Alternative *PDGFD* rearrangements in dermatofibrosarcomas protuberans without *PDGFB* fusions

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Abstract

Dermatofibrosarcoma protuberans is underlined by recurrent *collagen type I alpha 1 chain-platelet-derived growth factor B chain (COL1A1-PDGFB)* fusions but ~4% of typical dermatofibrosarcoma protuberans remain negative for this translocation in routine molecular screening. We investigated a series of 21 cases not associated with the pathognomonic *COL1A1-PDGFB* fusion on routine fluorescence in situ hybridization (FISH) testing. All cases displayed morphological and clinical features consistent with the diagnosis of dermatofibrosarcoma protuberans. RNA-sequencing analysis was successful in 20 cases. The classical *COL1A1-PDGFB* fusion was present in 40% of cases ($n = 8/20$), and subsequently confirmed with a *COL1A1* break-apart FISH probe in all but one case ($n = 7/8$). 55% of cases ($n = 11/20$) displayed novel *PDGFD* rearrangements; *PDGFD* being fused either to the 5' part of *COL6A3* (2q37.3) ($n = 9/11$) or *EMILIN2* (18p11) ($n = 2/11$). All rearrangements led to in-frame fusion transcripts and were confirmed at genomic level by FISH and/or array-comparative genomic hybridization. *PDGFD*-rearranged dermatofibrosarcoma protuberans presented clinical outcomes similar to typical dermatofibrosarcoma protuberans. Notably, the two *EMILIN2-PDGFD* cases displayed fibrosarcomatous transformation and homozygous deletions of *CDKN2A* at genomic level. We report the first recurrent molecular variant of dermatofibrosarcoma protuberans involving *PDGFD*, which functionally mimic bona fide *COL1A1-PDGFB* fusions, leading presumably to a similar autocrine loop-stimulating PDGFRB. This study also emphasizes that *COL1A1-PDGFB* fusions can be cytogenetically cryptic on FISH testing in a subset of cases, thereby representing a diagnostic pitfall that pathologists should be aware of.

Introduction

Dermatofibrosarcoma protuberans is a superficial and locally invasive mesenchymal tumor, which undergoes fibrosarcomatous transformation, portending a metastatic potential in 10% of cases [1]. Giant cell fibroblastoma represents a morphological variant of dermatofibrosarcoma protuberans occurring in children. Both dermatofibrosarcoma protuberans and giant cell fibroblastoma are molecularly defined by the presence of translocation (17;22)(q21.3;q13.1), which fuses *collagen type I alpha 1 chain (COL1A1)* with *platelet-derived growth factor B chain (PDGFB)* [2]. Most dermatofibrosarcomas protuberans display typical morphological features including a dense storiform proliferation of cells

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expressing diffusely and strongly CD34 [1]. Fibrous morphological variants of dermatofibrosarcoma protuberans may be confused with benign cellular fibrous histiocytomas or dermatofibromas [1]. A diagnosis of dermatofibrosarcoma protuberans warrants complete surgical excision that may require large resection owing to the propensity of dermatofibrosarcoma protuberans to infiltrate adjacent tissues [3]. Imatinib and pazopanib therapies, which target PDGF downstream signaling, may be used in unresectable or metastatic cases [3, 4]. Molecular confirmation of dermatofibrosarcoma protuberans is therefore strongly recommended. *COL1A1-PDGFB* fusion gene is mostly screened with dual fusion or break-apart fluorescence in situ hybridization (FISH) in routine practice [5]. Reverse transcription polymerase chain reaction (RT-PCR) assays are slightly less sensitive than FISH owing to the variability of *COL1A1* breakpoints and require non-degraded RNA, although some studies report comparable sensitivity [6, 7]. Cytogenetic studies have shown that t(17;22) is mostly unbalanced, associated with supernumerary ring chromosomes, and may therefore be evidenced by array-comparative genomic hybridization [8, 9]. Altogether, routine molecular screening remains negative in ~4% of otherwise typical dermatofibrosarcomas protuberans, raising clinical uncertainties and leading to render a diagnosis of dermatofibrosarcoma protuberans lacking the canonical translocation and therefore “molecularly unconfirmed” [5]. Single reports of molecular variants of dermatofibrosarcoma protuberans have been reported but no systematic study has been performed so far [10–13].

