in vivo *37*: 2459-2463 (2023)
doi:10.21873/invivo.13352

Fusion of the Genes for Interferon Regulatory Factor 2 Binding Protein 2 (*IRF2BP2*) and Caudal Type Homeobox 1 (*CDX1*) in a Chondrogenic Tumor

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Abstract. *Background/Aim: Chondrogenic tumors are benign, intermediate or malignant neoplasms showing cartilaginous differentiation. In 2012, we reported a mesenchymal chondrosarcoma carrying a t(1;5)(q42;q32) leading to an IRF2BP2::CDX1 fusion gene. Here, we report a second chondrogenic tumor carrying an IRF2BP2::CDX1 chimera. Case Report: Radiological examination of a 41 years old woman showed an osteolytic lesion in the os pubis with a large soft tissue component. Examination of a core needle biopsy led to the diagnosis chondromyxoid fibroma, and the patient was treated with curettage. Microscopic examination of the specimen showed a tumor tissue in which a pink-bluish background matrix was studded with small spindled to stellate cells without atypia, fitting well the chondromyxoid fibroma diagnosis. Focally, a more cartilage-like appearance was observed with cells lying in lacunae and areas with calcification. G-banding analysis of short-term cultured tumor cells yielded the karyotype 46,XX,der(1)inv(1)(p33~34q42) add(1)(p32)?ins(1;?)(q42;?),del(5)(q31),der(5)t(1;5)(q42;q35)[12]/46,XX[3]. RT-PCR together with Sanger sequencing showed the presence of two IRF2BP2::CDX1 chimeric transcripts in which exon 1 of the IRF2BP2 reference sequence NM_182972.3 or NM_001077397.1 was fused to exon 2 of CDX1. Both chimeras were predicted to code for proteins containing the zinc finger domain of IRF2BP2 and homeobox domain of CDX1. Conclusion: IRF2BP2::CDX1 chimera is recurrent in chondrogenic tumors. The data are still too sparse to conclude whether it is a hallmark of benign or malignant tumors.*

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Key Words: IRF2BP2::CDX1, chromosome band 1q42, chromosome band 5q32, caudal type homeobox 1, interferon regulatory factor 2 binding protein 2, *IRF2BP2, CDX1,* chondrogenic tumor.

Chondrogenic tumors are cartilaginous matrix-producing neoplasms which may be benign, intermediate (locally aggressive) or malignant (1-3). In 2012, our group reported a mesenchymal chondrosarcoma carrying a t(1;5)(q42;q32) and a fusion of the gene coding for interferon regulatory factor 2 binding protein 2 (*IRF2BP2*) from chromosomal band 1q42 with the gene coding for caudal type homeobox 1 protein (*CDX1*) from 5q32 (4). Here, we report a second chondrogenic tumor with rearrangements of chromosome arms 1q and 5q leading to an *IRF2BP2::CDX1* chimera.

The study was approved by the Regional Ethics Committee (Regional komité for medisinsk forskningsetikk Sør-Øst, Norge, http://helseforskning.etikkom.no). All clinical information has been de-identified.

Case Report

The patient was a 41-year-old woman with a five-year history of hip pain. Radiological examination showed an osteolytic lesion in the os pubis with a large soft tissue component. Examination of a core needle biopsy led to the diagnosis chondromyxoid fibroma, and the lesion was removed by curettage. Histological examination of the operation specimen showed a chondroid lesion but with an unusual histological appearance. The tumor tissue was dominated by a pink-bluish matrix in which small spindled to stellate cells without atypia were observed (Figure 1A), fitting the diagnosis chondromyxoid fibroma. However, focally there were areas with a more cartilage-like appearance (Figure 1B) with cells lying in lacunae and areas with calcification.

