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Fusions involving protein kinase C and membrane-associated proteins in benign fibrous histiocytoma☆

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ABSTRACT

Benign fibrous histiocytoma (BFH) is a mesenchymal tumor that most often occurs in the skin (so-called dermatofibroma), but may also appear in soft tissues (so-called deep BFH) and in the skeleton (so-called non-ossifying fibroma). The origin of BFH is unknown, and it has been questioned whether it is a true neoplasm. Chromosome banding, fluorescence in situ hybridization, single nucleotide polymorphism arrays, RNA sequencing, RT-PCR and quantitative real-time PCR were used to search for recurrent somatic mutations in a series of BFH. BFHs were found to harbor recurrent fusions of genes encoding membrane-associated proteins (podoplanin, CD63 and LAMTOR1) with genes encoding protein kinase C (PKC) isoforms PRKCB and PRKCD. PKCs are serine–threonine kinases that through their many phosphorylation targets are implicated in a variety of cellular processes, as well as tumor development. When inactive, the amino-terminal, regulatory domain of PKCs suppresses the activity of their catalytic domain. Upon activation, which requires several steps, they typically translocate to cell membranes, where they interact with different signaling pathways. The detected *PDPN-PRKCB*, *CD63-PRKCD* and *LAMTOR1-PRKCD* gene fusions are all predicted to result in chimeric proteins consisting of the membrane-binding part of PDPN, CD63 or LAMTOR1 and the entire catalytic domain of the PKC. This novel pathogenetic mechanism should result in constitutive kinase activity at an ectopic location. The results show that BFH indeed is a true neoplasm, and that distorted PKC activity is essential for tumorigenesis. The findings also provide means to differentiate BFH from other skin and soft tissue tumors.

This article is part of a Directed Issue entitled: Rare cancers.

1. Introduction

Benign fibrous histiocytoma (BFH) is a distinct tumor entity that usually occurs in the skin. It is then often referred to as dermatofibroma, possibly the most prevalent mesenchymal skin tumor in man (Weyers et al., 2006). Tumors with the same morphology can appear subcutaneously or, rarely, intramuscularly or in visceral soft tissue, known as deep BFH, or in the skeleton, then called non-ossifying fibroma or BFH of bone (Fletcher and Gleason, 2013; Nielsen and Kyriakos, 2013). These terminologic intricacies notwithstanding, all entities usually appear in young to middle aged adults, and they are essentially benign. However, they may recur locally if resected with inadequate margins, and metastases are seen in exceptional cases (Weyers et al., 2006; Weiss and Goldblum, 2008; Fletcher and Gleason, 2013; Nielsen and Kyriakos, 2013). Important differential diagnoses include dermatofibrosarcoma protuberans and solitary fibrous tumor when occurring in the skin or soft tissues, and giant cell tumor of bone when skeletal.

The neoplastic nature of BFH has been debated (Calonje, 2000; Weiss and Goldblum, 2008), but data supporting a monoclonal origin have been obtained at least for a subset of investigated tumors

Abbreviations: BFH, benign fibrous histiocytoma; SNP, single nucleotide polymorphism; RNA-Seq, mRNA sequencing; FISH, fluorescence in situ hybridization.

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(Hui et al., 2002). However, no characteristic genetic aberrations have been described. Here, we present the results of cytogenetic, single nucleotide polymorphism (SNP) array and mRNA sequencing (RNA-Seq) studies of BFH, revealing recurrent gene fusions involving protein kinase C (PKC) genes with genes encoding membrane-associated proteins. BFH is the first tumor type with recurrent gene fusions involving the PKC family of genes.

2. Materials and methods

2.1. Patients

The study comprised BFHs from eight patients from whom fresh tumor samples had been obtained for cytogenetic analysis; from five of them, frozen samples were available for further molecular studies (Table 1). The sampling, storage and analysis of the tumor samples included in this study were approved by the Regional Ethics Committee of Lund University; all samples were obtained after informed consent. Four tumors were subcutaneous, two were cutaneous and two were located both cutaneously and subcutaneously. The diagnoses were based on published criteria (Vanni et al., 2000; Weyers et al., 2006; Weiss and Goldblum, 2008; Fletcher and Gleason, 2013; Fig. 1). Local recurrences were seen in Cases 4 and 8. No metastases were reported after 4–24 years of follow-up.

