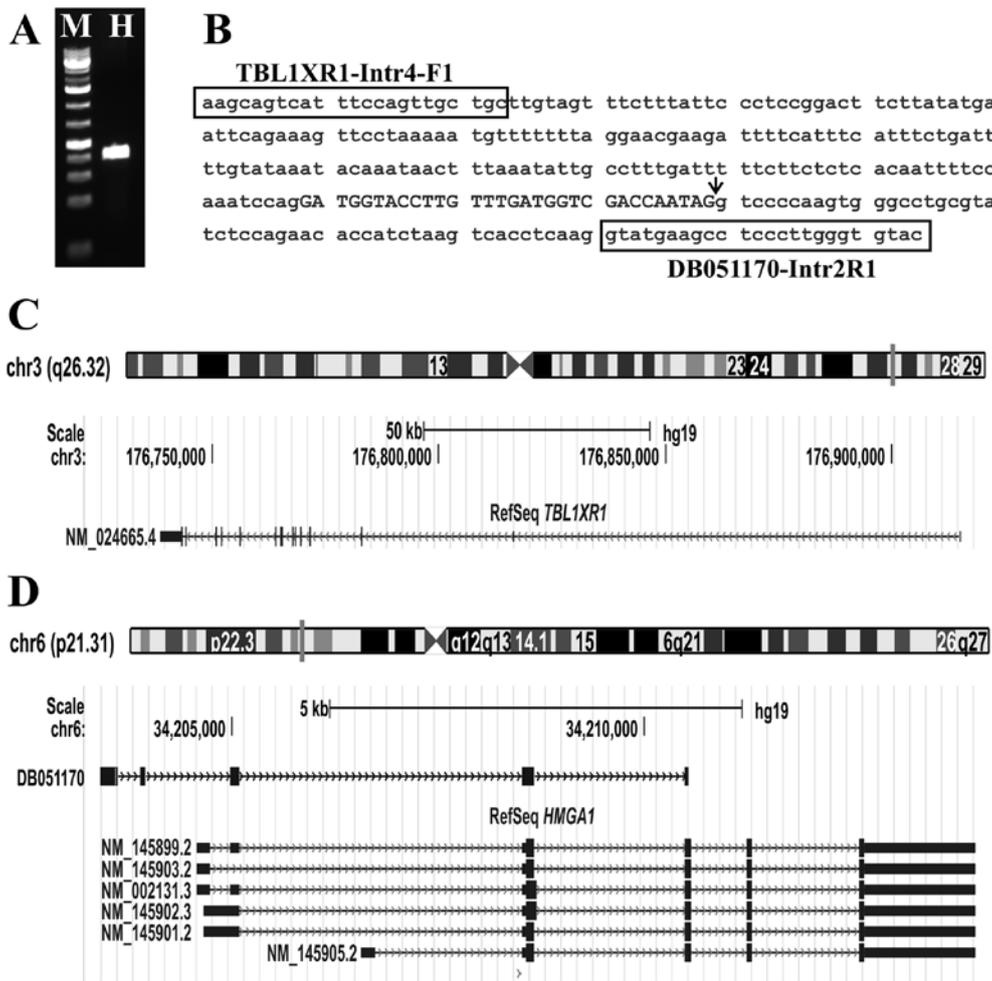


Figure 4. Genomic PCR analysis of splenic hemangioma (H). (A) Amplification of genomic DNA fragment using the primers *TBLIXR1*-intron4-F1 and *DB051170*-intr2R1. (B) Direct Sanger sequencing showed that the amplicon is a genomic hybrid DNA fragment with sequences from *TBLIXR1* and *HMGA1*. The primers are shown in boxes. Arrow indicates the genomic breakpoint. Capital letters show part of exon 5 of *TBLIXR1*. (C) The *TBLIXR1* gene. The location of *TBLIXR1* on chromosome 3 (vertical line) and the reference sequence *NM\_024665* are shown. (D) The *HMGA1* gene. The location of *HMGA1* on chromosome 6 (vertical line), the 6 reference sequences, and the sequence with the accession number *DB051170* are shown.

