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Fusion of the *COL1A1* and *FYN* Genes in Epithelioid Osteoblastoma

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Abstract. *Background/Aim: Epithelioid osteoblastoma is a rare benign tumor of the bone. Its pathogenesis is unknown and little is known regarding its genetic features. Materials and Methods: Cytogenetic, RNA sequencing, reverse transcription polymerase chain reaction (RT-PCR), genomic PCR, and Sanger sequencing analyses were performed on an epithelioid osteoblastoma. Results: G-banding analysis of short-term cultured tumor cells yielded a normal male karyotype in all examined metaphases. RNA sequencing detected a fusion of COL1A1 from 17q21 with FYN from 6q21. Both RT-PCR and genomic PCR together with Sanger sequencing verified the presence of a COL1A1-FYN fusion gene. In the COL1A1-FYN chimeric transcript, exon 43 of COL1A1 was fused to exon 2 of FYN. The genomic junction occurred in introns 43 and 1 of COL1A1 and FYN, respectively. Conclusion: A COL1A1-FYN fusion gene was found in an epithelioid osteoblastoma resulting in deregulation of FYN. Whether COL1A1-FYN represents a consistent genetic feature of epithelioid osteoblastomas, remains to be seen.*

Osteoblastoma is a rare benign tumor which accounts for about 1% of all bone tumors. It is most often found in the age range of 10-30 years and is 2.5 more common in males than females (1). The tumor was first described in 1956 in two different publications, one by Jaffe and the other by Lichtenstein (2, 3).

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Key Words: Epithelioid osteoblastoma, *COL1A1, FYN, COL1A1-FYN* fusion gene, RNA sequencing.

In the 1970s, a more aggressive type of osteoblastoma was described with a higher recurrence rate and, upon microscopy, many epithelioid osteoblasts; various names were given to this type of tumor, such as malignant osteoblastoma (4), aggressive osteoblastoma (5), and epithelioid osteoblastoma (6, 7). The initial reports emphasized that the aggressive behavior was associated with an epithelioid morphology (4, 5, 8); however, studying 306 osteoblastomas, Lucas *et al.* (9) did not find any difference in the aggressiveness between epithelioid and conventional tumors and concluded that "Aggressive behavior is within the biologic spectrum of osteoblastomas, and histopathology alone does not appear to be a reliable predictor of aggressiveness". Examining 55 cases of osteoblastomas, Della Rocca *et al.* (10) also concluded that "clinically aggressive behavior of osteoblastoma is not related to particular histological features, but rather to the skeletal location".

The cytogenetic information on osteoblastomas is limited. According to the Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer, only three epithelioid (aggressive) osteoblastomas and four of the so-called conventional osteoblastomas have been karyotyped and no consistent cytogenetic pattern has emerged (http://cgap.nci.nih.gov/Chromosomes/Mitelman, database last updated on February 19, 2019). Recently, recurrent rearrangements of *FOS* and *FOSB* were found in so-called conventional osteoblastoma. Examining six tumors by whole genome and RNA sequencing, Fitall *et al.* (11) found *FOS* and *FOSB* rearrangements in five and one tumors, respectively. Extending the investigation to 55 additional cases using fluorescence *in situ* hybridization (FISH) and immunohistochemical methodologies, they found that 51 carried *FOS* and one *FOSB* rearrangements, respectively.

In the present study, we used RNA sequencing and other molecular genetic techniques to find fusion of the collagen type I alpha 1 (*COL1A1*) and FYN proto-oncogene, Src family tyrosine kinase (*FYN*) genes in an epithelioid osteoblastoma.

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

Name	Sequence (5'->3')	Position	Reference number
COL1A1-3197F1	CTGGACGAGACGGTTCTCCTGG	3197-3218	NM_000088.3
FYN-Intr2-R1	CATCCTAGGTTCCAACAGGAAGCC	111846515-111846538	NC_000006.12
COL1A1-3221F1	CCAAGGGTGACCGTGGTGAGAC	3221-3242	NM_000088.3
FYN-Intr2-R2	TGCTCTCAGTGCAAAACTTGCCA	111846560-111846582	NC_000006.12
COL1A1-genF1	CTGCTGGCAAGAGTGGTGATCG	3302-3323	NM_000088.3
FYN-genR1	AAGGCAGCCCCCAATAATTCCT	111849708-111849729	NC_000006.12
COL1A1-genF2	TGAGACTGTAAGTAGCTGGGCTCCA	50188536-50188512	NC_000017.11
FYN-genR2	GCTATGCCTTGTCATTCCAATCTCA	111849738-111849762	NC_000006.12

