

An Unbalanced Chromosome Translocation Between 7p22 and 12q13 Leads to *ACTB-GLI1* Fusion in Pericytoma

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Abstract. *Background/Aim:* Since the first description of five pericytomas with the *t(7;12)/ACTB-GLI1* fusion gene, only three new tumors were studied by both cytogenetics and molecular techniques. We report here genetic data on another case of this rare tumor. *Materials and Methods:* Cytogenetic, fluorescence *in situ* hybridization (FISH), reverse transcription polymerase chain reaction (RT-PCR), and Sanger sequencing analyses were performed. *Results:* The pericytoma carried two structurally rearranged chromosomes: *der(7)t(7;12)(p22;q13)* and *der(12)t(1;12)(q12;q13)*. In FISH experiments with a break-apart probe for *GLI1*, the distal part of the probe hybridized to *der(7)* whereas the proximal part to *der(12)*. RT-PCR and Sanger sequencing detected an *ACTB-GLI1* fragment in which exon 2 of *ACTB* was fused to exon 6 of *GLI1*. *Conclusion:* The *ACTB-GLI1* fusion gene was mapped at *der(7)t(7;12)(p22;q13)* and coded for a putative *ACTB-GLI1* protein in which the first 41 amino acid (aa) of *ACTB* replaced the first 177 aa of *GLI1*.

Fusion of the genes actin beta (*ACTB* on 7p22.1) and glioma associated oncogene homologue 1 (*GLI1* on 12q13.3) was first reported by Dahlén *et al.* (1, 2) in five benign spindle cell tumors with distinctive pericytic features and a *t(7;12)(p21-22;q13-15)* chromosome translocation. The tumors defined a discrete group of previously uncharacterized neoplasms for which the term

“pericytoma with *t(7;12)*” was proposed in order to reflect both the morphological and cytogenetic features (1). Until now, the *ACTB-GLI1* fusion gene has been reported in fifteen such cases, four of which had developed metastases (1-7) (Table I). Because of the apparent rarity of tumors carrying a *t(7;12)/ACTB-GLI1*, we here present the genetic and clinical features of another pericytoma with an unbalanced chromosome translocation between 7p22 and 12q13 leading to an *ACTB-GLI1* fusion gene.

Materials and Methods

Case description and pathology. An 83-year-old woman presented with a painless palpable mass in her left thigh. Magnetic resonance imaging (MRI) revealed a 4×11×10 cm encapsulated, mainly cystic tumor in the adductor magnus muscle. There was contrast enhancement in the capsule and a small intracapsular part of the tumor. Staging studies revealed no evidence of metastatic disease. Examination of the core needle biopsy showed a small blue round cell tumor with low mitotic activity. Immunohistochemistry showed that the tumor cells were positive for CD99 (Figure 1A), and fluorescence *in situ* hybridization (FISH) showed no rearrangement of *EWSR1*.

Wide tumor excision was performed. Gross examination revealed a cystic tumor with a maximum diameter of 9.5 cm. The cystic tumor was filled with serous fluid and grey, necrotic appearing material. In the internal part of the fibrous capsule, there was a white and solid layer 3-15 mm thick (Figure 1B). The tumor demonstrated sheetlike growth with an intricate network of capillary-sized vessels (Figure 1C). In the fibrous capsule there was evidence of tumor cells around larger vessels (Figure 1D). The neoplastic cells were small and ovoid with sparse eosinophilic cytoplasm and round nuclei with dense chromatin and scattered small white pseudoinclusions. The ratio of mitoses was 1/10 high-power field (HPF), no atypical mitotic figures were noted. Immunohistochemical staining showed that the tumor cells were positive for cluster of differentiation (CD) CD99 (strong membranous, weak cytoplasmic positivity), alpha-smooth muscle actin (SMA weak), cytokeratin AE1/AE3 (CK ae1/ae3 weak and focal), epithelial membrane antigen (EMA weak and focal), and berep4 (weak and focal). Staining for CD34 and CD31 highlighted

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Table I. The currently reported pericytomas with the *ACTB-GLI1* fusion gene including the present tumor.