and recurrent mutations have been identified in the *TBL1XR1* gene and are linked to intellectual disability (18). *TBL1XR1* plays an important role in tumorigenesis, invasion, metastasis, and the development of therapy resistance (18). *TBL1XR1* is overexpressed in primary lung squamous cell carcinoma, breast cancer, cervical cancer, nasopharyngeal carcinoma, esophageal squamous cell carcinoma, and invasive prostate cancer (reviewed in ref. 18). Mutation of *TBL1XR1* was found in primary central nervous system lymphomas (22,23). Deletions of *TBL1XR1* were described in 15% of *ETV6-RUNX1* positive acute lymphoblastic leukemias (24). In frame *TBL1XR1* chimeric transcripts which code for chimeric proteins, were found in different neoplasias. A recurrent *TBL1XR1-TP63* fusion gene was reported in diffuse large B-cell lymphoma, peripheral T-cell lymphoma, and follicular lymphoma which was the result of a chromosomal rearrangement between 3q26 (*TBL1XR1*) and 3q28 (*TP63*) (25,26). In most of the cases, the exons 1-7 of *TBL1XR1* were fused in frame to exons 4-8 or 4-10 of *TP63*. Exon 14 or exon 4 of *TBL1XR1* was involved in the remaining cases (25,26).

*TBL1XR1* is fused to *RARA* in an acute promyelocytic leukemia carrying a variant t(3;17)(q26;q21) translocation (27). The *TBL1XR1-RARA* fusion protein was predominantly localized in the nucleus, formed homodimers or heterodimers with retinoid X receptor  $\alpha$ , and acted as transcriptional activator in the presence of ligand. In the presence of pharmacologic doses of ATRA, *TBL1XR1-RARA* protein could be degraded, and its homodimerization was abrogated (27).

Fusions of *TBL1XR1* resulting in promoter swapping were also found (28,29). In the breast carcinoma MCB7 cell line, the untranslated (5'-UTR) exon 1 of *TBL1XR1* is fused with the start codon-bearing exon 2 of *RGS17*, generating a fusion gene that encodes the full-length *RGS17* protein under the control of *TBL1XR1* gene promoter (28). The same 5'-UTR exon 1 of *TBL1XR1* was also found to be fused with the *PIK3CA* gene in breast cancer and prostate adenocarcinomas. The result was again the expression of *PIK3CA* which came under the control of the *TBL1XR1* promoter (29).

The *HMGAI* gene encodes a non-histone protein involved in many cellular processes, including regulation of inducible gene transcription, integration of retroviruses into chromosomes, and the metastatic progression of cancer cells (30-32). The encoded protein preferentially binds to the minor groove of A+T-rich regions in double-stranded DNA. It has little secondary structure in solution but assumes distinct conformations when bound to substrates such as DNA or other proteins. The encoded protein is frequently acetylated and is found in the nucleus. Six transcript variants were identified encoding two different isoforms (<http://www.ncbi.nlm.nih.gov/gene/3159>). However, expressed sequence tags (EST) indicate that additional alternative splicing exists with various 5'-UTR sequences occurring in different tissues. For example, the EST with accession numbers DB051170 and DB03591 are found in testis, EST BM845429 in lymph node, CN350278 in embryonic stem cells, DC338100 in brain, and DA921912 is found in the small intestine. These splicing variants may be regulated by alternative promoters of *HMGAI* (17).

*HMGAI* is overexpressed in poorly differentiated cancers originating from all three germ layers, suggesting that it plays a fundamental role in tumorigenesis regardless of the cell type

from which the tumor begins. High expression of the *HMGAI* gene or high levels of HMGA1 protein were found in cancers of the thyroid, lung, breast, bladder, prostate, colon, pancreas, uterine corpus, uterine cervix, kidney, head and neck, nervous system, stomach, liver, and hematopoietic system. Expression studies reveal that *HMGAI* overexpression correlates with adverse clinical outcomes in cancers (33-35).