Materials and Methods

Ethics statement. The study was approved by the Regional Committee for Medical and Health Research Ethics, South-East Norway (REK Sør-Øst; http://helseforskning.etikkom.no) and written informed consent was obtained from the patient's parents for publication of the case details. The ethics committee's approval included a review of the consent procedure. All patient information has been de-identified.

Case description. The patient was a 10-year old boy, who experienced ongoing pain in his left knee for more than 6 months. X-ray and CT scan showed an osteolytic lesion in the proximal fibula, first thought to be osteomyelitis. The local hospital performed curettage of the lesion. Histologically the lesion was bone forming, with trabeculae of osteoid, partly calcified, rimmed by osteoblasts (Figure 1A). In between the trabeculae there was fibrovascular tissue and sheets of epithelioid osteoblasts and scattered osteoclasts (Figure 1B). There was no cellular atypia and there were only few mitotic figures, none of them atypical. In addition, there were areas with "blue bone", where the osteoid was heavily calcified and some areas were cartilage like.

G-banding and karyotyping. Fresh tissue from a representative area of the tumor was short-term cultured and analyzed cytogenetically as previously described (12).

Fluorescence in situ hybridization (FISH). The BAC probes were purchased from BACPAC Resource Center located at the Children's Hospital Oakland Research Institute (Oakland, CA, USA) (https://bacpacresources.org/). FISH analyses were performed on interphase nuclei using *COL1A1* and *FYN* (see below) home-made dual-color single-fusion probes. Detailed information on the FISH procedure was given elsewhere (12). For the *COL1A1* gene on chromosome band 17q21, the BAC clone used was RP11-93L18 (Position: chr17:50219522-50388834). For the *FYN* gene on chromosome band 6q21, the BAC clones used were RP11-75C8 (Position: chr6:111639316-111835441), RP1-66H14 (accession number Z97989.1; Position: chr6:111582811-111738747), and RP3-487J7 (accession number AL008730.1, Position: chr6: 111497307-111613802; Band: 6q21). The probes for *COL1A1* and *FYN* were labelled with Fluorescein-12-dCTP (PerkinElmer, Boston, MA, USA) or Texas Red-5-dCTP (PerkinElmer) in order to obtain green and red signals, respectively. Fluorescent signals were captured and analyzed using the CytoVision system (Leica Biosystems, Newcastle, UK).

RNA sequencing. Total RNA was extracted from frozen (–80˚C) tumor tissue adjacent to that used for cytogenetic analysis and histologic examination using miRNeasy Mini Kit (Qiagen Nordic, Oslo, Norway). One μg of total RNA was sent to the Genomics Core Facility at the Norwegian Radium Hospital, Oslo University Hospital (http://genomics.no/oslo/) for high-throughput paired-end RNA-sequencing using the Illumina TruSeq Stranded mRNA protocol. The software FusionCatcher was used to find fusion transcripts (13, 14).

Reverse transcription (RT) and genomic PCR analyses. The primers used for PCR amplifications and Sanger sequencing analyses are shown in Table I. Genomic DNA was extracted using the Maxwell RSC Instrument and the Maxwell RSC Tissuel DNA Kit (Promega, Madison, WI, USA) and the concentration was measured using the Quantus Fluorometer and the QuantiFluor ONE dsDNA System (Promega). RT-PCR, genomic PCR, analysis of PCR products, and Sanger sequencing were performed as previously described (12).

For amplification of the *COL1A1-FYN* fusion transcript, the primer combinations were the forward COL1A1-3197F1 together with the reverse FYN-Intr2R1 and COL1A1-3221F1 together with the reverse FYN-Intr2R2. For amplification of genomic *COL1A1-FYN* fragments, the primer combinations were COL1A1-genF1/FYN-genR1 and COL1A1-genF2/FYN-genR2. The cycling was at 94˚C for 30 sec followed by 35 cycles of 7 sec at 98˚C, 30 sec at 60˚C, 30 sec at 72˚C, and a final extension for 5 min at 72˚C. The BLAST software (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used for computer analysis of sequence data.