Case	Gender/Age	Location	Karyotype	Fused exons in <i>ACTB-GLI1</i> transcript	Reference
1	M/61	Calf	45,XY,t(7;12)(p22;q13),inv(10)(p11q21)c,der(15;16)(q10;p10)	Exon 2 – Exon 7	(1)
2	F/27	Tongue	46,XX,t(7;12)(p22;q13)	Exon 1 – Exon 6	(1)
3	M/11	Tongue	45,XY,der(1)t(1;7)(p36;p?22),add(2)(p25),add(5)(p15),add(6)(p?21),der(7)t(7;12)(p11;p11),der(7)t(7;12)(p21;q1?5),-12	Exon 3 – Exon 7	(1)
4	F/65	Stomach	46,XX,t(7;12)(?p22;?q15)	Exon 2 – Exon 6	(1)
5	F/12	Tongue	46,XX,t(1;3)(p22;q21),t(7;12)(p22;q13)	Exon 3 – Exon 6	(1)
6	M/67	Skeleton	46,XY,+X,der(1)t(1;14)(p13;q11),der(2)t(2;13)(q31;q31),der(3)t(2;13;3)(q31;q11-31;p13),t(7;12)(p22;q13),-13,der(?14)t(13;?14;?)(q11-12;?q11;?),der(15)t(3;15)(p13;q11)	Exon 3 – Exon 6	(3)
7	F/9	Stomach	46,XX,t(7;12)(p22;q13)	Exon 2 – Exon 6	(4)
8	F/11	Ovary	46,XX,t(7;12)(p22;q13)	Exon 3 – Exon 6	(7)
9	M/20	Thigh	No data	Exon 3 – Exon 6	(5)
10	F/30	Foot	No data	No data	(5)
11	F/79	Retroperitoneum	No data	Exon 1-Exon 5	(5)
12	F/38	Chest wall (skeletal muscle)	No data	No data	(5)
13	F/57	Tibia	No data	No data	(6)
14	M/62	Scapula	No data	No data	(6)
15	F/41	Ovary	No data	No data	(6)
16	F/83	Thigh	46,XX,der(7)t(7;12)(p22;q13),der(12)t(1;12)(q12;q13)[11]/46,idem,tas(X;8)(qter;qter)[3]/46,idem,tas(8;12)(qter;qter)[2]	Exon 2 – Exon 6	Present case

a prominent thin-walled vasculature, but the tumor cells were negative. The tumor cells were additionally negative for S100, Sox-10, desmin, H-caldesmon, synaptophysin, CD45, CD30, and ETS-related gene protein (ERG).

G-banding and FISH analyses. Fresh tissue from a representative area of the tumor was short-term cultured and analyzed cytogenetically as previously described (8).

FISH was performed on metaphase spreads using the Cytocell *CHOP* (*DDIT3* on 12q13.3) break-apart FISH Probe (Cytocell, Oxford Gene Technology, Begbroke, Oxfordshire, UK). According to the company's information, the *CHOP* break-apart probe consists of a proximal to the centromere red 146 kb probe and a distal to the centromere green 165 kb probe, which are positioned on each side of the *DDIT3* (*CHOP*) gene. However, the genes *INHBC*, *INHBE*, *GLI1*, *ARHGAP9*, *MARS*, *DDIT3*, and *MBD6* map between the red and the green probes. Thus, the probe is not specific for the *DDIT3* rearrangement but can be used to investigate possible rearrangements of any of the above-mentioned genes. In the present study, we used it to look for *GLI1* rearrangements. Fluorescent signals were captured and analyzed using the CytoVision system (Leica Biosystems, Newcastle, UK).