The basis for *HMGAI* overexpression is not well understood but it seems that *HMGAI* expression is induced through different pathways. For example, *HMGAI* expression was shown to be induced by cMYC and AP1 transcription factors (36,37). Recent studies suggest that microRNAs regulate *HMGAI* in some types of cancer (38-40). Another recent study showed that HMGA1 was an immediate target of the  $\beta$ -catenin/TCF-4 signaling pathway in colon cancer (41).

Dysregulation of *HMGAI* as a result of specific chromosomal rearrangements involving chromosomal region 6p21.3 has also been identified in a variety of common benign mesenchymal tumors such as lipomas, uterine leiomyomas, endometrial polyps, and pulmonary hamartomas (42-51). The breakpoints in most tumors are located 3' of *HMGAI*, but 5' or intragenic breakpoints have also been reported (42,43,46,47). The findings we present indicate that a similar pathogenetic mechanism may be operative also in splenic hemangioma. Whether any systematic tumorigenic differences exist between this and the other benign neoplasias is a question that can be answered only after many more splenic hemangiomas are genetically analyzed.

So far, only one *HMGAI*-fusion transcript has been described in the literature: an *HMGAI-LAMA4* found in a pulmonary hamartoma as a result of inv(6)(p21q21) (46). Fusion of *HMGAI* to the intergenic region between *LPP* and *TPRG1* in a lipoma carrying a t(3;6)(q27;p21) has also been reported (52).

The present t(3;6)(q26;p21) chromosomal rearrangement would have molecular consequences for both *TBL1XR1* and *HMGAI*. The *TBL1XR1* gene would have only a single functional copy of the gene left in the cell, while the other, rearranged allele would produce a putative truncated form of *TBL1XR1* protein containing the LiSH and F-box-like domains (NP\_078941; LiSH domain: 4-32, F-box-like domain: 41-86). Thus, the truncated form of *TBL1XR1*, through the LiSH and F-box-like domains, could participate in protein dimerization, affect protein half-life, and could influence specific cellular localizations (53,54).

With regard to *HMGAI*, the *TBL1XR1-HMGAI* fusion transcript leads untranslated exons of *HMGAI* to be replaced by the first 5 exons of the *TBL1XR1* gene. The result is that the entire coding region of *HMGAI* comes under the control of the *TBL1XR1* promoter leading to dysregulation of *HMGAI*.

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## References

1. Willcox TM, Speer RW, Schlinkert RT and Sarr MG: Hemangioma of the spleen: Presentation, diagnosis, and management. *J Gastrointest Surg* 4: 611-613, 2000.