2.2. Cytogenetic and FISH analyses

Cell culturing, harvesting and G-banding were performed as described (Vanni et al., 2000). Two of the karyotypes (Cases 7 and 8) were reported before (Vanni et al., 2000).

FISH was performed on metaphase spreads from Case 6, which had a supernumerary marker chromosome, using whole chromosome painting (WCP) probes for chromosomes 3, 8 and 12 (Applied Spectral Imaging, Migdal Haemek, Israel) and BAC clone RP11-134N13 (BAC PAC resources, Oakland, USA), spanning the *PRKCD* gene. Clone preparation, hybridization and analysis were performed as described (Jin et al., 2012).

2.3. SNP array analysis

Material for SNP array analysis was available from five cases. DNA was extracted from frozen tumor tissue using the DNeasy Blood & Tissue kit including the optional RNaseA treatment, according to the manufacturer's instructions (Qiagen, Hilden, Germany). Tumor DNA was hybridized on Affymetrix CytoScan HD arrays, containing more than 2.6 million markers (Affymetrix, Santa Clara, USA). Genomic aberrations were identified by visual inspection using the Chromosome Analysis Suite version CytoB-N1.2.2.271 (Affymetrix). The positions of SNPs were according to the GRCh37/hg19 build. Constitutional copy number variations were excluded through comparison with the Database of Genomic Variants (http://projects.tcag.ca/variation/).

2.4. RNA-Seq and data analysis

mRNA libraries from Cases 4–6 were prepared for sequencing using the Truseq RNA sample preparation kit v2 (Illumina, San Diego, USA) according to the manufacturer's protocol. In brief, 200–1000 ng of total RNA was enriched for poly-A tailed RNA using magnetic oligo-dT beads. The poly-A tailed enriched RNA was fragmented to a median size of 200 nucleotides by thermal fragmentation at 94 °C, 10 s in the "Elute, Prime, Fragment" buffer. The fragmented RNA was used as template for cDNA synthesis using Superscript II reverse transcriptase (Life Technologies). A second DNA strand was produced using DNA polymerase I and RNase H. After end repair and 3′ end-adenylation, indexed adapters were ligated to the double stranded cDNA fragments. The adapter-bound fragments were then enriched using a 15-cycle PCR. Paired end 101 bp reads were generated from the mRNA libraries using a HiscanSQ (Illumina).

To identify candidate fusion transcripts from the sequence data, analyses were performed on gunzipped fastq files using TopHat v2.0.7 with the *--fusion-search* and *--bowtie1* options, only allowing for detection of fusions within a minimum distance of 100,000 bp (*--fusion-min-dist* option). The GRCh37/hg19 build was used as the human genome reference. The mate inner distance (*-r* option) was set to 200 with a standard deviation (*--mate-std-dev* option) of 200. TopHat-fusion-post was run on the output files from TopHat v2.0.7 to filter for fusions with at least one fusion-spanning read and two fusion-spanning mate-pairs (Kim et al., 2013).

2.5. RT-PCR

Material for RT-PCR was available from Cases 2 and 4–6. RNA extraction and cDNA synthesis were performed as described (Jin et al., 2012). Primers for *CD63*, *LAMTOR1*, *PDPN*, *PRKCB* and *PRKCD* were designed to verify the transcripts identified at RNA-Seq analysis in Cases 4–6 and to assess whether Case 2, which at chromosome banding analysis displayed the same t(1;16) as Case 5, had a *PDPN-PRKCB* fusion (Supplementary Table S1). PCRs were performed in a 50 μl total reaction volume containing 1× PCR buffer, 1 mM dNTPs, 1.25 mM $MgCl_2$, 0.5 μM of each of the forward and reverse primers, 1 U Platinum Taq DNA Polymerase (Life Technologies, Foster City, USA) and 1 μl cDNA template. After denaturation for 2 min at 94 °C, 30 cycles of 30 s at 94 °C, 30 s at 56–58 °C and 2 min at 72 °C were run, followed by a final extension for 3 min at 72 °C. Amplified fragments were purified from agarose gels and directly sequenced using the Big Dye v1.1 cycle sequencing kit (Life Technologies) on an ABI-3100 Avant genetic analyzer (Life Technologies). Obtained sequence data were analyzed using the BLASTN software (http://blast.ncbi.nlm.nih.gov/Blast).

