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Fusion of Platelet Derived Growth Factor Receptor Alpha (*PDGFRA*) With Ubiquitin Specific Peptidase 8 (*USP8*) in a Calcified Chondroid Mesenchymal Neoplasm Harboring t(4;15)(q12;q21) as a Sole Aberration

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Abstract. *Background/Aim: The term "calcified chondroid mesenchymal neoplasm" was introduced in 2021 to describe a group of tumors characterized by various morphological features, including the formation of cartilage or chondroid matrix. These tumors frequently carry chimeric genes where the 5´-end partner gene is fibronectin 1 and the 3´-end partner gene codes for receptor tyrosine kinase. Our study explores fusion of the genes platelet-derived growth factor receptor alpha (PDGFRA) and ubiquitin-specific peptidase 8 (USP8) in calcified chondroid mesenchymal neoplasm. Case Report: Genetic investigations were conducted on a tumor located in the leg of a 71-year-old woman. G-banding analysis of short-term cultured tumor cells revealed the karyotype 46,XX,t(4;15)(q12;q21)[6]/46,XX[4]. RNA sequencing detected in-frame PDGFRA::USP8 and USP8::PDGFRA chimeric transcripts, which were validated by RT-PCR/Sanger sequencing. The PDGFRA::USP8 chimeric protein is predicted to have cell membrane location and functions as a chimeric ubiquitinyl hydrolase. The USP8::PDGFRA protein was predicted to be nuclear and function as a positive regulator of cellular metabolic process. Conclusion: We report, for the first time, a calcified chondroid mesenchymal neoplasm carrying a balanced t(4;15)(q12;q21) chromosomal translocation, resulting in the generation of both PDGFRA::USP8 and USP8::PDGFRA chimeras. The PDGFRA::USP8 protein is located on the cell membrane and functions as a chimeric ubiquitinyl hydrolase, activated by PDGFs. Conversely, USP8::PDGFRA is a nuclear protein regulating metabolic processes.*

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Key Words: Calcified chondroid mesenchymal neoplasm, chromosome translocation, t(4;15)(q12;q21), *PDGFRA, USP8, PDGFRA::USP8, USP8::PDGFRA.*

The term "calcified chondroid mesenchymal neoplasm" describes a group of tumors that exhibit a range of morphological features, including the formation of cartilage or chondroid matrix (1).

These tumors frequently carry chimeric genes where the 5´-end partner gene is fibronectin 1 (*FN1*, located on chromosome band 2q35) and the 3´-end partner gene codes for receptor tyrosine kinase (1). The original study of 12 calcified chondroid mesenchymal neoplasms showed a predilection for the distal extremities and temporomandibular joint, a multinodular architecture with chondroid/cartilaginous matrix, increased cellularity towards the periphery of the nodules, frequent dirty to lacy (chondroblastoma-like or chicken-wire) calcifications, and biphasic tumor cells that were polygonal to stellate in the matrix-rich areas and spindled in the fibrous septa (1). Additional features included osteoclast-like giant cells and, in a subset of cases, areas resembling tenosynovial giant cell tumors. *FN1* chimeras with various 3´-end partner genes were found in 10 out of the 12 tumors. However, the most frequent 3´-end partner gene was fibroblast growth factor receptor 2 (*FGFR2*) from chromosome band 10q26 (*FN1::FGFR2* chimera) (1).

Recently, four calcified chondroid mesenchymal neoplasms were reported to carry a fusion of the genes platelet derived growth factor receptor alpha (*PDGFRA*) from 4q12 and ubiquitin specific peptidase 8 (*USP8*) from

15q21 (*PDGFRA::USP8* chimera) (2). We present cytogenetic and molecular genetic findings of a calcified chondroid mesenchymal neoplasm.

Case Report

Ethics statement. The study was approved by the Regional Ethics Committee. The administrator of the approval application is the leader of the Section for Cancer Cytogenetics Dr. Francesca Micci (Regional komité for medisinsk forskningsetikk Sør-Øst, Norge, http://helseforskning.etikkom.no; 2010/1389/REK sør-øst A). Written informed consent was obtained from the patient. The Ethics Committee's approval included a review of the consent procedure. All patient information has been de-identified.

