Novel TNC-PDGFD fusion in fibrosarcomatous dermatofibrosarcoma protuberans: a case report

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Abstract

Background: Dermatofibrosarcoma protuberans (DFSP) is a superficial fibroblastic tumor characterized by high rate of local recurrence and low metastatic potential. Fibrosarcomatous transformation can rarely arise in DFSP either de novo or as recurrent, which represents a form of tumor progression and carries an increased risk of metastasis over classic DFSP. Cytogenetically, DFSP is characterized by a recurrent unbalanced chromosome translocation t (17; 22)(q22;q13), leading to the formation of COL1A1-PDGFB fusion transcript that is present in more than 90% of cases. Alternative fusions involving the PDGFD with partners of COL6A3 or EMILIN2 have recently been documented in less than 2% of cases. Herein, we report a DFSP with fibrosarcomatous morphology harboring a novel TNC-PDGFD fusion.

Case presentation: A 54-year-old female presented with a slowly growing mass in the right thigh. Excision demonstrated a 2-cm ovoid, well-circumscribed, gray-white, mass. Microscopic examination revealed a partially encapsulated subcutaneous nodule without dermal connection. The neoplasm was composed of cellular and fairly uniform spindle cells with brisk mitoses, arranged in elongated fascicles and herringbone patterns, with focal collagenized stroma. The neoplastic cells were positive for CD34 and smooth muscle actin. Fluorescence in-situ hybridization analyses showed negative for COL1A1-PDGFB fusion as well as NTRK1/2/3 rearrangements. A subsequent RNA sequencing detected an in-frame fusion between exon 15 of TNC and exon 6 of PDGFD. This fusion was further confirmed by nested reverse transcription polymerase chain reaction amplification followed by Sanger sequencing. A diagnosis of fibrosarcomatous DFSP was rendered and the patient was in good status at a follow-up of 12 months after the operation.

Conclusions: We report a fibrosarcomatous DFSP with novel TNC-PDGFD fusion, which adds to the pathologic and genetic spectrum of PDGFD-rearranged DFSP.

Keywords: Dermatofibrosarcoma protuberans, Fibrosarcomatous transformation, TNC, PDGFD, Fusion gene
Background
Dermatofibrosarcoma protuberans (DFSP) is a locally aggressive but rarely metastasizing, fibroblastic neoplasm that typically presents as a nodular and multinodular cutaneous mass on the trunk and proximal extremities of young to middle-aged adults [1, 2]. Classically, DFSP is composed of fairly uniform, mildly atypical spindle cells, often arranged in tight storiform, whorled, or cartwheel patterns. It usually originates in the dermal with subsequent infiltration the subcutaneous fat with a characteristic honeycomb appearance. By immunohistochemistry (IHC), the neoplastic cells usually express CD34 with focal expression of smooth muscle actin (SMA) sometimes observed [1, 2]. Fibrosarcomatous transformation can rarely arise in DFSP either de novo or as recurrent, which represents a form of tumor progression and carries an increased risk of metastasis over classic DFSP [3, 4]. The fibrosarcomatous component varies in proportion from less than 5% to more than 95%, and often arises in the subcutis with a nodular, rather well-demarcated growth pattern [3, 4]. The tumor cells in fibrosarcomatous areas often appear in fasicular and herringbone-like and frequently have greater cytologic atypia, increased cellularity and mitotic activity than those in the ordinary DFSP. By IHC, fibrosarcomatous DFSP commonly exhibits diminished, even loss of CD34 expression and increased P53 expression [3, 4]. Cytogenetically, the vast majority of DFSPs (including those with fibrosarcomatous change) harbor the recurrent unbalanced chromosome translocation t (17;22)(q21;q13), commonly in the form of supernumerary ring chromosomes, resulting in the fusion of genes COL1A1 on chromosome 17q21.3 and PDGFB on 22q13 [3, 4]. It has been proposed that constitutive expression of PDGFB is the fundamental mechanism of tumorigenesis in DFSP [5, 6]. However, rare variant fusion such as COLIA2-PDGFB fusion [7] and alternative rearrangements involving the related PDGFD gene with partners of either COL6A3 or EMILIN2 have also been documented recently [8, 9].

Herein, we report a fibrosarcomatous DFSP in which a novel fusion between TNC and PDGFD genes was detected by RNA sequencing and further confirmed by nested reverse transcription polymerase chain reaction (RT-PCR) amplification followed by Sanger sequencing.

Case presentation
A previously healthy 54-year-old female presented with a slowly growing mass in the right thigh for 1 year. Enhanced computed tomography scan demonstrated a 2.2 cm, well-defined, subcutaneous nodule in the right thigh with moderately heterogeneous enhancement. The tumor was surgically removed with narrow margins. No evidence of tumor recurrence or metastasis was noted at a follow-up of 12 months after the operation.