Immunohistochemistry. The FYN antibody was a mouse monoclonal antibody (2A10) purchased from ThermoFisher Scientific (Catalogue number MA5-15865) applied at 1:100 dilution. Formalin-fixed, paraffin-embedded sections from the epithelioid osteoblastoma were analyzed for FYN expression using the Dako EnVision Flex + System (K8012; Dako, Glostrup, Denmark) as previously described (15).

Results

The G-banding analysis of short-term cultured tumor cells revealed a normal karyotype, 46, XY, in all 25 examined metaphases (data not shown).

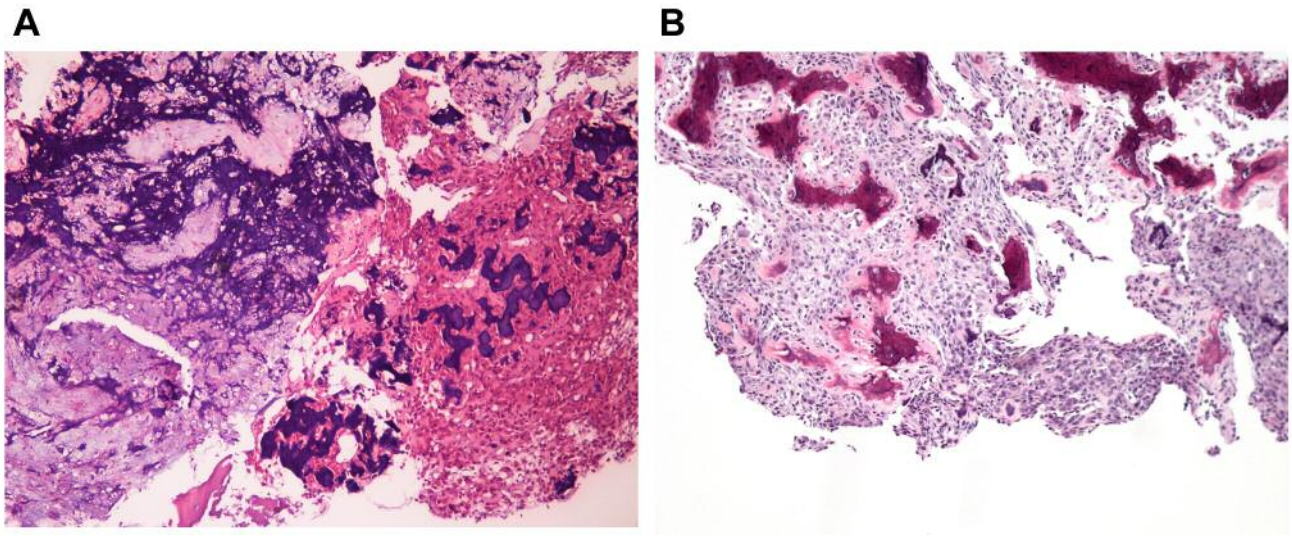

Figure 1. *Microscopic examination of the epithelioid osteoblastoma. A) H&E-stained section showing irregular trabeculae of woven bone with deep blue, calcified areas and areas with cartilage, ×20. B) H&E-stained section showing irregular trabeculae of woven bone rimmed by osteoblasts. In the intertrabecular space, fibrovascular tissue and sheets of epithelioid osteoblasts are shown, ×20.*

Using the FusionCatcher software with the fastq files from the RNA sequencing, four fusion genes were found with 17 fusion transcripts (Table II): a read-through *COL1A1-HILS1* fusion gene with eight fusion transcripts, a *COL1A1-FYN* fusion gene with seven fusion transcripts, an *EBP41L5-COL5A2* with one fusion transcript, and a read-through *CTBS-GNG5* fusion gene with one fusion transcript. Detailed information on the fusion genes and transcripts is given in Table II. Taking into consideration that *COL1A1* is fused to *PDGFB* in dermatofibrosarcoma protuberans (http://omim.org/entry/120150) and *FYN* is a tyrosine kinase protooncogene related to *SRC*, *FGR*, and *YES* (http://omim.org/entry/137025) we decided to investigate further with molecular techniques the presence of *COL1A1-FYN* fusion gene in the tumor. No other fusion transcripts were examined.