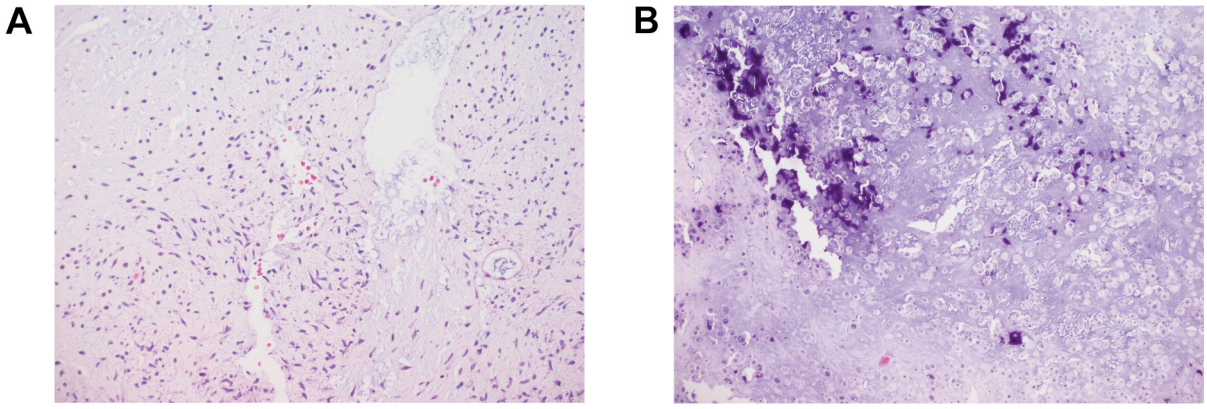

Figure 1. *Microscopic examination of the operation specimen of the chondrogenic tumor. (A) Hematoxylin and eosin stained section showing pink-bluish matrix in the background with small spindled to stellate cells without atypia. (B) Hematoxylin and eosin stained section showing area with more cartilage-like appearance with cells in lacunae and areas of calcification.*

Table I. *Designation, sequence (5'->3'), and position in reference sequences of the forward (F) and reverse (R) primers used for polymerase chain reaction amplification and Sanger sequencing analyses.*

Designation	Sequence (5'->3')	Reference sequence: position
IRF2BP2-895F	CAAGAGCCGCGGGTCTGGAGA	NM_001077397.1: 895-915
IRF2BP2-926F	GTCAACAGGCCCAAGACCGTGC	NM_001077397.1: 926-947
CDX1-650R	CCAGATTGGCAGCCAGCTCTGATT	NM_001804.3: 673-650
CDX1-774R	TGATGTCGTGGGCCATCGGC	NM_001804.3: 793-774
CDX1-827R	GAGGCTGGTGTTGCTGGGACACA	NM_001804.3: 849-827

A representative tumor area was investigated cytogenetically as previously described (5). After mechanical and enzymatic disaggregation of the tissue sample, the resulting cells were short-term cultured, harvested, and processed for cytogenetic examination. To obtain G-banding of chromosomes, Wright's stain was used (Sigma Aldrich; St Louis, MO, USA). The subsequent cytogenetic analysis and karyotype description followed the recommendations of the International System for Human Cytogenomic Nomenclature (ISCN) 2020 guidelines (6).

The methods used in the present study have been described in many previous publications (4, 7-10). Total RNA was isolated from tissue adjacent to that used for cytogenetic analysis and histologic examination, complementary DNA (cDNA) was synthesized from 400 ng of total RNA, and cDNA corresponding to 20 ng total RNA was used as template in a PCR assay containing 1X Premix Ex Taq DNA Polymerase Hot Start Version (Takara Bio, Kusatsu, Shiga, Japan) and 0.4 μM of each of the forward and reverse primers (Table I). The primer combination ABL1-91F1/ABL1-404R1 was used to amplify a 338 bp cDNA fragment from the *ABL1* gene (ABL proto-oncogene 1, non-receptor tyrosine kinase) to check the quality of cDNA synthesis (11). To detect the

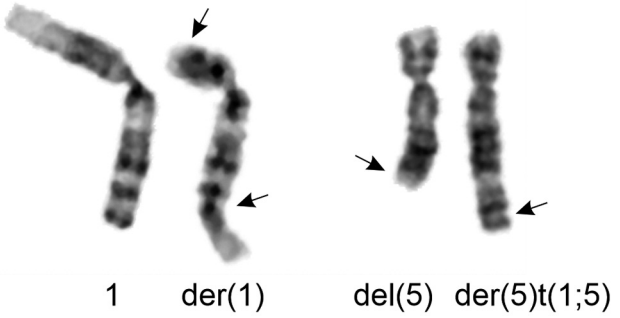

Figure 2. *Cytogenetic analysis of the chondrogenic tumor. Partial karyogram showing the abnormal chromosomes der(1)inv(1)(p33~34q42)add(1)(p32)?ins(1;?)(q42;?), del(5)(q31), and der(5)t(1;5)(q42;q35) together with the normal chromosome 1. Arrows indicate breakpoints.*