Patient. The patient, a 71-year-old woman, has had intermittent pain in her right calf for many years, with resent progression. Radiological examination revealed an elongated, fusiform lesion in the gastrocnemius muscle, subfascial. No apparent enlargement over time. Resected due to pain. On macroscopical examination, the tumor shows areas with calcification. The microscopical examination shows a lobulated mesenchymal neoplasm with a chondroid matrix with calcification and focal cartilage and bone formation (Figure 1).

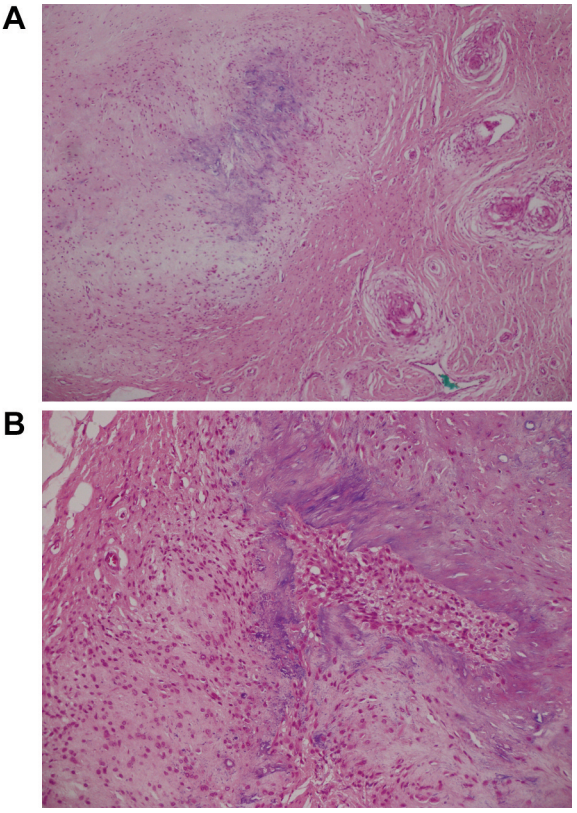

Figure 1. *Microscopic examination of the calcified chondroid mesenchymal neoplasm. Hematoxylin and eosin (H&E) stained section showing polygonal to epithelioid cells in a chondrohyaline matrix with areas of fine and coarse calcification. (A) Magnification ×100. (B) Magnification ×200.*

G-banding and karyotyping. Fresh tissue from a representative area of the tumor was cytogenetically analyzed as part of our diagnostic routine. The methodology for G-banding and karyotyping has been described previously (3, 4). The samples were 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's stain (Sigma-Aldrich, St Louis, MO, USA) and examined. Metaphases were analyzed and karyograms prepared using the CytoVision computer assisted karyotyping system (Leica Biosystems, Newcastle, UK). The karyotype was reported according to the International System for Human Cytogenomic Nomenclature (5).

RNA sequencing. Total RNA was extracted from frozen (-80 °C) tumor tissue adjacent to that used for cytogenetic analysis and histological examination using the miRNeasy Mini Kit and Qiacube (Qiagen, Hilden, Germany). The concentration was measured using a QIAxpert microfluidic UV/VIS spectrophotometer (Qiagen). RNA integrity was assessed with an Agilent 2100 bioanalyzer and DV200 index, which was found to be 70% (6). Total RNA (200 ng) was sent to the Genomics Core Facility at the Norwegian Radium Hospital, Oslo University Hospital, for high-throughput paired-end RNA-sequencing. FusionCatcher software was used to detect potential fusion transcripts (7, 8).

Confirmation of the fusion transcripts. The presence of fusion transcripts was confirmed by reverse transcription (RT) polymerase chain reaction (PCR) and Sanger sequencing. The primers used for RT-PCR and Sanger sequencing are listed in Table I. The methodology used in the present study has been described in detail in many of our previous publications (9-12). In brief, 200 ng of total RNA was reverse-transcribed in a 20 μl reaction volume using the iScript Advanced cDNA Synthesis Kit for RT-qPCR according to the manufacturer's instructions (Bio-Rad, Hercules, CA, USA). cDNA, corresponding to 20 ng total RNA, was used as a template in subsequent PCR assays. PCR amplifications were performed using the primer combinations PDGFRA-3033F1/USP8-1027R1 and USP8-616F1/PDGFRA-3443R1 (Table I). The quality of cDNA synthesis was assessed by amplification of a cDNA fragment from the ABL proto-oncogene 1, non-receptor tyrosine kinase (*ABL1*) gene using the primer combination ABL1-91F1/ABL1-404R1 (13). Three μl of the PCR products were stained with GelRed (Biotium, Hayward, CA, USA), analyzed by electrophoresis on 1.5 % agarose gel, and