Supplementary table related to this article found, in the online version, at http://dx.doi.org/10.1016/j.biocel.2014.03.027.

2.6. Quantitative real-time PCR

To determine expression levels of *PRKCB* and *PRKCD* in Cases 2 and 4–6, the 7500 quantitative real-time PCR (q-PCR) System (Life Technologies) was used. Five samples each of dermatofibrosarcoma proturberans and solitary fibrous tumor were included as controls. A human *PRKCD* (Hs01090047_m1) probe and a custom probe mapping to *PRKCB* exon 10 were used as targets and *TBP* (Hs00427620_m1) was used as an endogenous control gene (Life Technologies). Each reaction was done in triplicate using TaqMan Gene Expression Assays (Applied Biosystems) and the amount of cDNA was 50 ng per well. The PCR program used was 50 °C for 2 min, followed by 95 °C for 10 min, and 40 cycles of 95 °C for 15 s, and 60 °C for 1 min.

3. Results

3.1. Chromosome banding, FISH and SNP array analyses

Recurrent karyotypic features, each present in two tumors, included a t(1;16)(p36;p11) in Cases 2 and 5 (Fig. 2A), a supernumerary derivative chromosome 3 involved in unbalanced translocations with chromosome 12 in Cases 6 and 7 (Fig. 2B), and trisomy 20 in Cases 4 and 6 (Table 1). FISH on seven metaphase spreads from Case 6 verified that the supernumerary marker chromosome consisted of a derivative chromosome 3 with material

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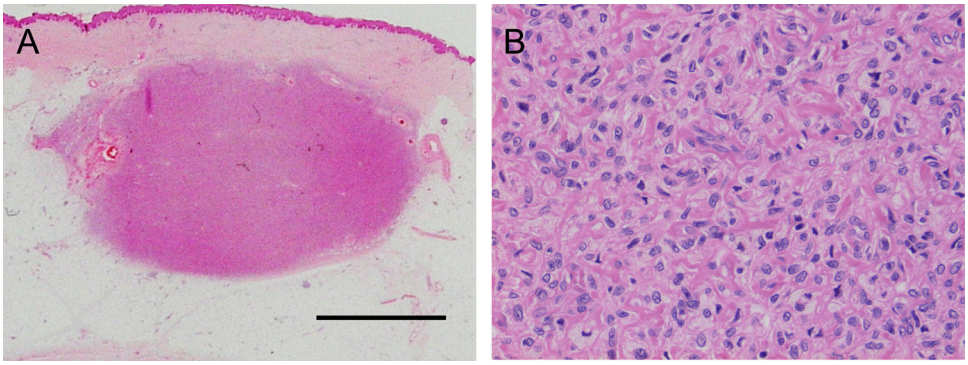

Fig. 1. Morphologic appearance of benign fibrous histiocytoma (BFH). (A) Low-power magnification of BFH with subcutaneous location (Case 5). Bar indicates 0.5 cm. (B) High-power magnification of BFH composed of fibroblasts, foamy histiocytes and entrapped collagen (Case 2). Hematoxylin and eosin, 50×.