Reverse transcription (RT) PCR analysis. In order to confirm the presence of an *ACTB-GLI1* fusion transcript (see below), reverse transcription (RT) polymerase chain reaction (PCR) and Sanger sequencing analyses were performed as previously described (9). The primers used were the forward ACT61F (5'-CCGCCAGCTCACC

ATGGATGATG-3') and the reverse GLI720R (5'-AGGTCCTCCGCCATCCAGC-3') (1). The quality of the cDNA synthesis was assessed by amplification of a cDNA fragment of the *ABL* protooncogene 1, non-receptor tyrosine kinase (*ABL1*) gene using the primers ABL1-91F1 (5'-CAGCGGCCAGTAGCATCTGACTTTG-3') and ABL1-404R1 (5'-CTCAGCAGATACTCAGCGGCATTGC-3'). PCR cycling was at 94°C for 30 s followed by 35 cycles of 7 s at 98°C, 30 s at 60°C, 30 s at 72°C, and a final extension for 5 min at 72°C.

Results

G-banding analysis of short-term cultured cells from the tumor yielded the karyotype 46,XX,der(7)t(7;12)(p22;q13),der(12)t(1;12)(q12;q13)[11]/46,idem,tas(X;8)(q28;q24)[3]/46,idem,tas(8;12)(q24;q24)[2] (Figure 2).

FISH analysis using the *CHOP* break-apart probe (Figure 3A and B) showed that the distal part of the probe (green signal) hybridized to the der(7)t(7;12)(p22;q13), whereas the proximal part of the probe (red signal) hybridized to der(12)t(1;12)(q12;q13) (Figure 3C).

RT-PCR amplified a 264 bp cDNA fragment. Sanger sequencing showed that it was an *ACTB-GLI1* chimeric fragment in which exon 2 of *ACTB* (nt 207 in the sequence with accession number NM_001101.5) fused with exon 6 of *GLI1* (nt 613 in the sequence with accession number NM_005269.2) (Figure 4).