In order to identify novel fusion genes in dermatofibrosarcoma protuberans, we investigated by RNA-sequencing and array-comparative genomic hybridization a series of 21 morphologically typical dermatofibrosarcomas protuberans, negative for *COL1A1-PDGFB* fusion on routine molecular testing.

Materials and methods

Sample selection

We identified 25 cases in our records diagnosed as dermatofibrosarcoma protuberans between 2006 and 2017 but negative on molecular testing. Cases were retrieved from the archives of the department of Genetics of University Hospital of Nice (Nice, France), and departments of Pathology of Centre Léon Bérard (Lyon, France) and Institut Bergonié (Bordeaux, France). Notably, all three participating institutions used FISH for molecular testing, but they applied two different screening strategies. First, *COL1A1-PDGFB* fusion probe followed by *PDGFB* break-

apart probe in case of negative or equivocal result. Second, a screening with *PDGFB* break-apart probe in the first place, followed if negative or ambiguous by a screening with a *COL1A1* break-apart probe. Owing to the evolution of molecular testing strategies over time, seven cases of our series had not been previously tested with a *PDGFB* break-apart probe. Hence, FISH analysis was completed in these cases, rendering positive results in four cases, which were therefore removed from the final cohort ($n = 21$).

All cases from the series were registered in the RREPS sarcoma database, a national clinical database approved by the Commission Nationale Informatique et Libertés (CNIL) and the Comité national d'éthique.

The material used in this publication was provided by the SarcomaBCB, the Conticanet database (<https://auth.sacomabcb.org>).

Clinical review

Clinical follow-up was obtained from the medical records of patients either available at our institutions or through corresponding clinicians. Follow-up duration was calculated from the date of the clinical detection of the lesion. Clinical data are provided in Supplementary Table 1.

Histopathological analyses

All cases of the final series were reviewed blindly by soft tissue expert pathologists (FLL, JMC, DRV). Fibrosarcomatous transformation was defined by the presence of a fascicular component with increased mitotic activity contrasting with the storiform pattern of the DFSP component [1]. All samples were studied with a panel of antibodies available in routine including AE1/E3, CD34, S100 protein, EMA, and Ki67. Pathological data are summarized in Supplementary Table 1.

FISH analyses

FISH analyses were performed on formalin-fixed paraffin-embedded tissue sections using commercial and custom bacterial artificial chromosome (BAC) probes. Commercial FISH probes used in the study included dual fusion probe *COL1A1/PDGFB* (#Z-2116-200, Zytovision, Bremerhaven, Germany), *PDGFB* Break-Apart probe (#Z-2119-200, Zytovision), and *COL1A1* Break Apart Probe (#Z-2121-200, Zytovision). *PDGFD*, *EMILIN2*, and *COL6A3* break-apart probes were prepared with BAC listed in Supplementary Table 2. FISH analysis was performed by assessing at least 100 non-overlapping intact nuclei by two independent operators. The positive threshold to call the FISH assay positive was 15%. BACs were cultured and labeled as previously described [14].

Table 1 Clinical and molecular features of the cohort of dermatofibrosarcoma protuberans variants