Table I. *Designation, sequence (5´->3´), and position in reference sequences of the forward (F1) and reverse (R1) primers of the platelet derived growth factor receptor alpha (PDGFRA) and the ubiquitin specific peptidase 8 (USP8) genes.*

Designation	Sequence (5´->3´)	Reference sequence: Position
PDGFRA-3033F1	CCTGGACTTCCTGAAGAGTGACCAT	NM_006206.6: 3033-3057
PDGFRA-3443R1	GTTTTCTGAACGGGATCCAGAGGT	NM_006206.6: 3466-3443
USP8-1027R1	TTGCGCAGGACAGTTTTACTTTCC	NM_005154.5: 1050-1027
USP8-616F1	CACATTGGCTAAAGGCTCTTTGGA	NM_005154.5: 616-639
M13For-PDGFRA-3075F1	*TGTAAAACGACGGCCAGT*GCGTGTGGACTCAGACAATGCATA	NM_006206.6: 3075-3098
M13Rev-USP8-984R1	*CAGGAAACAGCTATGACC*TCAGACTCCGGAGAGTTGTTCCAA	NM_005154.5: 1007-984
M13For-USP8-781F1	*TGTAAAACGACGGCCAGT*GGATGCTCGAAGAATGCAGGATT	NM_005154.5: 781-803
M13Rev-PDGFRA-3393R1	*CAGGAAACAGCTATGACC*AATCCGCCAGTTACAGGAAGCTG	NM_006206.6: 3415-3393

M13 forward primer (M13For: TGTAAAACGACGGCCAGT) and M13 reverse primer (M13Rev: CAGGAAACAGCTATGACC) sequences are in italics.

photographed. The remaining PCR products were purified using the MinElute PCR Purification Kit (Qiagen) and sequenced using the BigDye Direct Cycle Sequencing Kit following the manufacturer's recommendations (Thermo Fisher Scientific, Waltham, MA, USA). The primer combinations were M13For-PDGFRA-3075F1/M13Rev-USP8-984R1 and M13For-USP8-781F1/M13Rev-PDGFRA-3393R1 (Table I).

Sequencing was performed using an Applied Biosystems SeqStudio Genetic Analyzer system (Thermo Fisher Scientific). The Basic Local Alignment Search Tool (BLAST) was used to compare the sequences obtained by Sanger sequencing with the National Center for Biotechnology Information (NCBI) reference sequences (14). The reference sequences were NM_006206.6 for *PDGFRA* transcript variant 1 and NM_005154.5 for *USP8* transcript variant 1. In addition, the BLAST-like alignment tool (BLAT) and the human genome browser at University of California, Santa Cruz (UCSC) were used to map the sequences obtained by Sanger sequencing, on the Human GRCh37/hg19 assembly (15, 16).

For the prediction of subcellular localization of the proteins BUSCA and DeepLoc algorithms were used (17, 18). To predict the function of proteins the recently described deep neural network ProteInfer was used (19). For the visualization of the chimeric protein the Protter program was used (20).

Results

G-banding analysis yielded a karyotype with a single chromosome abnormality: 46,XX,t(4;15)(q12;q21)[6]/46, XX[4] (Figure 2A).

Using FusionCatcher software with fastq files from RNA sequencing, both *PDGFRA-USP8* and *USP8-PDGFRA* chimeric transcript sequences were detected, which corresponded to t(4;15)(q12;q21) (Table II). RT-PCR with the primer combinations PDGFRA-3033F1/USP8-1027R1 and USP8-616F1/PDGFRA-3443R1 amplified 414 bp and 455 bp long cDNA fragments, respectively (Figure 2B). Further sequencing of the cDNA-amplified fragments verified the above-mentioned *PDGFRA::USP8* and *USP8::PDGFRA* chimeric transcripts (Table II and Figure 2C and D). In *PDGFRA::USP8* chimeric transcript, exon 22 of *PDGFRA* fused to exon 8 of *USP8* (Figure 2C), whereas in *USP8::PDGFRA* chimeric transcript exon 7 of *USP8* fused to exon 23 of *PDGFRA* (Figure 2D).