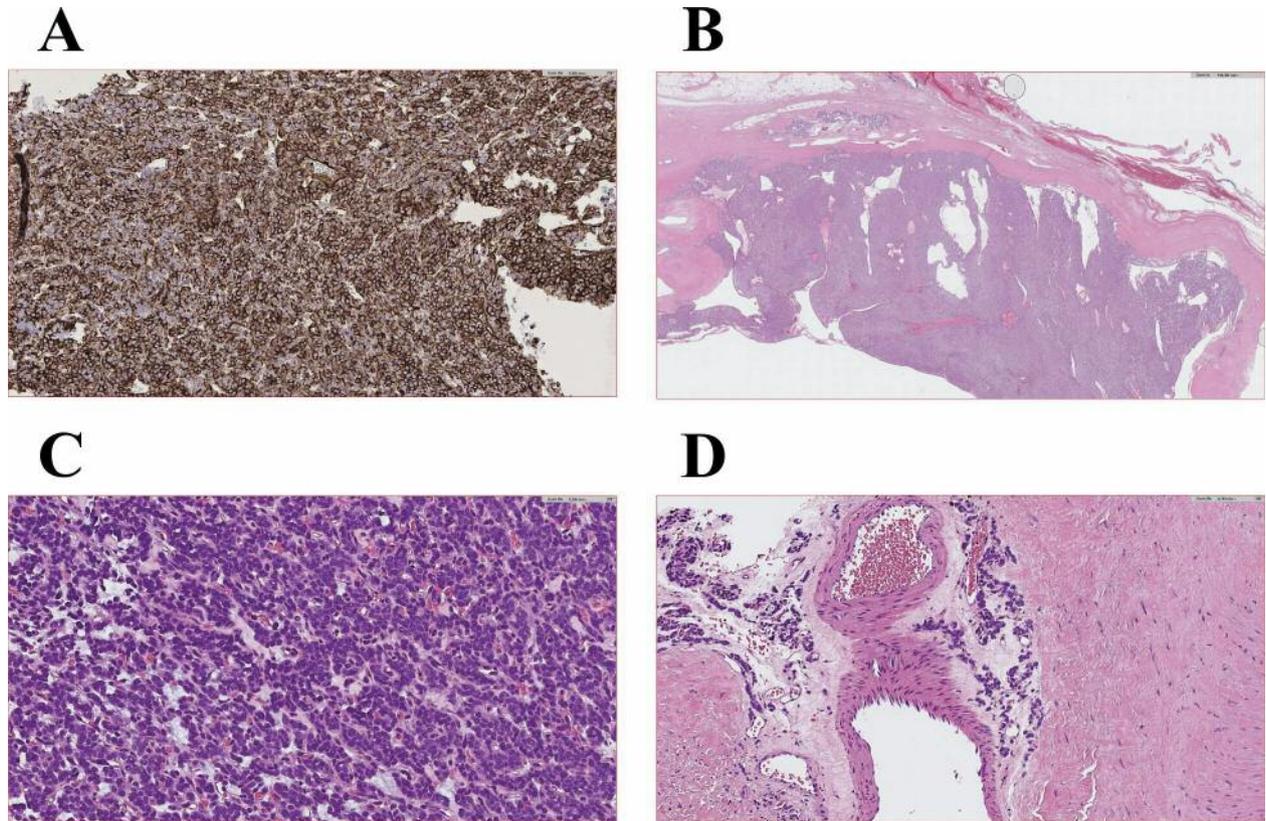


Figure 1. Microscopic examination of the pericytoma. A) Immunohistochemical examination showing expression of CD99 in the pericytoma, 40 \times . B) Hematoxylin and eosin (H&E)-stained section showing area of encapsulated tumor, 1 \times . C) H&E-stained section showing sheet-like growth with an intricate network of capillary-sized vessels, 40 \times . D) H&E-stained section showing perivascular orientation of tumor cells, 20 \times .

Discussion

Since the first description in 2004 of “pericytoma with t(7;12)” in five tumors (1), only three new pericytomas were studied by both cytogenetics and molecular techniques (Table I). On the chromosome level, all eight tumors reported in literature were in diploid range (45-46 chromosomes). Half of the pericytomas had a sole t(7;12), two more tumors carried 1-2 additional changes, whereas the other two had complex karyotypes. All but one tumor showed visibly balanced t(7;12) (Table I).

The present case also had a simple diploid karyotype, accompanied by two subclones with supplementary telomeric associations. The tumor did not carry a balanced t(7;12) chromosome translocation, but instead a der(7)t(7;12)(p22;q13) and a der(12)t(1;12)(q12;q13). The FISH analyses not only supported the cytogenetic data but further located the pathogenetic *ACTB-GLI1* fusion gene on the der(7).

The t(7;12) / der(7)t(7;12) places *GLI1* under the control of the strong *ACTB* promoter resulting in activation of *GLI1* and deregulation of its downstream targets (1-7, 10, 11). According to the NCBI database, *ACTB* is ubiquitously

expressed in 27 tissue types. The highest expression was found in the appendix having 2395.4 RPKM (Reads Per Kilobase of transcript, per Million mapped reads) whereas the lowest was in the pancreas with 95.5 RPKM (12). In the same 27 tissues, *GLI1* is weakly expressed. The highest *GLI1* expression was found in the endometrium with 5.6 RPKM followed by expression in the testis (2.9 RPKM) and the gall bladder (2.5 RPKM) (13).

GLI1 together with *GLI2* (on 2q14.2), *GLI3* (on 7p14.1), and *GLI4* (on 8q24.3) make up the GLI family of transcription factors (14). They bind to the DNA consensus sequence 5'-GACCACCCA-3' in the promoters of target genes regulating their expression (15). The *GLI1* protein is a transcription activator, *GLI2* is both an activator and a repressor, *GLI3* functions as a repressor, whereas there is no information on the function of *GLI4* (16). The members of the GLI family are main mediators of the highly conserved Hedgehog signaling pathway, which plays a critical role in embryonic development (17-20). Aberrant Hedgehog signaling is associated with the development and progression of various types of cancer and is implicated in multiple aspects of tumorigenesis (21-27).