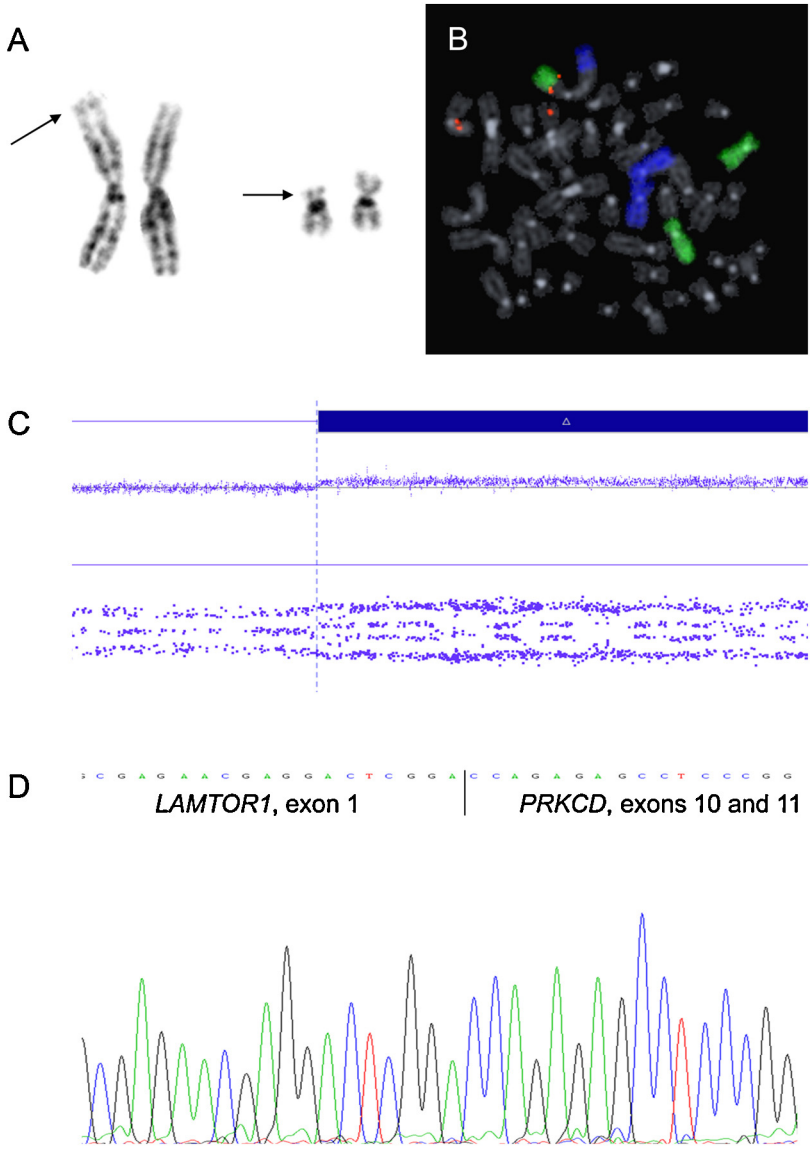

Fig. 2. Genetic findings in benign fibrous histiocytomas. (A) A partial karyogram showing the t(1;16)(p36;p11) in Case 2. Arrows indicate breakpoints. (B) Fluorescence in situ hybridization analysis of metaphase spread from Case 6, which at G-banding had a supernumerary chromosome 3 with material from the long arms of chromosomes 8 (blue) and 12 (green) attached to its q- and p-arms, respectively. A BAC probe (red) covering the *PRKCD* locus in 3p21 gave a signal at the junction between 3p21 and the added chromosome 12 material, as well as on the two normal copies of chromosome 3. (C) SNP array profile from Case 6, showing the transition from two to three copies of chromosome 3 material. The dotted line indicates the location of *PRKCD*. (D) Chromatogram from the sequenced RT-PCR product in Case 4, showing fusion of exon 1 of *LAMTOR1* with exon 10 of *PRKCD*. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

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Table 1
Clinical and genetic findings in eight benign fibrous histiocytomas.

Case no.	Sex/age	Location[a]/ depth[b]/size[c]	Karyotype	RNA-Seq[d]	RT-PCR[d]	SNP array[d,e]	q-PCR[d,f]
1.	M/54	L leg/S/4	45,XY,der(1)t(1;14)(q4?4;q11) t(14;22)(q32;q12),?del(14)(q11), -22/46,XY,t(6;11;20)(p21;q21;p13)	ND	ND	ND	ND
2.	F/61	L leg/CS/6	46,XX,t(1;16)(p36;p11), inv(9)(p11q12)c	ND	*PDPN* ex5-*PRKCB* ex8	Normal	*PRKCB*+
3.	F/49	U leg/S/3	45,XX,der(1;9)(q10;q10),add(4)(p15),add(9)(p?11)	ND	ND	Failure	ND
4.	M/39	U arm/S/4	48,XY,+der(11)ins(11;?3)(q13;p13p21),+20	*LAMTOR1-PRKCD*	*LAMTOR1* ex1-*PRKCD* ex10	+3p13-21,+11, +20	*PRKCD*(+)
5.	F/37	L leg/S/3.5	46,XX,t(1;16)(p36;p11)	*PDPN-PRKCB*	*PDPN* ex5-*PRKCB* ex8	Normal	*PRKCB*+
6.	F/41	U leg/CS/3.5	48,XX,+der(3)t(3;8)(q29;q12) t(3;12)(p21;q13),+20	*RDH5-PRKCD*[g]	*CD63* ex8-*PRKCD* ex9	+3p21-qter, +8q12-qter, +12q13-qter, +20	*PRKCD*+
7.	F/20	Forehead/C/1.5	47,XX,+der(3)t(3;12)(p23;q15)[h]	ND	ND	ND	ND
8.	F/47	Shoulder/C/1	93,XXXX,+r[h]	ND	ND	ND	ND