RT-PCR with the primer combinations COL1A1-3197F1/FYN-Intr2R1 and COL1A1-3221F1/FYN-Intr2R2 amplified a 252 bp fragment and a 183 bp fragment, respectively (Figure 2A). Sanger sequencing of the PCR fragments showed that they were *COL1A1-FYN* chimeric cDNA fragments in which exon 43 of *COL1A1* (nucleotide 3333 of the *COL1A1* sequence with accession number NM_000088.3) was fused to the untranslated exon 2 of *FYN* (nucleotide 486 of the *FYN* sequence with accession number NM_002037.5) (Figure 2B). The fusion point was identical to one of the 7 fusion points found by FusionCatcher analysis of the RNA sequencing data: CCCCTGGCCCCGTTGGCCCTGC T G G C A A G A G T G G T G A T C G T G G T G A G A C T-TTTTTTTGAAGAAGCAGGATGCTGATCTAAACGTGGA AAAAGTAAGTTGG.

Genomic PCR with the primer combinations COL1A1-genF1/FYN-genR1 and COL1A1-genF2/FYN-genR2 amplified a 285 bp fragment and a 230 bp fragment, respectively (Figure 2C). Direct sequencing showed that they were genomic *COL1A1-FYN* chimeric fragments in which a sequence from intron 43 of *COL1A1* was fused to a sequence of intron 1 from *FYN* (Figure 2D). The genomic junction point was identical to one of the fusion points found by FusionCatcher analysis of the RNA sequencing data: GGCCAGGGACTCTTCAGGCCTCCTTAGAGGCCTGGG GATGGGTGTCGGAC-GGAAATCTAGTCCTGCATGGG GGTGTGGGCAAAGGAAAACAAGAGTGAAA.

FISH analysis, using *COL1A1* and *FYN* home-made dual-color single-fusion probe, showed a fusion signal in 9 out of 106 (8%) examined interphase nuclei suggesting a *COL1A1-FYN* fusion gene in these cells (Figure 3).

FYN immunohistochemical examination was uninformative (data not shown) probably due to damage done to the tissue by the decalcification procedure (16, 17).

Discussion

Using the RNA sequencing methodology, we identified a *COL1A1-FYN* fusion gene in the cells of an epithelioid osteoblastoma. The fusion gene was further verified at both transcriptional (RNA) and genomic (DNA) levels using RT-PCR and genomic PCR together with Sanger sequencing. Interphase FISH showed that the short-term cultured cells from tumor biopsy contained a small clone of cells (8%) carrying the *COL1A1-FYN* fusion. The cells carrying the