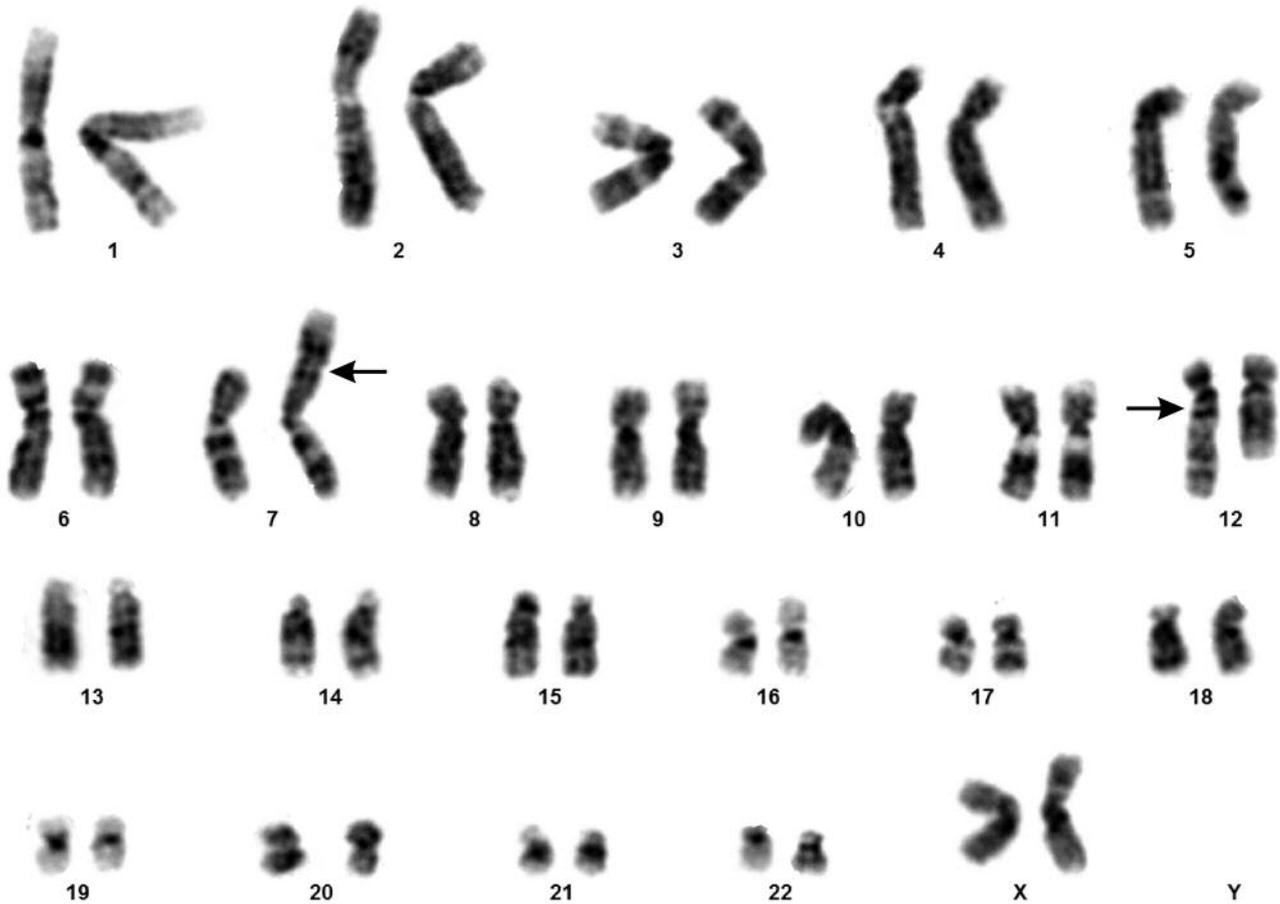


Figure 2. Karyogram of the pericytoma showing two abnormal chromosomes, $der(7)t(7;12)(p22;q13)$, and $der(12)t(1;12)(q12;q13)$. Breakpoint positions are indicated by arrows.