Case number	Age (years)/gender	Location	Diagnosis	RNA Sequencing	Array-comparative genomic hybridization	FISH validation	RT-PCR validation
1	33/M	Abdominal wall	Dermatofibrosarcoma protuberans	<i>COL6A3-PDGFD</i> fusion	<i>COL6A3</i> breakpoint: 3' <i>COL6A3</i> heterozygous deletion	Split <i>PDGFD</i> : +; Split <i>COL6A3</i> : +	<i>COL6A3-PDGFD</i> fusion
2	38/F	Breast	Dermatofibrosarcoma protuberans	<i>COL6A3-PDGFD</i> fusion	<i>PDGFD</i> breakpoint: <i>PDGFD</i> intragenic deletion	Split <i>PDGFD</i> : +; Split <i>COL6A3</i> : +	<i>COL6A3-PDGFD</i> fusion
3	38/F	Chest wall	Dermatofibrosarcoma protuberans	<i>COL6A3-PDGFD</i> fusion	<i>PDGFD</i> breakpoint: <i>PDGFD</i> intragenic deletion	Split <i>PDGFD</i> : +; Split <i>COL6A3</i> : +	<i>COL6A3-PDGFD</i> fusion
4	48/F	Chest wall	Dermatofibrosarcoma protuberans	<i>COL6A3-PDGFD</i> fusion	<i>COL6A3</i> breakpoint: 3' <i>COL6A3</i> heterozygous deletion; <i>PDGFD</i> breakpoint: <i>PDGFD</i> intragenic deletion	Split <i>PDGFD</i> : Failure; Split <i>COL6A3</i> : Failure	<i>COL6A3-PDGFD</i> fusion
5	33/F	Shoulder	Dermatofibrosarcoma protuberans	<i>COL6A3-PDGFD</i> fusion	<i>COL6A3</i> breakpoint: 3' <i>COL6A3</i> heterozygous deletion; <i>PDGFD</i> breakpoint: 5' <i>PDGFD</i> heterozygous deletion	ND	<i>COL6A3-PDGFD</i> fusion
6	39/F	Back	Dermatofibrosarcoma protuberans	<i>COL6A3-PDGFD</i> fusion	<i>COL6A3</i> breakpoint: 3' <i>COL6A3</i> heterozygous deletion; <i>PDGFD</i> breakpoint: 5' <i>PDGFD</i> heterozygous deletion	Split <i>PDGFD</i> : +; Split <i>COL6A3</i> : +	<i>COL6A3-PDGFD</i> fusion
7	29/F	Breast	Dermatofibrosarcoma protuberans	<i>COL6A3-PDGFD</i> fusion	Failure	Split <i>PDGFD</i> : +; Split <i>COL6A3</i> : +	<i>COL6A3-PDGFD</i> fusion
8	41/F	Chest wall	Dermatofibrosarcoma protuberans	<i>COL6A3-PDGFD</i> fusion	ND	Split <i>PDGFD</i> : +; Split <i>COL6A3</i> : Failure	<i>COL6A3-PDGFD</i> fusion
9	21/F	Leg	Fibrosarcoma	<i>EMILIN2-PDGFD</i> fusion <i>COL5A1-UBAP1L</i> fusion	No breakpoint within <i>PDGFD</i> or <i>EMILIN2</i>	Split <i>PDGFD</i> : +; Split <i>EMILIN2</i> : +	<i>EMILIN2-PDGFD</i> fusion
10	50/M	Leg	Fibrosarcoma	<i>EMILIN2-PDGFD</i> fusion	<i>PDGFD</i> breakpoint: <i>PDGFD</i> intragenic deletion	Split <i>PDGFD</i> : +; Split <i>EMILIN2</i> : +	<i>EMILIN2-PDGFD</i> fusion
11	64/M	Back	Atypical dermatofibrosarcoma protuberans	<i>COL1A1-PDGFB</i> fusion	<i>COL1A1</i> breakpoint: 5' <i>COL1A1</i> gain	Split <i>COL1A1</i> : +	
12	24/M	Thigh	Fibrosarcoma	<i>COL1A1-PDGFB</i> fusion	<i>COL1A1</i> breakpoint: 5' <i>COL1A1</i> gain	Split <i>COL1A1</i> : +	
13	43/F	Thigh	Dermatofibrosarcoma protuberans	<i>COL1A1-PDGFB</i> fusion	<i>COL1A1</i> breakpoint: 5' <i>COL1A1</i> gain	Split <i>COL1A1</i> : +	
14	40/M	Forehead	Dermatofibrosarcoma protuberans	<i>COL1A1-PDGFB</i> fusion	<i>COL1A1</i> breakpoint: 5' <i>COL1A1</i> gain	Split <i>COL1A1</i> : +	
15	37/F	Cheek	Dermatofibrosarcoma protuberans	<i>COL1A1-PDGFB</i> fusion	<i>COL1A1</i> breakpoint: 5' <i>COL1A1</i> gain	Split <i>COL1A1</i> : +	
16	38/M	Neck	Dermatofibrosarcoma protuberans	<i>COL1A1-PDGFB</i> fusion	ND	Split <i>COL1A1</i> : +	
17	73/F	Arm	Dermatofibrosarcoma protuberans	<i>COL1A1-PDGFB</i> fusion	ND	Split <i>COL1A1</i> : +	
18	29/F	Shoulder	Fibrosarcoma	<i>COL1A1-PDGFB</i> fusion	ND	Split <i>COL1A1</i> : -	
19	29/F	Breast	Dermatofibrosarcoma protuberans	<i>COL6A3-PDGFD</i> fusion (frozen)	<i>COL6A3</i> breakpoint: 5' <i>COL6A3</i> gain	Split <i>PDGFD</i> : +; Split <i>COL6A3</i> : +	<i>COL6A3-PDGFD</i> fusion
20	4/M	Calf	Pigmented dermatofibrosarcoma protuberans	No fusion transcript	No breakpoint	Split <i>PDGFD</i> : -; Split <i>COL6A3</i> : -; Split <i>EMILIN2</i> : -	