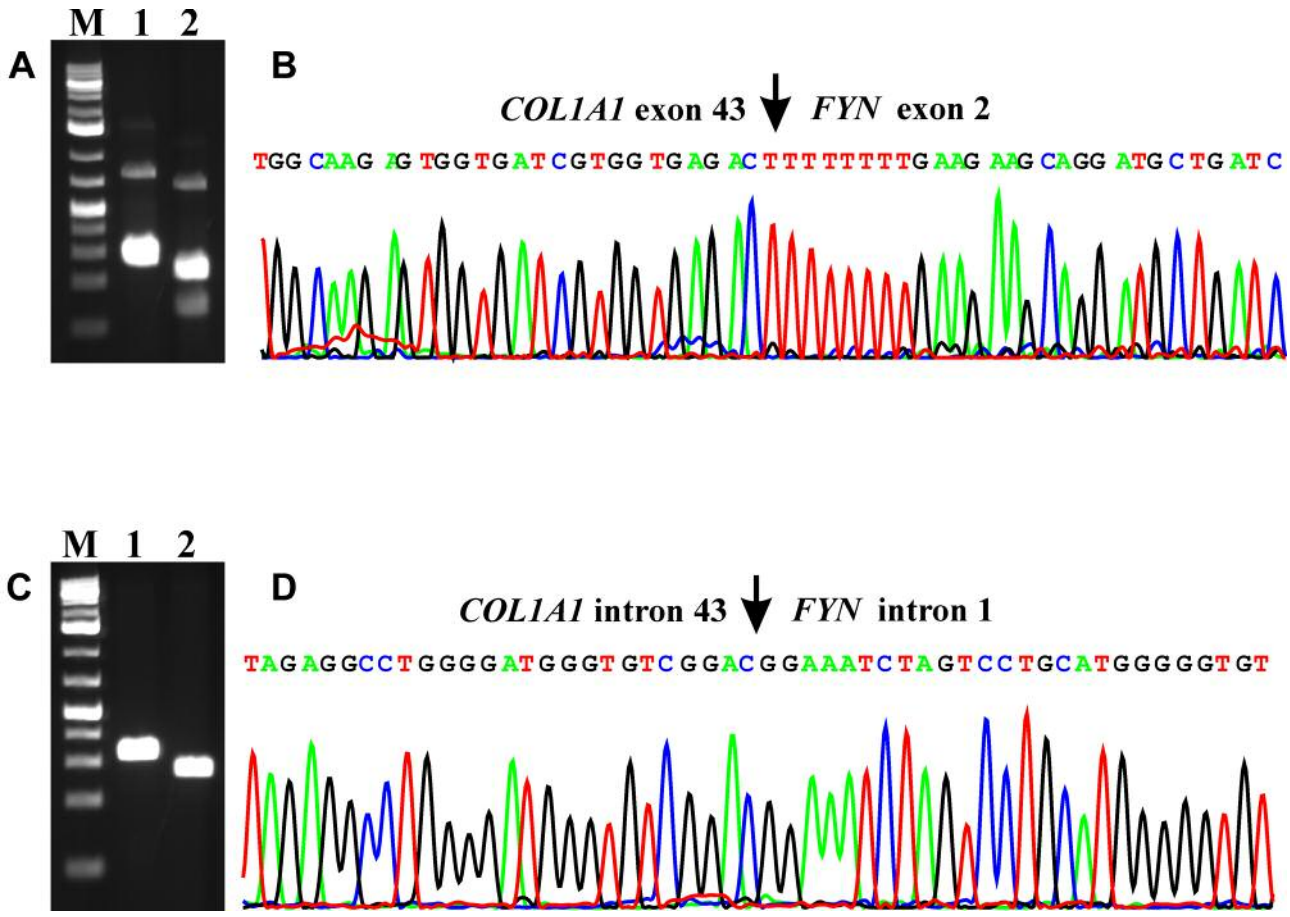

Figure 2. *Molecular genetic analysis of the epithelioid osteoblastoma. (A) Gel electrophoresis showing the amplified COL1A1-FYN cDNA fragment using the COL1A1-3197F1/FYN-Intr2R1 (lane 1) and COL1A1-3221F1/FYN-Intr2R2 (lane 2) primer combinations. (B) Partial sequence chromatogram of the cDNA amplified fragment showing the fusion (arrow) of exon 43 of COL1A1 with exon 2 of FYN. (C) Amplification of genomic COL1A1-FYN fragments using the primer combinations COL1A1-genF1/FYN-genR1 (lane 1) and COL1A1-genF2/FYN-genR2 (lane 2). (D) Partial sequence chromatogram of the genomic DNA amplified fragment showing the fusion (arrow) of intron 43 of COL1A1 with intron 1 of FYN. M, Thermo Scientific GeneRuler 1 kb Plus DNA Ladder.*

rearrangement most probably were not able to divide *in vitro*.

The *FYN* gene on 6q21 codes for a non-receptor tyrosine kinase, FYN, which is a member of the SRC family of kinases (18, 19). The FYN protein is found in the inner layer of the cell membrane and is involved in signal transduction pathways. It phosphorylates tyrosine residues of many proteins regulating numerous functions including control of cell growth, cell-cell adhesion, and cytoskeletal remodeling (18, 19). *FYN* is implicated in cancer, too (18, 19). *In vitro* studies have shown that overexpression of *FYN* in NIH-3T3 cells induced morphologic transformation and anchorage-independent growth (20). *FYN* was found to be overexpressed in prostate, breast, pancreas, and thyroid cancer (2, 21-24). In neuroblastoma, high expression of *FYN* and high FYN kinase activity was found in tumors of stage I, whereas *FYN* was down-regulated in stage 4 neuroblastomas (25). In chronic myeloid leukemia, *FYN* expression was significantly higher in blast crisis compared to chronic phase, and overexpression of *FYN* was an important determinant for resistance to BCR-ABL1 inhibitors (26-28).