Three transcript variants have been reported for *GLI1*. Transcript variant 1 (NM_005269) codes for a 1106 amino acid (aa) protein (NP_005260; isoform 1).

GLI1 isoform 1 contains (from N to C terminal) a Snail/Gfi-1(SNAG) domain, an N-terminal degradation signal, D_N degron, an N-terminal binding site for Suppressor of Fused protein (SUFU binding site), a forkhead box protein (FOXP) coiled-coil domain, 5 Cys2-His2 (C2H2) Zn-finger motifs, a nuclear localization signal, a C-terminal degradation signal, D_C degron, a nuclear export signal, a C-terminal SUFU binding site, and the transactivation domain (28-31).

Transcript variant 2 (NM_001160045) lacks the first two coding exons of transcript variant 1, transcription initiation occurs from an internal AUG site, and codes for N-terminally truncated protein isoform 2 (NP_001153517), also known as *GLI1*DeltaN (32). The *GLI1* isoform 2 lacks the first 128 amino acids of *GLI1* isoform 1 which contain the SNAG domain, the D_N degron, and the SUFU binding site

(29, 31, 32). Inhibition of *GLI1* activity by the SUFU protein has been reported (29, 30).

The *GLI1* transcript variant 3 (NM_001167609) has an in-frame deletion of 123 bases (41 codons) spanning the entire exon 3 and part of exon 4 of the *GLI1* gene (33). This transcript codes for the *GLI1* isoform 3 protein (NP_001161081), also known as t*GLI1* (33). *GLI1* transcript variant 3 was highly expressed in glioblastoma multiforme and other cancers but has not been detected in normal cells. It promotes cancer cell migration and activates a different set of genes from those activated by *GLI1* isoform 1 (33).

The chimeric *ACTB-GLI1* transcript described here is an in-frame fusion of exon 2 of *ACTB* with exon 6 of *GLI1* coding for a putative 969 aa protein in which the first 177 aa of *GLI1* are replaced with the first 41 aa of *ACTB*. In fact, in all reported *ACTB-GLI1* chimeric transcripts, exons 1, 2, or 3 of *ACTB* are fused to exons 5, 6 (most commonly), or 7 of *GLI1* (1, 3-5, 7).