M male, *F* female, *RNA sequencing* ribonucleic acid sequencing, *FISH* fluorescence in situ hybridization, *ND* not done

Array-comparative genomic hybridization analyses

Genomic DNA was extracted from formalin-fixed paraffin-embedded tissue using QIAamp, DNA micro kit (Qiagen, Hilden, Germany). Genomic DNA and human reference DNA (Promega) were labeled with cyanin 5 (Cy5) and cyanine 3 (Cy3), respectively, using the Genomic DNA High-Throughput ULS Labeling Kit (Agilent Technologies, Santa Clara, California) and co-hybridized onto a Sureprint G3 Human CGH microarray 4 × 180K (Agilent) following manufacturer's recommendations. Data were analyzed by Agilent Genomic Workbench software v7.0 or by Cytogenomics software (v2.9.2.4, Agilent) and expressed according to the human reference hg19 (GRCh37, Genome Reference Consortium Human Reference 36). The identification of aberrant copy number segments was based on ADM-2 segmentation algorithm with a threshold of 6.0.

RNA-sequencing analyses

RNA sequencing was performed with formalin-fixed paraffin-embedded material in all cases. Total RNA was extracted from formalin-fixed paraffin-embedded tissue section using Trizol reagent (Thermo Fisher Scientific, Courtaboeuf, France) following manufacturers' recommendations. DNA was removed using RNase-free DNase set (Qiagen) followed by second Trizol extraction. Quantity and quality of total RNA were evaluated using NanoDrop (Thermo Fisher Scientific) and Tape Station with Hs RNA Screen Tape (Agilent) using a cutoff of DV₂₀₀ (defined as the percentage of RNA fragments above 200 nucleotides) above 13%. All samples passed quality criteria. Libraries were prepared with 100 ng of total RNA using TruSeq RNA Access Library Prep Kit (Illumina, San Diego, USA). Libraries were pooled by group of 14 samples at 4 nM with 1% PhiX. Sequencing was performed (75 cycles paired end) using a NextSeq 500/550 High Output V2 kit on Illumina NextSeq 500 platform (Illumina, San Diego, CA). RNA-sequencing was successful in 20 out of 21 cases. The case with failure was ruled out from the final series as no material was available to complete its characterization by array-comparative genomic hybridization and FISH (final series $n = 20$, Table 1).

Sequencing data (up to 15 million reads per sample) were analyzed with BaseSpace sequence Hub (Illumina). Reads were aligned with STAR and TopHat2 on GRCh38 reference genome. The fusion transcripts were called with Manta, STAR-Fusion, FusionMap and TopHat2 fusion and validated if the Manta score was up to 0.7 and present in fusion list with TopHat2 fusion and/or STAR-Fusion and/or FusionMap [15–17].

To perform the clustering analysis, gene expression values were extracted using Kallisto v0.42.5 tool [18] with GENCODE release 23 genome annotation based on GRCh38 genome reference. Kallisto TPM expression values were transformed in $\log_2(\text{TPM} + 2)$ and all samples were normalized together using the quantile method from the R limma package within R (version 3.1.1) environment. Clustering was performed with the R package Cluster v2.0.3 using Pearson correlation distance and Ward's clustering method. Significance of clusters was assessed using SigClust as previously described [19].

RT-PCR and Sanger sequencing

In 11 cases, we performed reverse transcriptase-polymerase chain reaction (RT-PCR) to confirm either the *COL6A3-PDGFD* fusion transcript or the *EMILIN2-PDGFD* fusion transcript, using the following custom primers (designed using Primer 3 program): *COL6A3* forward primer 5'-GCAAGGTCAGCTTCTAGTTCA-3', *PDGFD* reverse primer 5'-TGGCCAACTTCAGCTCTTCT-3' (in case number 1, 2, 4, 5, 6, 7, 8, 19), *COL6A3* forward primer 5'-CAGCAAGGTCAGCTTCTAGTT-3' and *PDGFD* reverse primer 5'-CAGTTCCACAGCCACAATTTC-3' (in case number 3) and *EMILIN2* forward primer 5'-GCCACGCTTCCAGATTCTTA-3' and *PDGFD* reverse primer 5'-CAGTTCCACAGCCACAATTTC-3' (in case number 9, 10). Fusion transcripts were sequenced by Sanger after extraction and enzymatic purification of RT-PCR product using High pure PCR product purification kit (Roche Diagnostics, Meylan, France).