[a] L = lower; U = upper.
[b] S = subcutaneous; CS = cutaneous/subcutaneous; C = cutaneous.
[c] Largest diameter in cm.
[d] ND = not done.
[e] Detailed SNP array results are shown in Supplementary Table S2.
[f] q-PCR = quantitative real-time PCR; + = strong transcriptional up-regulation; (+) = moderate transcriptional up-regulation.
[g] Genes transcribed in opposite directions; the fusion transcript was between the antisense strand of *RDH5*, i.e., *CD63*, and the sense strand of *PRKCD*.
[h] Karyotypes previously published (Cases Lu 317 and Lu 342) by Vanni et al. (2000).

from chromosome 12 attached to 3p and material from chromosome 8 attached to 3q. In all seven metaphase spreads, BAC probe RP11-134N13 (spanning the *PRKCD* gene) hybridized to the junction between chromosomes 3 and 12 on the der(3), but with a weaker signal, suggesting a partial deletion, than on the two normal homologues of chromosome 3 (Fig. 2B).

Copy number aberrations were found in two (Cases 4 and 6) of the five tumors subjected to SNP array analysis (Table 1 and Supplementary Table S2). Case 4 displayed one extra copy of a 17 Mb region in 3p13-21 and trisomies for chromosomes 11 and 20. The breakpoint in 3p21 could be assigned to a 300 kb region containing the three genes *PRKCD*, *TKT*, and *DCP1A*. Case 6 displayed gain of 3p21-qter, most of 8q and 12q, and the entire chromosome 20. The breakpoint in 3p21 was mapped to a 45 kb region that harbors a single gene (*PRKCD*; Fig. 2C) and the breakpoint in 12q to an 18 kb region that contains the three genes *BLOC1S1*, *RDH5*, and *CD63*.

Supplementary table related to this article found, in the online version, at http://dx.doi.org/10.1016/j.biocel.2014.03.027.

3.2. RNA-Seq

All three tumors (Cases 4–6) subjected to RNA-Seq showed potential fusion transcripts (Table 1 and Supplementary Table S3): *LAMTOR1-PRKCD* in Case 4, *PDPN-PRKCB* in Case 5, and *RDH5-PRKCD* in Case 6. The fusion in Case 5 was seen in two different forms, with the same breakpoint in *PRKCB*, but two different breakpoints in exon 5 of *PDPN*. Looking specifically at the results for the last two exons of *PRKCB*, it was found that all reads ended with exon 17, i.e., the transcript variant encoding the βII isoform (data not shown). Additional potential fusion transcripts identified by TopHat2 were interpreted as read-through transcripts (Supplementary Table S3).