2. Husni EA: The clinical course of splenic hemangioma with emphasis on spontaneous rupture. *Arch Surg* 83: 681-688, 1961.
3. Arber DA, Strickler JG, Chen YY and Weiss LM: Splenic vascular tumors: A histologic, immunophenotypic, and virologic study. *Am J Surg Pathol* 21: 827-835, 1997.
4. Kutok JL and Fletcher CD: Splenic vascular tumors. *Semin Diagn Pathol* 20: 128-139, 2003.
5. Abbott RM, Levy AD, Aguilera NS, Gorospe L and Thompson WM: From the archives of the AFIP: primary vascular neoplasms of the spleen: radiologic-pathologic correlation. *Radiographics* 24: 1137-1163, 2004.
6. Halgrimson CG, Rustad DG and Zeligman BE: Calcified hemangioma of the spleen. *JAMA* 252: 2959-2960, 1984.
7. Kaplan J and McIntosh GS: Spontaneous rupture of a splenic vascular malformation. A report of three cases and review of the literature. *J R Coll Surg Edinb* 32: 346-347, 1987.
8. Rao RC, Ghose R, Sawhney S and Berry M: Hemangioma of spleen with spontaneous, extra-peritoneal rupture, with associated splenic tuberculosis - an unusual presentation. *Australas Radiol* 37: 100-101, 1993.
9. Pachtl M, Elmalik K, Cohen M, Kamupira S, Walker J and Murthi G: Ruptured splenic cavernous hemangioma in a neonate. *J Pediatr Surg* 43: 407-409, 2008.
10. Carta G, D'Alfonso A, Nallbani A, Palermo P, Franchi V and Patacchiola F: Spontaneous rupture of splenic hemangioma in puerperium. *Clin Exp Obstet Gynecol* 39: 407-408, 2012.
11. Kang LY, Huang FD and Liu YY: Blunt abdominal injury with rupture of giant hepatic cavernous hemangioma and laceration of the spleen. *Hepatobiliary Pancreat Dis Int* 14: 109-110, 2015.
12. Hoeger PH, Helmke K and Winkler K: Chronic consumption coagulopathy due to an occult splenic haemangioma: Kasabach-Merritt syndrome. *Eur J Pediatr* 154: 365-368, 1995.
13. Schaffer LG, Slovic ML and Campbell LJ (eds): *ISCN 2009: an International System for Human Cytogenetic Nomenclature*. Karger, Basel, 2009.
14. Kim D, Perle G, Trapnell C, Pimentel H, Kelley R and Salzberg SL: TopHat2: Accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome Biol* 14: R36, 2013.
15. Kim D and Salzberg SL: TopHat-Fusion: An algorithm for discovery of novel fusion transcripts. *Genome Biol* 12: R72, 2011.
16. Panagopoulos I, Gorunova L, Bjerkehagen B and Heim S: Fusion of the genes *EWSR1* and *PBX3* in retroperitoneal leiomyoma with t(9;22)(q33;q12). *PLoS One* 10: e0124288, 2015.
17. Kimura K, Wakamatsu A, Suzuki Y, Ota T, Nishikawa T, Yamashita R, Yamamoto J, Sekine M, Tsuritani K, Wakaguri H, *et al*: Diversification of transcriptional modulation: Large-scale identification and characterization of putative alternative promoters of human genes. *Genome Res* 16: 55-65, 2006.
18. Li JY, Daniels G, Wang J and Zhang X: *TBL1XR1* in physiological and pathological states. *Am J Clin Exp Urol* 3: 13-23, 2015.
19. Xu C and Min J: Structure and function of WD40 domain proteins. *Protein Cell* 2: 202-214, 2011.
20. Li D and Roberts R: WD-repeat proteins: Structure characteristics, biological function, and their involvement in human diseases. *Cell Mol Life Sci* 58: 2085-2097, 2001.
21. Neer EJ, Schmidt CJ, Nambudripad R and Smith TF: The ancient regulatory-protein family of WD-repeat proteins. *Nature* 371: 297-300, 1994.
22. Nakamura T, Tateishi K, Niwa T, Matsushita Y, Tamura K, Kinoshita M, Tanaka K, Fukushima S, Takami H, Arita H, *et al*: Recurrent mutations of *CD79B* and *MYD88* are the hallmark of primary central nervous system lymphomas. *Neuropathol Appl Neurobiol*: Jun 25, 2015 (Epub ahead of print). doi: 10.1111/nan.12259.
23. Gonzalez-Aguilar A, Idbaih A, Boisselier B, Habbita N, Rossetto M, Laurence A, Bruno A, Jouveta A, Polivka M, Adam C, *et al*: Recurrent mutations of *MYD88* and *TBL1XR1* in primary central nervous system lymphomas. *Clin Cancer Res* 18: 5203-5211, 2012.
24. Parker H, An Q, Barber K, Case M, Davies T, Konn Z, Stewart A, Wright S, Griffiths M, Ross FM, *et al*: The complex genomic profile of *ETV6-RUNX1* positive acute lymphoblastic leukemia highlights a recurrent deletion of *TBL1XR1*. *Genes Chromosomes Cancer* 47: 1118-1125, 2008.
25. Scott DW, Mungall KL, Ben-Neriah S, Rogic S, Morin RD, Slack GW, Tan KL, Chan FC, Lim RS, Connors JM, *et al*: *TBL1XR1/TP63*: A novel recurrent gene fusion in B-cell non-Hodgkin lymphoma. *Blood* 119: 4949-4952, 2012.