The *COL1A1* gene codes for the pro-alpha1 chain of type I collagen, a fibril-forming collagen which is abundant in bone, cornea, dermis, and tendon (29, 30). Mutations in the *COL1A1* gene are associated with osteogenesis imperfecta types I-IV, Ehlers-Danlos syndrome type VIIA, Ehlers-Danlos syndrome Classical type, Caffey Disease, and idiopathic osteoporosis (http://omim.org/entry/120150). Simon *et al.* (31) showed that in dermatofibrosarcoma protuberans, the t(17;22)(q21;q13) and supernumerary ring chromosomes characteristic of these tumors, contain a

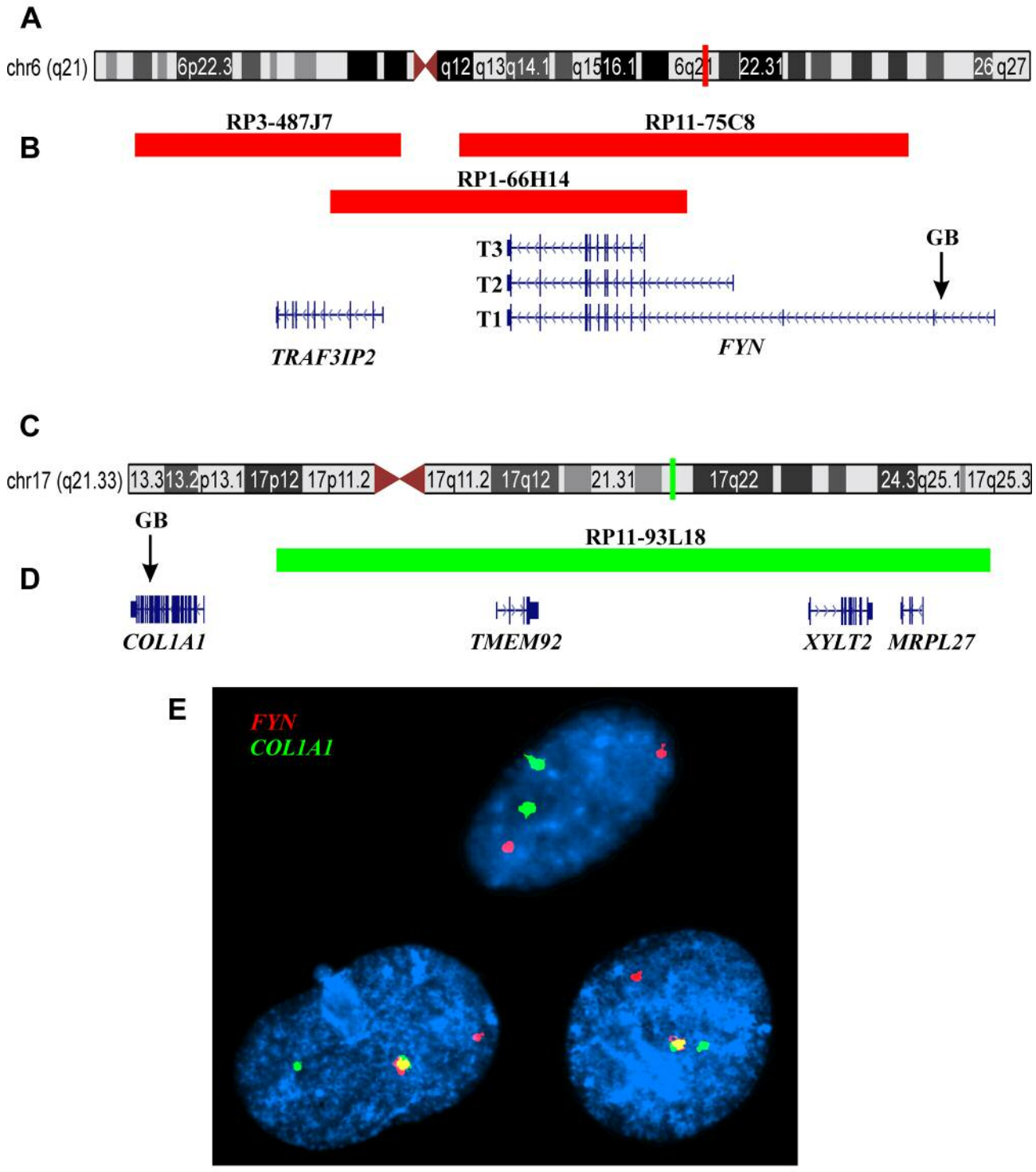

Figure 3. *FISH analysis of epithelioid osteoblastoma using COL1A1 and FYN home-made dual-color single-fusion probe. (A) Ideogram of chromosome 6 showing the mapping position of the FYN gene (vertical red line). (B) Diagram showing the FISH probes RP3-487J7, RP1-66H14, and RP11-75C8 for FYN. Arrow indicates the genomic breakpoint (GP) in the intron 1 of FYN. The neighbor TRAF3IP2 gene in this region is also shown. (C) Ideogram of chromosome 17 showing the mapping position of the COL1A1 gene (vertical green light). (D) Diagram showing the FISH probe RP11-93L18 for COL1A1. The TMEM92, XYLT2, and MRPL27 genes mapped in this region, distal to COL1A1, are also shown. Arrow indicates the genomic breakpoint (GP) in the intron 43 of COL1A1. (E) FISH results with the FYN (red signal) and COL1A1 (green signal) probes on three interphase nuclei (composite photo) showing a red signal, a green signal, and one yellow-fusion signal in two nuclei and two green and two red signals in one nucleus.*

Table II. *Fusion transcripts detected using FusionCatcher.*

Fusion transcript	Spanning pairs	Spanning unique reads	Fusion sequence
COL1A1-HILS1	828	84	CCCGTGGCCTGCCTGGTGAGAGAGGTCGCCCTGGAGCCCCTGGCCCTGCT-CACCTTAACTCTCAGCTTCCCAGACTTTCTCCAATGACAGAGCTGGGTGG