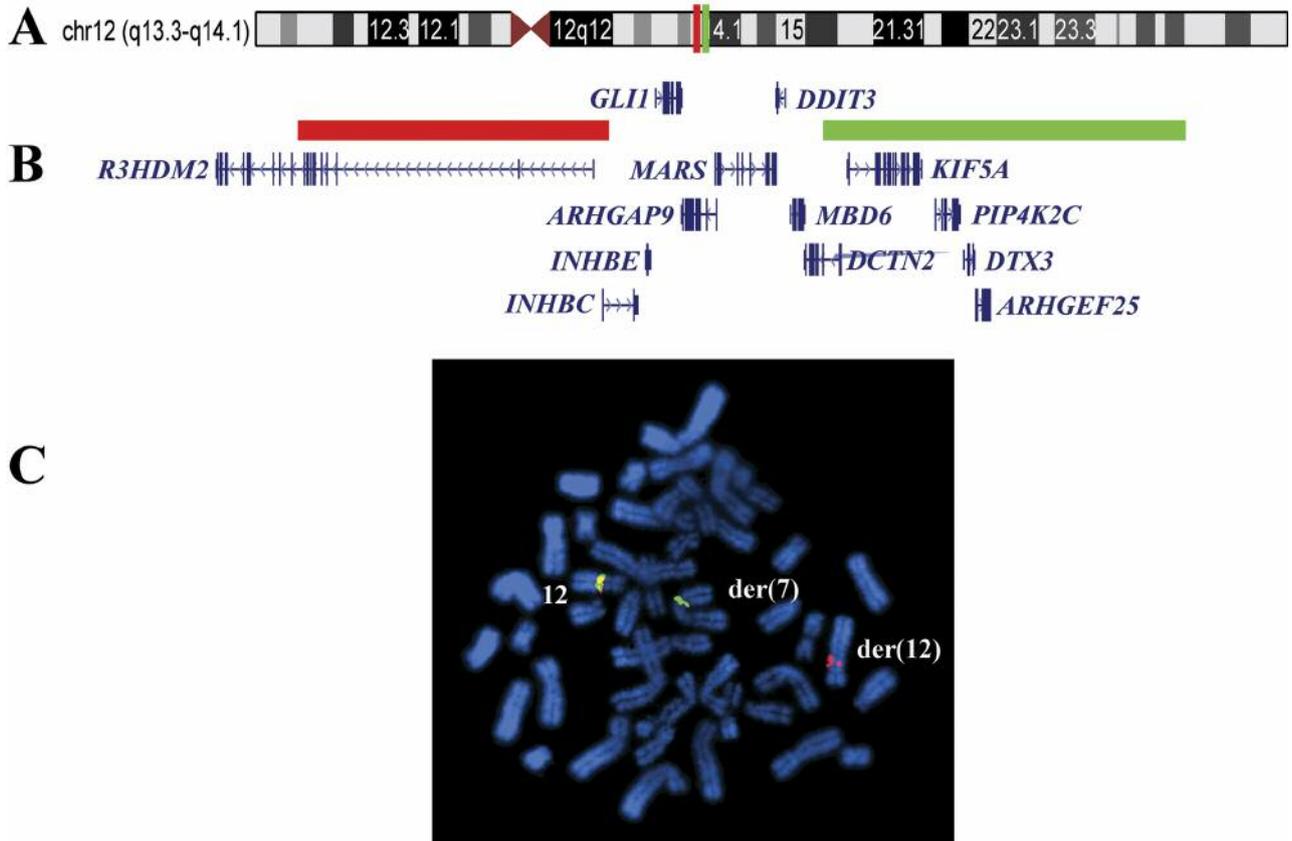


Figure 3. FISH analysis of the pericytoma with the Cytocell CHOP (DDIT3) (12q13.3) break-apart FISH Probe. A) Ideogram of chromosome 12 showing the mapping position of the FISH probe. B) Diagram showing the FISH probe and the genes covered by the probe. C) FISH on a metaphase spread showing that the distal part of the probe (green signal) hybridized to the *der(7)t(7;12)(p22;q13)* whereas the proximal part of the probe (red signal) hybridized to *der(12)t(1;12)(q12;q13)*. Both distal and proximal parts of the probe hybridized to the normal chromosome 12.