Results

Clinicopathological features

All cases were located in classical anatomical sites including trunk ($n = 11/20$), limbs ($n = 6/20$), and head and neck ($n = 3/20$) (Table 1). Patients' age ranged from 4 to 73 years old. Clinically, lesions presented with a classical scar-like appearance ($n = 2$), slow-growing cutaneous plaques ($n = 1$) or cutaneous plaques with red discoloration ($n = 2$). In addition, one case had a multinodular presentation. All tumors displayed outcomes in line with the local malignant potential of dermatofibrosarcoma protuberans with late local recurrences occurring up to 12 years after initial resection (Supplementary Table 1). All cases presented morphological features either typical of dermatofibrosarcoma protuberans ($n = 12/20$) (Figs. 1 and 2) or consistent with a morphological variant of dermatofibrosarcoma protuberans including fibrous pattern ($n = 5/20$), pigmented pattern

Fig. 1 Cryptic *COL1A1*-*PDGFB* fusion in dermatofibrosarcoma protuberans. **a–b** Band-like infiltration of hypodermis and subcutaneous adipose tissue (case number 15, HES staining, $\times 2.5$ and $\times 6.5$ magnifications, respectively) **c** Storiform proliferation infiltrating hypodermis with a honeycomb pattern (HES, $\times 200$ magnification). **d–e** High-power field view of tumor cell nuclei displaying ovoid shape and monomorphism **d** and focal interspersed multinucleated cells **e** (HES, $\times 200$ magnification) **f** Fluorescence in situ hybridization (FISH) using

COL1A1 break-apart probe. FISH analysis shows an unbalanced translocation with a gain of the 5' part of *COL1A1* (probe labeled in red). **g** Array-comparative genomic hybridization profile harbors a genomic breakpoint at *COL1A1* locus with a 5' *COL1A1* gain but no breakpoint at *PDGFB* locus (chromosome 22 view not shown). Additional copy number alterations include gains of 4q28-q35, 6p, and whole chromosome 7

(also referred to as Bednar tumor) ($n = 1/20$) or hybrid Bednar/dermatofibrosarcoma protuberans variant with focal presence of pigments or giant cells ($n = 2/20$) (Supplementary Table 1). Four cases displayed fibrosarcomatous transformation (Fig. 3). All but two cases showed classical honeycomb infiltrative borders. Five cases were deeply located in hypodermis without dermal infiltration.

RNA sequencing

Classical *COL1A1*-*PDGFB* fusions were found in eight cases ($n = 8/20$). *PDGFB* breakpoint was constantly found in exon 2, whereas breakpoints in *COL1A1* varied from exons 11 to 45 (Supplementary Table 3).

PDGFB rearrangements were found in 11 cases ($n = 11/20$), *PDGFB* being fused either to collagen type VI alpha 3 chain (*COL6A3*) at 2q37 ($n = 9/11$) or elastin microfibril Interface 2 (*EMILIN2*) at 18p11 ($n = 2/11$). Breakpoints in *COL6A3* involved either exon 42 ($n = 8/9$) or exon 43 ($n = 1/9$), whereas breakpoint in *PDGFB* constantly involved exon 6 (Fig. 2) and breakpoints in *EMILIN2* were located within exon 4 (Fig. 3).

One case (case number 20) failed to show a fusion transcript by RNA-sequencing with the different algorithms.

FISH and RT-PCR

COL6A3-*PDGFB* transcript and *EMILIN2*-*PDGFB* transcript were confirmed by RT-PCR in all cases (case number 1–10 and 19). Regarding cases with *COL1A1*-*PDGFB* fusions, FISH analyses were completed with a *COL1A1* break-apart probe, which was positive in 7/8 cases (Fig. 1). Regarding cases with *PDGFB* fusions, FISH analyses were carried out with *PDGFB* break-apart probe, which was positive in nine cases out of 11 ($n = 9/11$). Gene partners fused to *PDGFB* were also assessed with *COL6A3* and *EMILIN2* break-apart probes (Figs. 2 and 3, respectively), showing rearrangements in 5/7 and 2/2 cases, respectively (Supplementary Table 3).