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3.3. RT-PCR and qPCR

RT-PCR could be performed on four cases, all of which showed fusion transcripts involving a PKC gene (Table 1). Primers are given in Supplementary Table S1. Cases 2 and 5 both had in-frame *PDPN-PRKCB* fusion transcripts, in which the last nucleotide of exon 5 (nt 759; NM_006474.4) of the *PDPN* gene in chromosome 1 was fused with the first nucleotide of exon 8 (nt 1019; NM_002738.6) of *PRKCB* in chromosome 16. Both of them simultaneously showed a six nucleotides shorter transcript, in agreement with the RNA-Seq results on Case 4, as well as with a known splicing variant (e.g., NM_198389.2) of *PDPN* exon 5. Case 4 had an in-frame *LAMTOR1-PRKCD* fusion transcript in which all but four nucleotides of exon 1 of *LAMTOR1* (nt 201; NM_017907.2) were fused with the last three nucleotides of exon 10 of *PRKCD* (nt 1238; NM_006254.3; Fig. 2D). In Case 6, RNA-Seq had indicated the existence of an *RDH5-PRKCD* fusion transcript. These two genes are transcribed in opposite directions, but *CD63*, which on the genomic level is partly overlapping with *RDH5* on the opposite strand and is transcribed in the same direction as *PRKCD*, maps immediately telomeric to *RDH5*. Indeed, RT-PCR with a forward primer for *CD63* and reverse primers for *PRKCD* disclosed a fusion transcript joining the last coding nucleotide in exon 8 of *CD63* (nt 1238; NM_001257389.1) with the first nucleotide (nt 1011; NM_006254.3) of exon 9 of *PRKCD*. Inserted between the two genes, there was a 370 nt sequence that perfectly corresponded to the genomic sequence immediately following the last exon of *CD63*. In none of the four cases could the reciprocal fusion transcript be detected (data not shown).qPCR for the expression of *PRKCB* and *PRKCD* was performed on four BFHs and five cases each of solitary fibrous tumor and dermatofibrosarcoma protuberans (Table 1 and Supplementary Fig. S1). Both BFHs (Cases 2 and 5) with *PDPN-PRKCB* fusions showed high expression of the *PRKCB* gene, and the BFH with a *CD63-PRKCD* (Case 6) fusion showed high expression of *PRKCD*. The BFH with a *LAMTOR1-PRKCD* fusion (Case 4) showed moderately increased expression of *PRKCD*.

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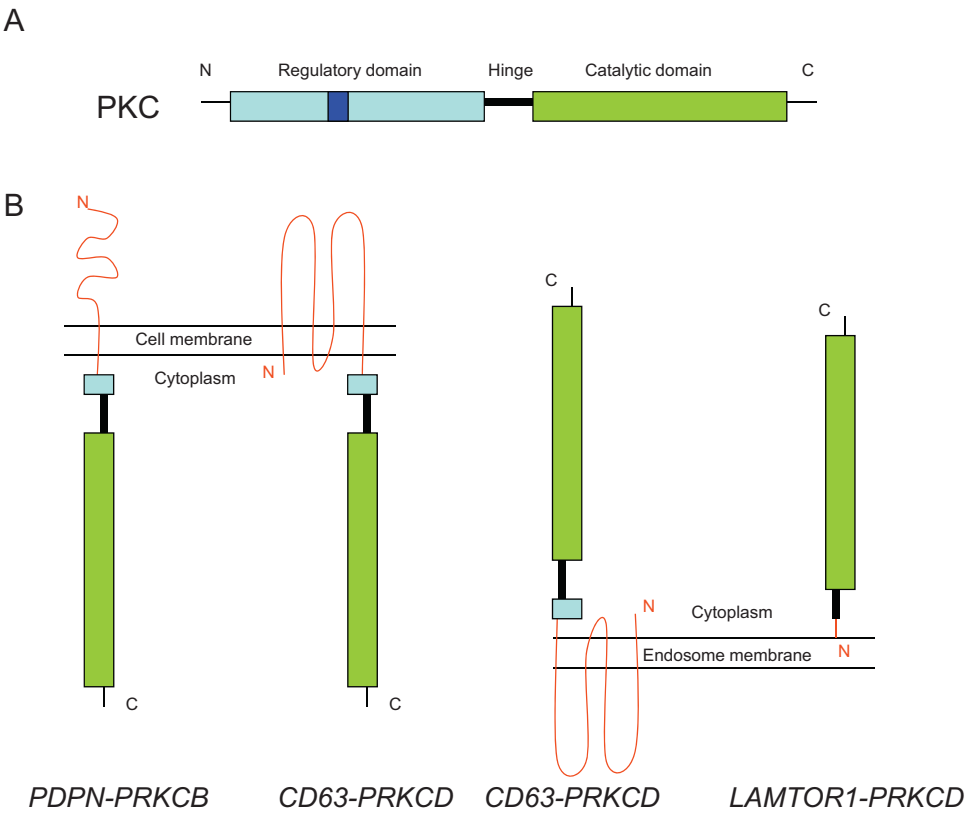