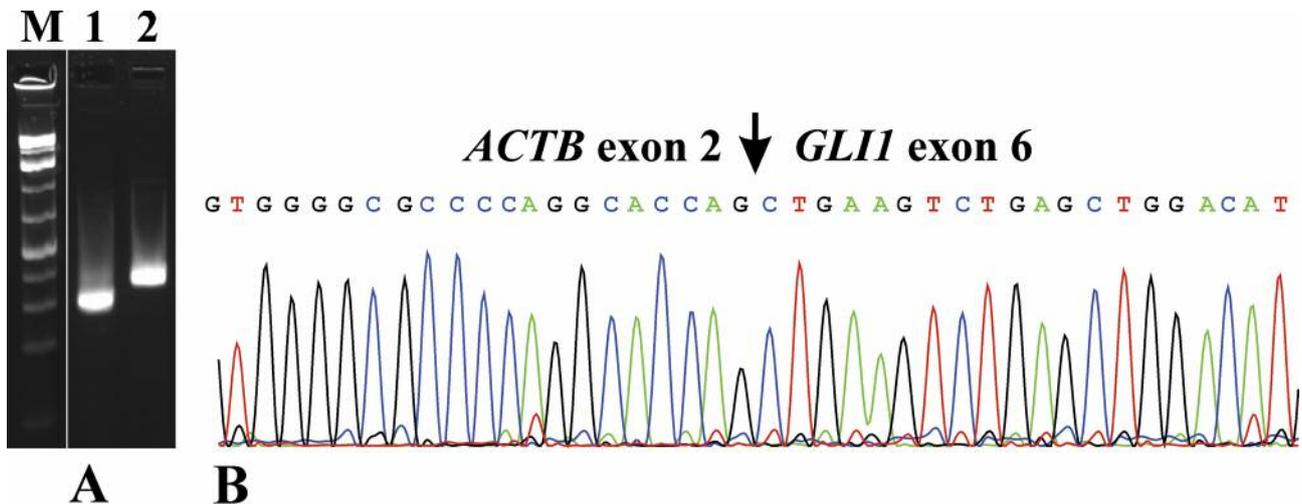


Figure 4. RT-PCR and Sanger sequencing. A) Gel electrophoresis showing the amplified *ACTB-GLI1* cDNA fragment using the primer combination *ACT61F/ GLI720R* (lane 1) and an amplified *ABL1* cDNA using the primers *ABL1-91F1* and *ABL1-404R1* (lane 2). M, GeneRuler 1 Kb Plus DNA ladder (ThermoFisher Scientific). B) Partial sequence chromatograms of the cDNA amplified fragment showing the junction position of exon 2 of *ACTB* with exon 6 of *GLI1*.

Recently, the *MALAT1-GLI1* fusion gene was reported in a subset of plexiform fibromyxomas, in gastroblastomas, and in epithelioid tumors with metastatic potential (5, 34, 35). The *MALAT1* gene (on 11q13.1) is ubiquitously expressed and produces a long non-coding RNA (36, 37). Part of *MALAT1* was fused to exon 6 (plexiform fibromyxomas) or to the last 19 nt of the 145 bp long exon 5 (gastroblastoma) of *GLI1* (34, 35). Spans *et al.* (34) showed that the truncated *GLI1* protein (lacking exons 1–5) was transcriptionally active.

A *PTCH1-GLI1* fusion has also been found in an epithelioid tumor with metastatic potential (5). In that case, exon 1C of *PTCH1* fused to exon 7 of *GLI1* (*PTCH1* transcript variant 1a, accession number NM_001083602.2) (38, 39). The only contribution of exon 1C *PTCH1* was the initiation ATG codon. Expression of exon 1C of *PTCH1* was reported to be tissue specific, found in the adult brain, placenta, lung, kidney, pancreas, and in fetal kidney (38).

Taking the information reviewed above into consideration, we conclude that the *ACTB-GLI1*, *MALAT1-GLI1*, and *PTCH1-GLI1* fusion genes code for proteins which are similar to the *GLI1* isoform 2 protein. They contain the *GLI1* FXP coiled-coil domain, the 5 C2H2 Zn-finger motifs, the nuclear localization signal, the C-terminal degradation signal, D_C degron, nuclear export signal, the C-terminal SUFU binding site, and the transactivation domain. They will lack the SNAG domain, the N-terminal degradation signal, D_N degron, and the N-terminal SUFU binding site. The expression of these proteins will be controlled by strong (*ACTB* and *MALAT1*) or tissue specific (*PTCH1*) promoters.

Conflicts of Interest

The Authors declare that no potential conflicts of interest exist.

Authors' Contributions

IP designed and supervised the research, performed molecular genetic experiments, bioinformatics analysis, and wrote the article. LG performed cytogenetic analysis and evaluated the FISH data. TVR performed the pathological examination. KA performed molecular genetic experiments, FISH analysis, and evaluated the data. FM supervised the research. SH assisted with experimental design and writing of the article. All Authors read and approved the final article.

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