Array-comparative genomic hybridization

On profiles, all cases with cryptic *COL1A1*-*PDGFB* highlighted genomic breakpoints within *COL1A1* with a genomic gain in flanking region 17q21.33-qter ($n = 5/5$). Additional non-recurrent copy number alterations were present in all cases. Genomic gains were seen in 1q21-q25.3, 4q28-q35, 6p, 7pter, 22q11 in one case each. Whole-chromosome gains involved chromosomes 6, 7, 10, and

Fig. 2 *COL6A3-PDGFD* dermatofibrosarcoma protuberans. **a** Biopsy specimen highlighting deep-seated dense nodular proliferation (case number 6, HES staining, $\times 20$ magnification). **b** Storiform proliferation with honeycomb infiltrative pattern typical of dermatofibrosarcoma protuberans (HES, $\times 200$). **c** Fibrous stromal changes focally interposed between tumor cells (HES, $\times 250$) **d** Tumor cells display ovoid monomorphic nuclei (HES, $\times 400$). **e** Array-comparative genomic hybridization profile showing deletions and breakpoints involving *COL6A3* and *PDGFD*. Additional copy number alteration includes

gain of chromosome 12. **f–g** Fluorescence in situ hybridization (FISH) using *COL6A3* break-apart probe **f** and *PDGFD* break-apart probe **g** showing an unbalanced rearrangement with loss of the 3' *COL6A3* and the 5' *PDGFD*, respectively. **h** Structure of *COL6A3-PDGFD* fusion transcript. From top to bottom: scheme representing locus and chromosomal position of *COL6A3* and *PDGFD*; schematic of breakpoints involving *COL6A3* exon 42 and *PDGFD* exon 6; nucleotide sequence of adjointed sequences

Fig. 3 *EMILIN2-PDGFD* dermatofibrosarcoma protuberans. **a** Biopsy specimen highlighting densely cellular tumor infiltrating hypodermis (case number 9, HES staining, $\times 2$ magnification). **b** Focal collagenized stroma arranged in elongated fascicles suspicious for fibrosarcomatous transformation ($\times 200$ magnification). **c** Tumor cells display ovoid monomorphic nuclei embedded in a collagenous stroma ($\times 400$ magnification). **d** CD34 staining in this case ($\times 250$ magnification). **e** Array-comparative genomic hybridization profile showing a homozygous deletion of *CDKN2A* (P16) but no breakpoint within

EMILIN2 or *PDGFD* loci. **f–g** Fluorescence in situ hybridization (FISH) using *EMILIN2* break-apart probe **f** and *PDGFD* break-apart probe **g** showing a rearrangement of the *EMILIN2* and *PDGFD* loci, respectively. **h** Structure of *EMILIN2-PDGFD* fusion. From top to bottom scheme representing locus and chromosomal position of *EMILIN2* and *PDGFD*; schematic of breakpoints positions which constantly occurred in exon 4 of *EMILIN2* and in exon 6 of *PDGFD*; nucleotide sequence of adjoined sequences

genomic loss were seen in 1q22–23 6q12–q26 and 8p in one case each (Supplementary Table 3).

Array-comparative genomic hybridization analysis of the dermatofibrosarcomas protuberans with *COL6A3-PDGFD*

fusion highlighted no breakpoint within *PDGFB* nor *COL1A1* but within *COL6A3* and *PDGFD* in 4/7 and 6/8 cases, respectively. Copy number alterations were present in the regions flanking the breakpoints in all but one case ($n =$