Discussion

Herein, we report a novel t(4;15)(q12;q21) chromosomal translocation as the only cytogenetic aberration in a calcified chondroid mesenchymal neoplasm. The translocation rearranged *PDGFRA* on chromosome band 4q12 and *USP8* on 15q21 and generated *PDGFRA::USP8* and *USP8::PDGFRA* chimeras (Figure 2). Because both *PDGFRA* and *USP8* are transcribed from the centromere to the telomere, the chimeric *PDGFRA::USP8* gene is predicted to be on der(4)t(4;15)(q12;q21) whereas *USP8::PDGFRA* is predicted to be on der(15)t(4;15)(q12;q21). Because in *PDGFRA-USP8* chimeric transcript exon 22 of *PDGFRA* fused to exon 8 of *USP8* whereas in *USP8::PDGFRA* chimeric transcript exon 7 of *USP8* fused to exon 23 of *PDGFRA* the genomic breakpoints are predicted to occur in intron 22 of *PDGFRA* and intron 7 of *USP8*. Chimeric *PDGFRA::USP8* transcripts have been reported recently in four cases of calcified chondroid mesenchymal neoplasm (2) and in a sarcoma (21) which is registered in cBioPortal database (22) as retroperitoneal undifferentiated pleomorphic sarcoma/malignant fibrous histiocytoma/high-grade spindle cell sarcoma (https://www.cbioportal.org/patient?sampleId=TCGA-Z4-AAPG-01&studyId=sarc_tcga_pan_can_atlas_2018). In all the above-mentioned tumors, exon 22 of *PDGFRA* fused to exon 5 of *USP8* (*PDGFRA::USP8* chimeric transcript) (2, 21).

The *PDGFRA* gene codes for a cell surface receptor (Figure 2E), belonging to the receptor tyrosine kinase family, that plays