IRF2BP2::CDX1 fusion transcript, the three primer combinations IRF2BP2-895F/CDX1-774R, IRF2BP2-926F/CDX1-650R, and IRF2BP-895F/CDX1-827R were used. The cycling program was as follows: 30 s at 94˚C followed by 35 cycles of 7 s at 98˚C, 2 min at 68˚C, and a final extension step for 5 min at 72˚C. PCR products were

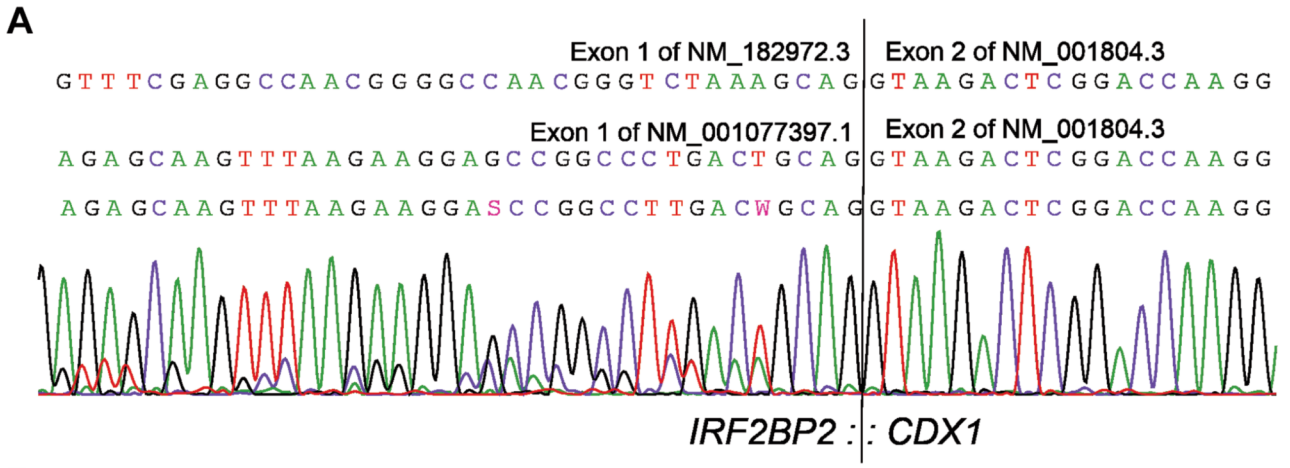

Figure 3. *Molecular genetic analysis of the chondrogenic tumor. (A) Partial chromatogram showing the fusion point of exon 1 of IRF2BP2 (reference sequences NM_182972.3 and NM_001077397.1) with exon 2 of CDX1 (reference sequence NM_001804.3). (B) The putative IRF2BP2::CDX1 fusion protein. The N-terminal Cys4 zinc finger domain of IRF2BP2 is in green background, the IRF3BP2::CDX1 fusion point is in red background, and the homeobox domain of CDX1 is in yellow background. The underlined amino acids are those which are translated only by exon 1 of IRF2BP2 reference sequence NM_182972.3.*

Sanger sequenced with the dideoxy procedure using the BigDye Terminator v1.1 Cycle Sequencing Kit in accordance with the company's recommendations (ThermoFisher Scientific, Waltham, MA, USA). The basic local alignment search tool (BLAST) was used to compare the sequences obtained using Sanger sequencing with the NCBI reference sequences NM_001804.3 of *CDX1* and NM_182972.3 and NM_0010 77397.1 corresponding to transcript variants 1 and 2 of *IRF2BP2*, respectively (12).

G-banding analysis of tumor cells detected complex rearrangements of chromosomes 1 and 5 (Figure 2) described by the karyotype 46,XX,der(1)inv(1)(p33~34q42) add(1)(p32)?ins(1;?)(q42;?),del(5)(q31),der(5)t(1;5)(q42;q35)[12]/46,XX[3].

RT-PCR together with Sanger sequencing showed the presence of two *IRF2BP2::CDX1* chimeric transcripts (Figure 3A). In the first chimera, exon 1 of the reference sequence NM_182972.3 of *IRF2BP2* was fused to exon 2 of *CDX1 r*eference sequence NM_001804.3. In the second *IRF2BP2::CDX1* chimeric transcript, exon 1 of the reference sequence NM_001077397.1 of *IRF2BP2* was fused to exon 2 of *CDX1*. Both chimeric transcripts are in-frame and hence predicted to code for a 466 and 450 amino acids IRF2BP2::CDX1 chimeric protein, respectively (Figure 3B). The IRF2BP2::CDX1 proteins contain the zinc finger domain of IRF2BP2 and the homeobox domain of CDX1.