26. Vasmatzis G, Johnson SH, Knudson RA, Ketterling RP, Braggio E, Fonseca R, Viswanatha DS, Law ME, Kip NS, Ozsan N, *et al*: Genome-wide analysis reveals recurrent structural abnormalities of *TP63* and other p53-related genes in peripheral T-cell lymphomas. *Blood* 120: 2280-2289, 2012.
27. Chen Y, Li S, Zhou C, Li C, Ru K, Rao Q, Xing H, Tian Z, Tang K, Mi Y, *et al*: *TBLR1* fuses to retinoic acid receptor  $\alpha$  in a variant t(3;17)(q26;q21) translocation of acute promyelocytic leukemia. *Blood* 124: 936-945, 2014.
28. Hahn Y, Bera TK, Gehlhaus K, Kirsch IR, Pastan IH and Lee B: Finding fusion genes resulting from chromosome rearrangement by analyzing the expressed sequence databases. *Proc Natl Acad Sci USA* 101: 13257-13261, 2004.
29. Stransky N, Cerami E, Schalm S, Kim JL and Lengauer C: The landscape of kinase fusions in cancer. *Nat Commun* 5: 4846, 2014.
30. Benecke AG, Eilebrecht S, Benecke A and Eilebrecht S: RNA-mediated regulation of *HMGA1* function. *Biomolecules* 5: 943-957, 2015.
31. Reeves R and Beckerbauer L: *HMGI/Y* proteins: Flexible regulators of transcription and chromatin structure. *Biochim Biophys Acta* 1519: 13-29, 2001.
32. Resar LM: The high mobility group A1 gene: Transforming inflammatory signals into cancer? *Cancer Res* 70: 436-439, 2010.
33. Zhang Z, Wang Q, Chen F and Liu J: Elevated expression of *HMGA1* correlates with the malignant status and prognosis of non-small cell lung cancer. *Tumour Biol* 36: 1213-1219, 2015.
34. Huang R, Huang D, Dai W and Yang F: Overexpression of *HMGA1* correlates with the malignant status and prognosis of breast cancer. *Mol Cell Biochem* 404: 251-257, 2015.
35. Liao SS, Rocha F, Matros E, Redston M and Whang E: High mobility group AT-hook 1 (*HMGA1*) is an independent prognostic factor and novel therapeutic target in pancreatic adenocarcinoma. *Cancer* 113: 302-314, 2008.
36. Wood LJ, Mukherjee M, Dolde CE, Xu Y, Maher JF, Bunton TE, Williams JB and Resar LM: *HMG-I/Y*, a new c-Myc target gene and potential oncogene. *Mol Cell Biol* 20: 5490-5502, 2000.
37. Dhar A, Hu J, Reeves R, Resar LM and Colburn NH: Dominant-negative c-Jun (*TAM67*) target genes: *HMGA1* is required for tumor promoter-induced transformation. *Oncogene* 23: 4466-4476, 2004.
38. Zhao XX, Yuan QZ, Mu DP, Sun DW, Bo QA, Pan GZ, Li GQ, Cui T, Ding PP, You FP, *et al*: MicroRNA-26a inhibits proliferation by targeting high mobility group AT-hook 1 in breast cancer. *Int J Clin Exp Pathol* 8: 368-373, 2015.
39. Liu K, Zhang C, Li T, Ding Y, Tu T, Zhou F, Qi W, Chen H and Sun X: *Let-7a* inhibits growth and migration of breast cancer cells by targeting *HMGA1*. *Int J Oncol* 46: 2526-2534, 2015.
40. Xu G, Wang J, Jia Y, Shen F, Han W and Kang Y: *Mir-142-3p* functions as a potential tumor suppressor in human osteosarcoma by targeting *HMGA1*. *Cell Physiol Biochem* 33: 1329-1339, 2014.
41. Bush BM, Brock AT, Deng JA, Nelson RA Jr and Sumter TF: The *Wnt*/ $\beta$ -catenin/T-cell factor 4 pathway up-regulates high-mobility group A1 expression in colon cancer. *Cell Biochem Funct* 31: 228-236, 2013.
42. Kazmierczak B, Bol S, Wanschura S, Bartnitzke S and Bullerdiek J: PAC clone containing the *HMGI(Y)* gene spans the breakpoint of a 6p21 translocation in a uterine leiomyoma cell line. *Genes Chromosomes Cancer* 17: 191-193, 1996.
43. Kazmierczak B, Wanschura S, Rommel B, Bartnitzke S and Bullerdiek J: Ten pulmonary chondroid hamartomas with chromosome 6p21 breakpoints within the *HMG-I(Y)* gene or its immediate surroundings. *J Natl Cancer Inst* 88: 1234-1236, 1996.
44. Dal Cin P, Wanschura S, Christiaens MR, Van den Berghe I, Moerman P, Polito P, Kazmierczak B, Bullerdiek J and Van den Berghe H: Hamartoma of the breast with involvement of 6p21 and rearrangement of *HMGIY*. *Genes Chromosomes Cancer* 20: 90-92, 1997.
45. Williams AJ, Powell WL, Collins T and Morton CC: *HMGI(Y)* expression in human uterine leiomyomata. Involvement of another high-mobility group architectural factor in a benign neoplasm. *Am J Pathol* 150: 911-918, 1997.
46. Xiao S, Lux ML, Reeves R, Hudson TJ and Fletcher JA: *HMGI(Y)* activation by chromosome 6p21 rearrangements in multipotential mesenchymal cells from pulmonary hamartoma. *Am J Pathol* 150: 901-910, 1997.
47. Kazmierczak B, Dal Cin P, Wanschura S, Borrmann L, Fusco A, Van den Berghe H and Bullerdiek J: *HMGIY* is the target of 6p21.3 rearrangements in various benign mesenchymal tumors. *Genes Chromosomes Cancer* 23: 279-285, 1998.