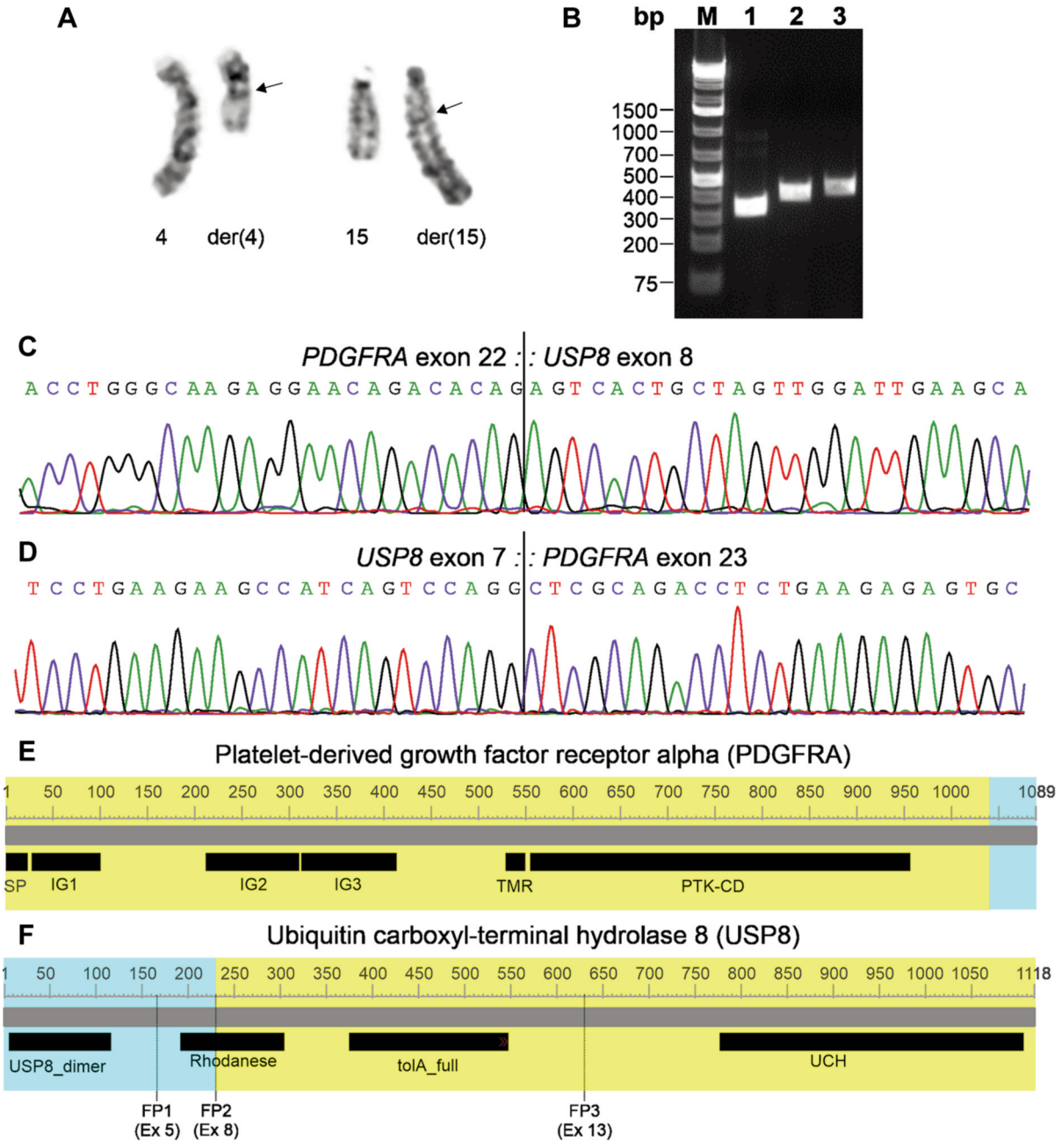

Figure 2. *Genetic examination of the calcified chondroid mesenchymal neoplasm. (A) Partial karyogram showing the der(4)t(4;15)(q12;q21) and der(15)t(4;15)(q12;q21) together with the corresponding normal chromosome homologs. Breakpoint positions are indicated by arrows. (B) Gel electrophoresis showing the amplified ABL1 (lane 1), PDGFRA::USP8 (lane 2) and USP8::PDGFRA (lane 3) cDNA fragments using the primer combinations ABL1-91F1/ABL1-404R1, PDGFRA-3033F1/USP8-1027R1, and USP8-616F1/PDGFRA-3443R1, respectively. M: GeneRuler 1kb Plus DNA ladder (Thermo Fisher Scientific). (C) Partial sequence chromatogram showing the junction position in the PDGFRA::USP8 cDNA amplified fragment. (D) Partial sequence chromatogram showing the junction position in the USP8::PDGFRA cDNA amplified fragment. The exon numbers are based on the sequences with accession numbers NM_006206.6 for PDGFRA and NM_005154.5 for USP8. (E). Illustration showing the coding protein PDGFRA. SP: Signal peptide; IG1: Ig_3 immunoglobulin domain; IG2: immunoglobulin-like domain of platelet-derived growth factor receptors (PDGFR), alpha and beta, member of the I-set of IgSF domains; IG3: fourth immunoglobulin-like domain of PDGFR; TMR: transmembrane region; PTK-CD: catalytic domain of the protein tyrosine kinase. (F) Illustration showing the coding protein USP8. USP8-dimer: USP8 dimerization domain; Rhodanese: Rhodanese-like domain; tolA_full: TolA protein; UCH: ubiquitin carboxyl-terminal hydrolase; FP1: fusionpoint reported by Fisher et al. (2) in calcified mesenchymal neoplasm with PDGFRA::USP8; FP2: fusionpoint reported in the present study in a calcified mesenchymal neoplasm with PDGFRA::USP8; FP3: breakpoint reported in B-cell lineage acute lymphoblastic leukemia carrying KMT2A::USP8 chimera which was generated by chromosomal translocation t(11;15)(q23;q21) (50). The parts of the PDGFRA and USP proteins in yellow compose the chimeric PDGFRA::USP8 protein while the parts in cyan compose the chimeric USP8::PDGFRA protein.*