Gross examination revealed a 2 cm, well-circumscribed nodular mass with a firm, gray-white cut surface. Low power magnification showed a well-defined, partially encapsulated subcutaneous tumor without dermal connection (Fig. 1a). The tumor was composed of cellular, fairly uniform, slightly rounded spindle cells containing scant cytoplasm and ovoid, mildly atypical nuclei with brisk mitoses (up to 10 mitotic figures per 10 high power fields). The tumor cells were arranged predominantly in elongated fascicles and herringbone architectures (Fig. 1b, c). The stroma was typical minimal with scattered round small vessels and focal depositions of thick and band-like collagen bundles (Fig. 1d). No tumor necrosis was noted. At the periphery of the mass, minor areas showing vague storiform growth of less cellular and more bland-appearing tumor cells setting in a more collagenized stroma, with occasionally entrapped mature adipose tissues, were observed (Fig. 1e).

Immunohistochemical staining revealed the tumor cells to be positive moderately and diffusely for CD34 (Fig. 1f) and focally for SMA and negative for Cam5.2, epithelial membrane antigen (EMA), desmin, calponin, TLE-1, CD99, Stat6, anaplastic lymphoma kinase (ALK, 1A4), SOX10, and S100 protein. The Ki67 proliferation index was approximately 20%. Fluorescence in-situ hybridization (FISH) analysis was negative for fusion of the COL1A1 and PDGFB using the dual spanning probe set (Fig. 2a). Assessment for rearrangements of the NTRK1/2/3 locus using the corresponding break-apart probe sets were all negative (Fig. 2b).

RNA sequencing was performed on formalin-fixed paraffin-embedded (FFPE) tissue as described previously [10]. Specifically, total RNA was extracted from FFPE samples with a RNeasy FFPE kit (QIAGEN). The quantity and quality of total RNA were assessed with the KAPA Library Quantification Kit (KAPA Biosystems), and the Agilent High Sensitivity DNA Kit and Bioanalyzer 2100 (Agilent Technologies), respectively. Sequencing was performed on the Illumina HiSeq next-generation sequencing platform (Illumina). The results were then analyzed using the BLAT aligner, Factera and Socrates, respectively, as previously described [10]. A fusion product between TNC exon 15 and PDGFD exon 6 with the variant allele frequency (VAF) of 85.37% was identified. The fusion result was confirmed through manually reviewing on the Integrative Genomics Viewer (Fig. 2c). This fusion was further confirmed by nested reverse transcription polymerase chain reaction (RT-PCR) amplification (TNC forward primer 5′-TGGCTA CCGATGGGATCTTC – 3′ and PDGFD reverse primer...
Discussion
Historically DFSP has genetically been featured by t(17; 22)(q22;q13) translocation, leading to the fusion of the COL1A1 gene with the PDGFB gene [5, 6]. The COL1A1-PDGFB fusion results in the constitutive up-regulation of PDGFB expression, leading to autocrine activation of PDGF receptor β (PDGFR-β) receptor tyrosine kinase signaling and consequently drives the tumorigenesis [2, 11]. These provide a rationale for targeted therapy with tyrosine kinase inhibitors (TKIs) for unresectable or metastatic DFSPs [12]. The COL1A1-PDGFB fusion has been detected in up to 96% of DFSPs and represents a quite useful tool for the differential diagnosis of DFSP with its mimickers [1, 2, 6, 7, 13]. With the application of more sensitive detection assays, the fusion incidence appears to increase and rare cryptic fusions and alternative rearrangements involving the related PDGFD gene have also been documented [7–9]. In the few cases of DFSP that were negative for COL1A1-PDGFB fusion through routine FISH analysis, two recent studies using Next generation sequencing found that 40% of cases indeed had the classical COL1A1-PDGFB fusion, while more than half of cases harbored a fusion between PDGFD and either COL6A3 or EMILIN2, with the COL6A3-PDGFD fusion much more frequently encountered than the EMILIN2-PDGFD fusion [8, 9]. In this report, we describe a novel TNC-PDGFD gene fusion in a DFSP with fibrosarcomatous morphology, enhancing the genetic spectrum of DFSP.