Fig. 3. Schematic of the structure of protein kinase C (PKC) proteins and of the predicted fusion proteins in benign fibrous histiocytoma (BFH). (A) PKC proteins are composed of an amino-terminal part, containing regulatory elements, that is linked through a hinge region to the carboxy-terminal, catalytic, serine–threonine kinase domain. Activation of the kinase requires several steps, including translocation from the cytoplasm to the cell membrane. (B) Three different gene fusions were detected: *PDPN-PRKCB*, *CD63-PRKCD* and *LAMTOR1-PRKCD*. All 5′-partner genes encode proteins localized to the cell membrane and/or endosome membrane. All predicted fusion proteins retain the amino-terminal part of the 5′-partner, which is critical for binding to membranes, to which the entire catalytic domain from a PKC protein is attached. Various portions of the hinge and regulatory domains are included in the predicted chimeric proteins.

Supplementary figure related to this article found, in the online version, at http://dx.doi.org/10.1016/j.biocel.2014.03.027.

4. Discussion

All tumors that could be investigated at the RNA level had gene fusions involving a PKC gene – *PRKCB* in two cases and *PRKCD* in two. The fusions were identified at the genomic level by chromosome banding, FISH and/or SNP array, as well as at the transcript level by RNA-Seq and/or RT-PCR, in all cases (Table 1 and Fig. 2). The PKCs form a multigene family encoding serine/threonine kinases (Nishizuka, 1989; Liu and Heckman, 1998; Breitkreutz et al., 2007). All proteins in the PKC family share a similar structure: an amino-terminal part, containing regulatory domains that are necessary for activation and recruitment to cellular membranes, is linked through a hinge region to a catalytic serine/threonine kinase domain in the carboxyl-terminal part. When stimulated, the proteins translocate from the cytoplasm to various subcellular locations, typically the plasma membrane, as active kinases (Liu and Heckman, 1998; Breitkreutz et al., 2007; Bosco et al., 2011).

The complex regulation and function of the PKC proteins can be exemplified by PRKCD. Several studies have shown that it in many cell types have pro-apoptotic properties, while in other cells the effects are clearly anti-apoptotic. To some extent, this could be explained by differences in phosphorylation patterns or cellular context, e.g., differences in subcellular locations or absence/presence of proteins with which it interacts (Sitailo et al., 2004; Steinberg, 2004; Jelacic and Linnekin, 2005; Okhrimenko et al., 2005). Of particular interest in the present context, caspase-dependent cleavage of the hinge region of the protein generates a constitutively active catalytic fragment. Experimental studies have shown that overexpression of only this fragment may have both pro- and anti-apopototic effects (DeVries et al., 2002; Okhrimenko et al., 2005).

Also PRKCB has been implicated in carcinogenesis (Herbst et al., 2007). For instance, the *PRKCB* gene has been identified as an important target of the EWSR1-FLI1 fusion protein in Ewing sarcoma, where it is among the top up-regulated genes. Specific silencing of *PRKCB* gene expression by shRNA or pharmacologically with enzastaurin, a selective PRKCB inhibitor, resulted in significant growth reduction of tumor cells in vitro as well as in vivo in SCID mice (Surdez et al., 2012).

The finding of increased expression of *PRKCB* and *PRKCD* mRNA in three of the four cases with gene fusions coupled with the finding of three different fusion partners could possibly suggest that the essential outcome of the fusions is transcriptional up-regulation of the catalytic domain of the PRKC proteins. However, as all three 5′-partners encode membrane-associated proteins, we find it more likely that it is the structure and subcellular localization of the chimeric protein that is pathogenetically important.