COL1A1-HILS1	828	8	CCTGCCTGGTGAGAGAGGTCGCCCTGGAGCCCCTGGCCCTGCT-GTAAAGGTGAGGGCCGGAGCTCAAGAGGAAGCCTCAGCGAGGA
COL1A1-HILS1	828	3	CCCGTGGCCTGCCTGGTGAGAGAGGTCGCCCTGGAGCCCCTGGCCCTGCT-AGAATTTGAGAAAACAGCAAAATGCCAAGAGTCCAAAGGGCAGGCCGGCA
COL1A1-HILS1	828	3	CCCCGTGGCCTGCCTGGTGAGAGAGGTCGCCCTGGAGCCCCTGGCCCTGC-CACCTTAACTCTCAGCTTCCCAGACTTTCTCCAATGACAGAGCTGGGTGG
COL1A1-HILS1	828	2	CCCGTGGCCTGCCTGGTGAGAGAGGTCGCCCTGGAGCCCCTGGCCCTGCT-CCTTAACTCTCAGCTTCCCAGACTTTCTCCAATGACAGAGCTGGGTGGGT
COL1A1-HILS1	828	2	CCCCCGTGGCCTGCCTGGTGAGAGAGGTCGCCCTGGAGCCCCTGGCCCTG-CACCTTAACTCTCAGCTTCCCAGACTTTCTCCAATGACAGAGCTGGGTGG
COL1A1-HILS1	828	2	CCCGTGGCCTGCCTGGTGAGAGAGGTCGCCCTGGAGCCCCTGGCCCTGCT-GCGTGAGCGTGGGCCGCGTGGGAAAGAATGCGAGGCTGCCGTCAAGCCCA
COL1A1-HILS1	828	2	CAAAGATGGAGAGGCTGGAGCTCAGGGACCCCCTGGCCCTGCT-GTAAAGGTGAGGGCCGGAGCTCAAGAGGAAGCCTCAGCGAGGA
COL1A1-FYN	39	36	CCCCTGGCCCCGTTGGCCCTGCTGGCAAGAGTGGTGATCGTGGTGAGACT-TTTTTTTGAAGAAGCAGGATGCTGATCTAAACGTGGAAAAAGTAAGTTGG
COL1A1-FYN	39	17	GGCCAGGGACTCTTCAGGCCTCCTTAGAGGCCTGGGGATGGGTGTCGGAC-GGAAATCTAGTCCTGCATGGGGGTGTGGGCAAAGGAAAACAAGAGTGAAA
COL1A1-FYN	39	14	GCCCCTGGCCCCGTTGGCCCTGCTGGCAAGAGTGGTGATCGTGGTGAGAC-TTTTTTTGAAGAAGCAGGATGCTGATCTAAACGTGGAAAAAGTAAGTTGG
COL1A1-FYN	39	10	CCCCTGGCCCCGTTGGCCCTGCTGGCAAGAGTGGTGATCGTGGTGAGACT-GTTTTTTTGAAGAAGCAGGATGCTGATCTAAACGTGGAAAAAGTAAGTTG
COL1A1-FYN	39	2	CCTGGCCCCGTTGGCCCTGCTGGCAAGAGTGGTGATCGTGGTGAGACTGT-TTTTTTTGAAGAAGCAGGATGCTGATCTAAACGTGGAAAAAGTAAGTTGG
COL1A1-FYN	39	2	CCCCTGGCCCCGTTGGCCCTGCTGGCAAGAGTGGTGATCGTGGTGAGACT-GTGAGAAATCAGAGGCCCGGGGAGAGTACTACTTGTTCAAGACCACCCTG
COL1A1-FYN	39	2	GGCCAGGGACTCTTCAGGCCTCCTTAGAGGCCTGGGGATGGGTGTCGGAC-AAATCTAGTCCTGCATGGGGGTGTGGGCAAAGGAAAACAAGAGTGAAAAA
EPB41L5-COL5A2	12	2	CTCGGCTCTTCCCCGCTCTGGTCGCCGGGGCTGCGCCGTCCCCAGCTCAG-AAGGATATGGTGAAGAAATAGCCTGCACTCAGAATGGCCAGATGTACTTA
CTBS-GNG5	3	2	CCTATGGGATAAAGATCAGCGGGCTCCTTATTATAACTATAAA-GTTTCCCAGGCAGCTGCAGACTTGAAACAGTTCTGTCTGCAGA