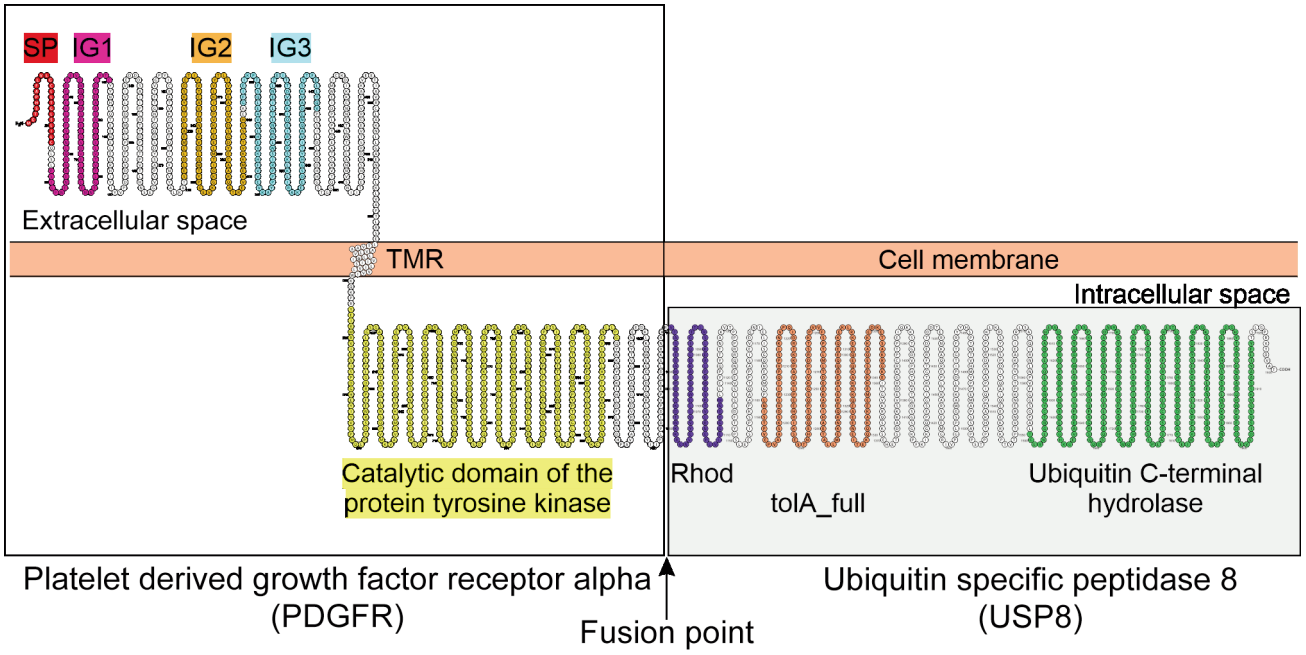

Figure 3. *Illustration showing the cell membrane location of the chimeric PDGFRA::USP8 protein. The illustration is based on the reference sequences NM_006206.6/NP_006197.1 for PDGFRA and NM_005154.5/ NP_005145.3 for USP8. SP: Signal peptide; IG1: Ig_3 immunoglobulin domain; IG2: immunoglobulin-like domain of platelet-derived growth factor receptors (PDGFR), alpha and beta, member of the I-set of IgSF domains; IG3: fourth immunoglobulin-like domain of PDGFR; TMR: transmembrane region; Rhod: Rhodanese-like domain; tolA_full: TolA protein. Illustration was made with the Protter program (20).*

Table II. *The PDGFRA::USP8 and USP8::PDGFRA fusion transcripts detected in calcified chondroid mesenchymal neoplasm carrying a t(4;15)(q12;q21) chromosome translocation after analysis of RNA sequencing data with FusionCatcher.*

Fusion transcript	Spanning reads	Fusion sequence (5´->3´)
PDGFRA::USP8 (exon 22-exon 8)	25	ATTGACCCTGTCCCTGAGGAGGAGGACCTGGGCAAGAGGAACAGACACAG::AGTCACTGCTAGTTGGATTGAAGCACACCTGCCAGATGATTCTAAAGACA
USP8::PDGFRA (exon 7-exon 23)	5	TTTTACATTCTCTCAGTGTTCCTGAAGAAGCCATCAGTCCAGG::CTCGCAGACCTCTGAAGAGAGTGCCATTGAGACGGGTTCCAGC

Exons are based on the reference sequences NM_006206.6 for *PDGFRA* and NM_005154.5 for *USP8*.

a crucial role in various cellular processes, including cell growth, proliferation, and differentiation (23-26). PDGFRA is activated by binding to its specific ligands, platelet-derived growth factors (PDGFs) (27). Upon ligand binding, the receptor undergoes autophosphorylation, initiating downstream signaling cascades that regulate diverse cellular functions (23-26). Aberrant PDGFRA signaling has been implicated in numerous pathological conditions, including cancer, cardiac fibrosis, and diabetes 2 (23, 26, 28-30). Mutations, overexpression, and fusion genes of *PDGFRA* have been associated with the development and progression of certain cancers, such as gastrointestinal stromal tumors (31-33), brain tumors (34-36), and hematological neoplasms (37, 38), making it a potential therapeutic target (24, 25, 33, 39-41).