PDPN, encoding the 162 amino acid mucin-type transmembrane protein podoplanin, is expressed in a variety of cell types, as well as in soft tissue tumors and cancer-associated fibroblasts (Xu et al., 2011; Astarita et al., 2012; Krishnan et al., 2013). Expression is regulated by the transcription factor activator protein (AP-1), which mainly consists of FOS-JUN heterodimers (Durchdewald et al., 2008). It contains a small (nine amino acids) cytoplasmic region that is encoded by exons 5 and 6; exon 6 contributes only

four nucleotides to the translated mRNA (Martín-Villar et al., 2005; Fernández-Muñoz et al., 2011). PDPN has no known enzymatic activity but binds, e.g., ezrin and moesin proteins, thereby linking it to the actin cytoskeleton and implicating it in cell adhesion and motility. In the plasma membrane, PDPN preferentially localizes to so-called lipid rafts, specialized micro-domains that are enriched for cholesterol, sphingolipids and proteins, including tetraspanins (Astarita et al., 2012; Head et al., 2013). Lipid rafts are thought to be critical in transmitting extra-cellular signals to the cytoskeleton (Head et al., 2013).

Also LAMTOR1 (late endosomal/lysosomal adaptor, MAPK and MTOR activator 1, also known as p18) is predominantly found in lipid rafts, but in late endosome/lysosome membranes (Nada et al., 2009). It binds the cytosolic aspect of the endosome membrane through its amino-terminal part, where it is an essential anchoring protein for the p14-MP1 (also known as LAMTOR2-LAMTOR3) complex, which in turn serves as a scaffolding complex in the RAF/MEK/ERK and mTORC1 pathways (Nada et al., 2009; Magee and Cygler, 2011; Soma-Nagae et al., 2013). CD63 belongs to the large family of tetraspanins, proteins with four transmembrane domains and certain conserved amino acids (Pols and Klumperman, 2009). Tetraspanins organize laterally with themselves and with other transmembrane and intracellular proteins such as integrins and PKCs into so-called tetraspanin-enriched microdomains (TEMs); TEMs share certain features with lipid rafts, and are also involved in cytoskeletal reorganization (Hemler, 2005, 2008; Israels and McMillan-Ward, 2007). Like LAMTOR1, CD63 is predominantly found in late endosomes where it is involved in intra-cellular trafficking of other proteins (Pols and Klumperman, 2009).

All predicted fusion proteins – PDPN-PRKCB, CD63-PRKCD, and LAMTOR1-PRKCD – will thus result in fusion of the entire catalytic domain of a PKC with a membrane-associated protein that has retained its membrane-binding/-spanning part (Fig. 3). Removed from their inhibitory amino-terminal domains, it can be expected that the chimeric proteins will display constitutive serine/threonine kinase activity. While neither *PRKCB* or *PRKCD* nor any of the three 5′-partner genes involved in BFH has been described in gene fusions before, it can be noted that two fusions involving the PKC epsilon gene – *MBOAT2-PRKCE* and *MAP4K3-PRKCE* – were reported in one case each of breast and lung cancer, respectively (Stephens et al., 2009; Seo et al., 2012). The pathogenetic significance of these fusions was not investigated further, but it could be noted that also *MBOAT2* encodes a membrane-associated protein, namely membrane-bound *o*-acyl transferase. This provides further support for the notion that translocation of the catalytic domain from a PKC to a novel membranous location is of pathogenetic importance. The finding of recurrent gene fusions on the basis of somatic chromosome aberrations also shows that BFH is not the result of a reactive process and thus settles the dispute about whether BFH is a true neoplasm or not (Calonje, 2000; Hui et al., 2002; Weiss and Goldblum, 2008). Further studies of cutaneous, visceral and skeletal BFHs are necessary to find out whether all BFHs, irrespective of anatomic location, have fusions involving PKC genes. Of particular interest are the rare metastasizing variants of BFH (Doyle and Fletcher, 2013), as clinically available PKC inhibitors could be of potential therapeutic benefit (Herbst et al., 2007; Surdez et al., 2012).

Conflict of interest

There are no potential conflicts of interest or competing interests known to the authors of this paper.

Authors' contributions

AP carried out PCR analyses and drafted the manuscript. JN and LM carried out SNP and FISH studies. OB, OL, FVvS, and HAD contributed material and clinicopathologic data. HL, TF, and JT carried out the RNA-Seq analyses. NM and KHN contributed cytogenetic and molecular genetic expertise. FM conceived the study and assisted in drafting the manuscript. All authors have read and approved the final manuscript.

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