COL1A1-PDGFB fusion gene consisting of *COL1A1* from 17q21 and *PDGFB* from 22q13 resulting in deregulation of *PDGFB*. In the *COL1A1-PDGFB* fusion gene, the exact site of the breakpoint in *COL1A1* was shown to be highly variable from exons 6 to 49, whereas the *PDGFB* breakpoint was consistently located in intron 2 so that exon 2 of the gene was always present in the *COL1A1-PDGFB* fusion transcript (31-33). No correlation was found between the genomic breakpoint in *COL1A1* and clinico-histopathologic dermatofibrosarcoma protuberans features (34, 35).

Fusion of *COL1A1* with *USP6* (from 17p13) was also described in a case of aneurysmal bone cyst and in a benign bone tumor (36, 37). In both instances, exon 1 of *COL1A1* was fused to exon 2 of *USP6* and the pathogenic consequence of the fusion gene appeared to be control of *USP6* expression by the *COL1A1* promoter (36, 37).

In the present case of epithelioid osteoblastoma, we believe that the *COL1A1-FYN* fusion gene results in regulation of *FYN* expression by the *COL1A1* promoter similar to what happens with *COL1A1-PDFGB* and *COL1A1-USP6*.

Both *FYN* (on chromosome band 6q21) and *COL1A1* (on 17q21) are transcribed from telomere to centromere. Hence, formation of a *COL1A1-FYN* fusion is possible through a simple t(6;17)(q21;q21). The *COL1A1-FYN* is predicted to lie in the breakpoint of the putative der(6)t(6;17).

To the best of our knowledge, this is the first time that a fusion gene is described in epithelioid osteoblastoma. Whether *COL1A1-FYN* represents a consistent genetic feature of these tumors, and whether additional clinico-pathological features, including the aggressive behavior, may be associated with this fusion, remains to be seen.

Conflicts of Interest

No potential conflicts of interest exist regarding this study.

Authors' Contributions

IP conceived the study, designed and performed the experiments, performed the bioinformatics analysis, and drafted the manuscript. LG performed cytogenetic analysis. IL performed pathological examination. ML-I performed pathological examination and evaluated immunohistochemical staining. KA performed cytogenetic and FISH analyses. AH performed immunohistochemistry examinations. BB performed pathological examination. SH evaluated the cytogenetic and FISH data and assisted with writing of the manuscript. All Authors read and approved the final manuscript.

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