Discussion

The chondrogenic tumor we describe here had the same *IRF2BP2::CDX1* fusion transcripts found by us also in a previously examined mesenchymal chondrosarcoma (4) demonstrating that this is a recurrent genetic event in a subset of chondrogenic tumors. The karyotypic changes as revealed by chromosome banding, however, were different in those two tumors, albeit with a telling similarity. The mesenchymal chondrosarcoma carried a t(1;5)(q42;q32) as the sole chromosomal aberration whereas the present tumor had more complex rearrangements of chromosomes 1 and 5, but again with band 1q42 being targeted. *IRF2BP2* in 1q42 is transcribed

from telomere to centromere whereas *CDX1* in chromosome band 5q32 is transcribed from centromere to telomere. Hence, formation of an *IRF2BP2::CDX1* fusion is not possible through a simple t(1;5)(q42;q32) alone but would require an additional genomic aberration, such as a cytogenetically invisible insertion or inversion on either der(1) or der(5). Apart from the two chondrogenic tumors, an *IRF2BP2::CDX1* fusion has also been reported in a soft tissue myoepithelioma (13). Neither cytogenetic nor clinicopathological information was provided for the latter tumor.

The *IRF2BP2* gene is ubiquitously expressed and encodes a protein that is involved in transcriptional regulation (14, 15). The IRF2BP2 protein contains a Cys4 zinc finger domain at the N-terminal end, a nuclear localization signal in the middle, and a Cys3HisCys4 RING domain at the C-terminal end (15-17). Cys4 zinc finger domains are believed to be DNA binding motifs (18-20). The N-terminal Cys4 zinc finger domain was found to be essential for the interaction of IRF2BP2 with IRF2BPL and DLX4 proteins as well as the repression of transcription mediated by IRF2BP2 (16). The C-terminal Cys3HisCys4 RING domain was found to be important for the interaction of IRF2BP2 with IRF2, NFAT1, NRIF3, ETO2, VGLL4, and HNF4A proteins (15, 17, 21).

CDX1, *CDX2* on 13q12, and *CDX4* on Xq13 constitute the caudal-related homeobox transcription factor gene family (22-24). They are expressed during early development and are transcriptional regulators of axial elongation and antero-posterior patterning (25, 26). All of them have a biased expression in human adult tissues. *CDX1* and *CDX2* are specifically expressed in appendix, colon, duodenum, and small intestine whereas *CDX4* is expressed in skin and testis (27). The CDX proteins have a transactivation domain at the N-terminal end and a homeobox DNA binding domain at the C-terminal end (22-24, 28-30).

The IRF2BP2::CDX1 proteins are predicted to code for a chimeric transcription factor containing the N-terminal zinc finger domain of IRF2BP2 and homeobox domain of CDX1. Recently, an *IRF2BP2::CDX2* chimera was reported (31) in an intravascular spindle cell myoepithelioma with a similar structure of *IRF2BP2::CDX1*, *i.e.*, an in frame fusion of exon 1 of *IRF2BP2* with exon 2 of *CDX2* coding for a chimeric transcript with an N-terminal zinc finger domain of IRF2BP2 and the homeobox domain of CDX2. Rearrangement and fusion of *CDX2* with the *ETV6* gene was reported (32) in an acute myeloid leukemia with t(12;13)(p13;q12). The *ETV6::CDX2* chimera generated both in frame and out of frame alternative fusion transcripts. Subsequent experiments in a murine model showed that whereas the ectopic expression of *CDX2* induced myeloid leukemogenesis, expression of *ETV6::CDX2* failed to induced leukemia (33). Thus, in order to determine the role of *IRF2BP2::CDX1* and *IRF2BP2::CDX2* chimeras in oncogenesis, functional studies are required.

In conclusion, the present case shows that the *IRF2BP2::CDX1* chimera is a recurrent genetic event in chondrogenic tumors. It is too early to say whether the rearrangement is more associated with, let alone specific for, benign or malignant tumors.

Conflicts of Interest

The Authors declare that they have no potential conflicts of interest in relation to this study.

Authors' Contributions

IP designed and supervised the research, performed molecular genetic experiments, and wrote the manuscript. LG performed the cytogenetic analysis. IL performed the pathological examination. SH evaluated the cytogenetic data and assisted with writing of the manuscript. All Authors read and approved of the final manuscript.

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Received June 27, 2023
Revised August 6, 2023
Accepted August 7, 2023