Fig. 4 Transcriptomic classification of dermatofibrosarcoma protuberans. Unsupervised hierarchical clustering, using the top 10% most variant genes based on interquartile range, comparing *PDGFB*-rearranged dermatofibrosarcoma protuberans ($n = 8$, including 3 “conventional” dermatofibrosarcomas protuberans positive for *PDGFB* rearrangement using FISH analysis), *PDGFD*-rearranged dermatofibrosarcoma protuberans ($n = 9$), infantile fibrosarcomas ($n = 7$), clear cell sarcomas of soft tissue (superficially seated) ($n = 2$), *ALK*-

rearranged Spitzoid neoplasms ($n = 5$), and *NTRK1/3*-rearranged Spitzoid neoplasms ($n = 7$). Branches depicted with colors within the dendrogram were found significant (p value $< 10e-5$) by SigClust clustering significance assessment. Samples are listed in Table 1 and Supplementary Table 4. Infantile fibrosarcomas are highlighted in purple, low grade fibromyxoid sarcomas in orange, spitzoid neoplasms in yellow, clear cell sarcomas in light red, and dermatofibrosarcoma protuberans in red

6/7). Additional non-recurrent copy number alterations included gain of chromosome 12 ($n = 1/7$), loss of 3q ($n = 1/7$), gains of chromosomes 1, 7, and 17 ($n = 1/7$). The genomic profiles of the two cases of *EMILIN2-PDGFD* dermatofibrosarcoma protuberans displayed breakpoints within *PDGFD* ($n = 1/2$), without copy number alteration involving *EMILIN2*. Interestingly, both profiles presented homozygous deletion of *CDKN2A* locus ($n = 2/2$). None of these cases displayed copy number alterations in the genomic regions flanking the breakpoints.

Clustering analysis

To assess whether *PDGFD*-rearranged dermatofibrosarcoma protuberans were biologically similar to *PDGFB*-rearranged dermatofibrosarcoma protuberans, we compared the RNA-seq expression profiles of samples of our cohort (including five *PDGFB*- and nine *PDGFD*-rearranged dermatofibrosarcoma protuberans) to other spindle cutaneous neoplasms including infantile fibrosarcomas ($n = 7$), clear cell sarcomas of soft tissue ($n = 2$), *ALK*-rearranged Spitzoid neoplasms ($n = 5$), *NTRK1/3*-rearranged Spitzoid neoplasms ($n = 7$) and conventional *PDGFB*-rearranged dermatofibrosarcomas protuberans positive for gene rearrangement upon routine molecular screening ($n = 3$) (Supplementary Table 4). All cases

clustered together with transcriptional profiles distinct from *ALK*- and *NTRK1/3*-rearranged Spitzoid proliferations and infantile fibrosarcomas. The dermatofibrosarcoma protuberans cluster lumped together all dermatofibrosarcoma protuberans including those with *PDGFB* fusions (both cryptic and overt rearrangements) and *PDGFD* fusions (Fig. 4).

Discussion

Dermatofibrosarcoma protuberans has historically been defined by the presence of recurrent *COL1A1-PDGFB* fusions, which lead to the upregulation of PDGFRB signaling through an autocrine activating loop [2,20–22]. However, ~4% of dermatofibrosarcoma protuberans prove negative for the translocation upon routine FISH testing [5].

We evidenced by RNA-sequencing that molecularly unconfirmed dermatofibrosarcoma protuberans displayed “cryptic” *COL1A1-PDGFB* fusions in 40% of cases ($n = 8/20$). All dermatofibrosarcomas protuberans associated with “cryptic” fusions involved previously reported *COL1A1* breakpoints, which position have been shown to vary considerably from exon 7 to exon 49. Interestingly, all cases displayed visible genomic breakpoints within *COL1A1* on quantitative genomic profiles along with and/or gains of the

17q locus flanking the breakpoint ($n = 5/5$) (Fig. 1). Notably, subsequent FISH analyses with *COL1A1* break-apart probe were positive in all but one case ($n = 7/8$). Altogether, our findings support that array-comparative genomic hybridization or screening with *COL1A1* break-apart probe may support the diagnosis of cryptic dermatofibrosarcoma protuberans. This complementary screening strategy remains cost-effective as compared with the cost of RNA-sequencing performed in all cases suspicious of dermatofibrosarcoma protuberans. Cryptic rearrangements have also been reported in other translocation-related sarcomas and are thought to be related to structural variations of the translocation or induction of neo-exons secondary to splicing variations [23–27].