48. Kazmierczak B, Meyer-Bolte K, Tran KH, Wöckel W, Breightman I, Rosigkeit J, Bartnitzke S and Bullerdiek J: A high frequency of tumors with rearrangements of genes of the HMGI(Y) family in a series of 191 pulmonary chondroid hamartomas. *Genes Chromosomes Cancer* 26: 125-133, 1999.
49. Tallini G, Vanni R, Manfioletti G, Kazmierczak B, Faa G, Pauwels P, Bullerdiek J, Giancotti V, Van Den Berghe H and Dal Cin P: HMGI-C and HMGI(Y) immunoreactivity correlates with cytogenetic abnormalities in lipomas, pulmonary chondroid hamartomas, endometrial polyps, and uterine leiomyomas and is compatible with rearrangement of the HMGI-C and HMGI(Y) genes. *Lab Invest* 80: 359-369, 2000.
50. Medeiros F, Araujo AR, Erickson-Johnson MR, Kashyap PC, Dal Cin P, Nucci M, Wang X, Bell DA and Oliveira AM: HMGA1 and HMGA2 rearrangements in mass-forming endometriosis. *Genes Chromosomes Cancer* 49: 630-634, 2010.
51. Nezhad MH, Drieschner N, Helms S, Meyer A, Tadayyon M, Klemke M, Belge G, Bartnitzke S, Burchardt K, Frantzen C, *et al*: 6p21 rearrangements in uterine leiomyomas targeting HMGA1. *Cancer Genet Cytogenet* 203: 247-252, 2010.
52. Wang X, Zamolyi RQ, Zhang H, Pannain VL, Medeiros F, Erickson-Johnson M, Jenkins RB and Oliveira AM: Fusion of HMGA1 to the LPP/TPRG1 intergenic region in a lipoma identified by mapping paraffin-embedded tissues. *Cancer Genet Cytogenet* 196: 64-67, 2010.
53. Gerlitz G, Darhin E, Giorgio G, Franco B and Reiner O: Novel functional features of the Lis-H domain: Role in protein dimerization, half-life and cellular localization. *Cell Cycle* 4: 1632-1640, 2005.
54. Mateja A, Cierpicki T, Paduch M, Derewenda ZS and Otlewski J: The dimerization mechanism of LIS1 and its implication for proteins containing the LisH motif. *J Mol Biol* 357: 621-631, 2006.