The *USP8* gene codes for a protein (officially named ubiquitin carboxyl-terminal hydrolase 8) that belongs to the ubiquitin-specific processing protease family of proteins (Figure 2F). USP8 is a deubiquitinase enzyme that plays a key role in the regulation of several cellular processes by cleaving ubiquitin molecules from substrate proteins (42, 43). USP8 is particularly notable for its involvement in endosomal trafficking and receptor signaling (42, 43). It regulates the ubiquitination status of several important membrane proteins, including receptor tyrosine kinases such as epidermal growth factor receptor (EGFR). USP8 deubiquitinates these receptors, prevents their degradation, and promotes their recycling back to the cell surface. This activity contributes to the fine-tuning of signaling cascades involved in cell growth, proliferation,

and differentiation (42-45). Somatic mutations of the *USP8* gene have been found in adenomas causing Cushing's disease (46-48), and abnormal expression of *USP8* has been found in various neoplasms (43, 49, 50). The Mitelman database of chromosome aberrations and gene fusions in cancer has documented 14 chimeric genes involving *USP8*, including a fusion between *USP8* and lysine methyltransferase 2A (*KMT2A)* in B-cell lineage acute lymphoplastic leukemia. The *KMT2A::USP8* chimera was generated by chromosomal translocation t(11;15)(q23;q21) (51, 52). The genomic breakpoints occurred within introns 20 and 12 of *KMT2A* and *USP8* (NM_005154), respectively. This resulted in an in-frame *KMT2A::USP8* chimeric transcript comprising exons 1-20 of *KMT2A* and exons 13-20 of *USP8* (51).

Based on the reference sequences NM_006206.6/ NP_006197.1 for *PDGFRA and* NM_005154.5/NP_005145.3 for *USP8,* the chimeric *PDGFRA::USP8* transcript was predicted to code for a 1930-amino acid protein. This protein comprises the first 1040 amino acids of PDGFRA (amino acid 1-1040 in NP_006197.1), an arginine generated at the fusion point, and the last 889 amino acids of USP8 (amino acid 230-1118 in NP_005145.3) (Figure 2E and F). Therefore, the PDGFRA::USP8 protein contains all regions of PDGFRA, that is, the signal peptide, immunoglobulin domains, transmembrane region, and catalytic domain of the protein tyrosine kinase (Figure 2E). Additionally, it includes the USP8 regions rhodanase, tolA_full, and ubiquitin carboxyl-terminal hydrolase (Figure 2F). Based on BUSCA, DeepLoc, and ProteInfer, the PDGFRA::USP8 protein is predicted to have a cell membrane location and function as a chimeric ubiquitinyl hydrolase, which is activated by PDGFs (Figure 3). Using a monoclonal antibody corresponding to the PDGFRA extracellular domain, Fisher and coworkers (2) detected a strong distinct membranous staining of PDGFRA on the neoplastic chondrocytic cells. This result supports the predicted subcellular location of PDGFRA::USP8 protein, which we describe in the present work.

The anticipated product of the *USP8::PDGFRA* transcript is a 277-amino acid protein composed of the first 229 amino acids of USP8 (1-229 of NP_005145.3), which contains the USP8 dimerization domain, and the last 47 amino acids of the PDGFRA protein (amino acids1042-1089 of NP_006197.1) (Figure 2E and F). The predicted subcellular localization of the USP8::PDGFRA protein is in the nucleus and its function is to positively regulate cellular metabolic processes.

In conclusion, we report, for the first time, a calcified chondroid mesenchymal neoplasm carrying a balanced t(4;15)(q12;q21) chromosomal translocation, resulting in the generation of *PDGFRA::USP8* and *USP8::PDGFRA* chimeras. The PDGFRA::USP8 protein is predicted to be located on the cell membrane and function as a chimeric ubiquitinyl hydrolase, activated by PDGFs. Conversely, USP8::PDGFRA is predicted to be a nuclear protein that positively regulates cellular metabolic processes.

Conflicts of Interest

The Authors declare that they have no conflicts of interest regarding this study.

Authors' Contributions

IP designed and supervised the research, performed molecular genetic experiments and bioinformatic analysis, and wrote the article. KA performed molecular genetic experiments. LG performed cytogenetic analysis. IL performed pathological examination. All Authors have read and approved the final manuscript.

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