Second, we report herein that 55% ($n = 11/20$) of cytogenetically negative- dermatofibrosarcoma protuberans are associated with alternative rearrangements involving *PDGFD*. Two different types of fusion transcripts were evidenced with the 3' part of *PDGFD* being fused to the 5' part either of *COL6A3* ($n = 9/11$) or *EMILIN2* ($n = 2/11$). *PDGFD*, located at 11q22.3, encodes a protein belonging to the same family of *platelet-derived growth factor* than *PDGFB* [28]. *PDGFD* is able to bind to PDGF receptor B (PDGFRB) [29, 30]. *PDGFD* has oncogenic properties through promotion of cell proliferation and angiogenesis [28] and its overexpression has been linked to a variety of malignancies [31–34]. The breakpoint was constantly located within exon 6, preserving the binding domain to PDGF receptors in a similar manner than translocations involving *PDGFB*. Interestingly, the loss of exon 6 have been shown to induce truncation owing to a premature stop codon in mice, therefore a breakpoint located downstream exon 6 might jeopardize *PDGFD* signaling [35]. *COL6A3*, located at 2q37.3, encodes a protein of extracellular matrix involved in cellular adhesion belonging to the same superfamily as *COL1A1* [36]. *COL6A3* contains 3152 amino acids-long alpha 3 chains, which have the same sequence as alpha 1 chains and harbor the same triple-helical and von Willebrand factor A domains [36]. Notably, somatic rearrangements of *COL6A3* have been previously reported in tenosynovial giant cell tumors in which *COL6A3* is fused to colony stimulating factor 1 (CSF1) [37, 38]. In addition, overexpression of *COL6A3* has been described in a wide array of malignancies including gastric [39], ovarian [40], colorectal [41, 42], and pancreatic cancers [43]. It has also been correlated to resistance to chemotherapy [40, 44]. The breakpoint occurred in exon 42 of *COL6A3* in all but one case ($n = 8/9$), in contrast to the frequent variations seen in *COL1A1*. All *COL6A3-PDGFD* cases displayed genomic imbalances in regions flanking the breakpoints of *COL6A3* and/or *PDGFD* and three cases displayed additional copy number alterations ($n = 3/7$). The morphological and clinical features of *COL6A3*-rearranged dermatofibrosarcoma

protuberans did not differ from those seen in bona fide dermatofibrosarcoma protuberans. The second fusion partner *EMILIN2* was involved in 2/11 cases a. *EMILIN2*, located at 18p11.32–p11.31, encodes a glycoprotein related to the superfamily of collagen, which contains collagen-like domains [36]. *EMILIN2* assembles into multimers, involved in the composition of extracellular matrix [45, 46]. The genomic breakpoints were located constantly in exons 4 and 6 of *EMILIN2* and *PDGFD*, respectively. The fusion preserved preserves key structural domains of *EMILIN2*, including EMI domain, coiled-coil structures and leucine zippers and gC1q domain [45, 46]. *EMILIN2-PDGFD* dermatofibrosarcoma protuberans displayed homozygous deletion of *CDKN2A* in both cases ($n = 2/2$). Interestingly, both cases displayed fibrosarcomatous transformation and were deep-seated within hypodermis without dermal connection. It is notable that homozygous deletions of *CDKN2A* are linked to malignancy in varied tumor types including mesenchymal, melanocytic, and epithelial neoplasms [47–49]. Therefore, this alteration may portend an increased malignant potential in this subset of dermatofibrosarcoma protuberans, which warrants further studies on larger cohorts with longer follow-up to assess whether the behavior of cases of *EMILIN2-PDGFD* dermatofibrosarcoma protuberans is similar or not to classical and *COL6A3-PDGFD*-associated cases.

Altogether, *PDGFD* rearrangements may functionally mimic the biology of *COL1A1-PDGFB* fusions as it involves functionally related genes and display a similar structure. Rearrangements might therefore be targeted by imatinib as classical dermatofibrosarcoma protuberans.

In conclusion, we report herein new and recurrent molecular variants of dermatofibrosarcoma protuberans underlined by *PDGFD* rearrangements, which are clinically, morphologically and molecularly indistinguishable from *COL1A1-PDGFB* dermatofibrosarcoma protuberans. Furthermore, we showed that roughly half of cases of dermatofibrosarcoma protuberans negative upon routine FISH screening are associated with cryptic *COL1A1-PDGFB* rearrangements that may be identified by array-comparative genomic hybridization or FISH with a *COL1A1* break-apart probe. The functional similarities between the classical *COL1A1-PDGFB* and the alternative *COL6A3-PDGFD* or *EMILIN2-PDGFD* transcripts suggest they activate an analogous oncogenic autocrine loop involving PDGFRB signaling.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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