PDGFD, located at 11q22.3, encodes a protein belonging to the same family of platelet-derived growth factor as PDGFB [14]. It has been proposed that PDGFD displays an oncogenic activity specifically through binding to and activating its cognate receptor PDGFR-β, and plays an important role in regulating tumor cell growth, migration, invasion, angiogenesis and metastasis by cross-talk with many signaling pathways in a wide array of pathways.
of malignancies [14, 15]. In PDGFD-rearranged DFSP, the reported genomic breakpoint was constantly located in exon 6, which retained the PDGF domain in a manner similar to rearrangements involving PDGFB [5, 8, 9]. TNC, also known as Tenasin-C, located at 9q33.1, is a member of tenascin gene family and encodes an extracellular matrix glycoprotein TNC with a spatially and temporally restricted tissue distribution [16, 17]. TNC is homohexameric with disulfide-linked subunits, and contains multiple EGF-like and fibronectin type-III domains [16, 17]. TNC has oncogenic properties through promotion of cell proliferation, migration and angiogenesis and its over-expression has been linked to a variety of malignancies [18]. Recently, fusions involving TNC have rarely been documented to occur in other neoplasms, including TNC-NRG1 fusion in a non-small cell lung carcinoma [19] and in a papillary renal cell carcinoma [20], and TNC-USP6 fusion in a primary aneurysmal bone cyst [21]. In these scenarios, TNC is functioned as a strong promoter, leading to activation of oncogenes NRG1 and USP6, and subsequently induction of tumor formation.
According to the limited cases published to date, the PDGFD-rearranged DFSPs are commonly centered in subcutaneous fat without dermal involvement [8, 9], and the COL6A3-PDGFD fusion variant shows an apparent proclivity for the breast or chest wall locations in female patients and present with classic histology and immunophenotype [8], while both the two reported cases of DFSP harboring PDFGD-EMILIN2 fusion arise in the leg and demonstrate a fibrosarcomatous morphology [9]. Studies from 2 groups by Dickson et al. [8] and Dadone-Montaudié et al. [9] have showed that PDGFD-rearranged DFSP clustered together with the group of DFSP with the classic COL1A1-PDGFB fusion upon unsupervised hierarchical clustering analysis, and demonstrated increased expression of PDGFB mRNA by RNA sequencing. These evidence suggested that PDGFD rearrangements may function in a similar pattern of autocrine activation via PDGFR-β receptor tyrosine kinase signaling as COL1A1-PDGFB fusions, and rearrangements of PDGFD might therefore be targeted by TKIs as classical DFSP [8, 9].

Fibrosarcomatous DFSP represents morphological progression to a usually fascicular and herringbone pattern with increased risk of recurrence and metastatic potential [1–4]. The underlying oncogenic mechanism of fibrosarcomatous transformation in conventional DFSP is largely undetermined. Several different molecular genetic alterations have been proposed to account for this transformation, including genomic gains of COL1A1-PDGFB, losses of genomic material from 22q, mutations of TP53, activation of the PDGFR-β/Akt/mTOR pathway signaling, and microsatellite instability [22]. Most recently, single reports have suggested that overexpression of programmed cell death 1 ligand (PD-L1) [23] and fusion of MAP 3K7CL-ERG [24] may be implicated in the transformation of conventional DFSP to fibrosarcomatous DFSP. It’s worth noting that both the previously reported two cases of EMILIN2-PDGFD fusion DFSP exhibited a fibrosarcomatous histology and showed homozygous deletion of CDKN2A [9], which had also been identified in PGDFB-rearranged DFSPs and often observed in cases showing hypercellularity and fibrosarcomatous transformation morphology [25]. These suggest that, despite limited experiences, disruptions of the CDKN2A/CDK4/RB1 pathway may also represent an oncogenic mechanism in the clonal evolution of a subset of PDGFβ-rearranged DFSP with fibrosarcomatous transformation.

The case we described here harbors a novel TNC-PDGFD gene fusion, as detected by RNA sequencing and nested RT-PCR, and shares some features with EMILIN2-PDGFD fusion DFSP, including deep-seated without dermal connection, fibrosarcomatous transformation morphology with focally abundant collagenous stroma. Similar to other reported PGFD fusion, the PDGF receptors DNA binding domain of PDGFD is preserved in the currently documented TNC-PDGFD fusion [8, 9]. As with the EMILIN2-PDGFD fusion, it is difficult to determine with certainty if TNC-PDGFD plays a driving role in the transformation of this tumor from DFSP to fibrosarcomatous DFSP, but it is a possible candidate. Larger cohorts with functional studies will be needed to further assess the role of TNC-PDGFD and the behavior of DFSP containing this fusion. With regard to the differential diagnosis of this tumor, NTRK-rearranged tumors may be a consideration given the monomorphic spindle cells, CD34 expression, and focally sclerotic background. However, NTRK-rearranged tumors typically co-express S100 protein, often harbor NTRK1 rearrangement although rare rearrangements involving NTRK2, NTRK3, RAF1, and BRAF have been documented [1].

In summary, we report a fibrosarcomatous DFSP with novel TNC-PDGFD fusion, which adds to the pathologic and genetic spectrum of PDGFD-rearranged DFSP. The expanded molecular spectrum provides a novel insight into DFSP oncogenesis and carries important implications for molecular diagnostics as well as potential tailored therapies.

Abbreviations
ALK: anaplastic lymphoma kinase; DFSP: dermatofibrosarcoma protuberans; EMA: epithelial membrane antigen; FISH: fluorescence in-situ hybridization; IHC: immunohistochemistry; PDGFR: PDGF receptor; PD-L1: programmed cell death 1 ligand; RT-PCR: reverse transcription polymerase chain reaction; SMA: smooth muscle actin; TKIs: tyrosine kinase inhibitors; VAF: variant allele frequency

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Authors’ contributions
YC, YS, MZ: conception and design of the work, acquisition, analysis and interpretation of data, drafting the manuscript and revising it critically for important intellectual content and scientific integrity. YC, YS, XF, XW, XH: conception and design of the work, acquisition, analysis and interpretation of data, reading and revising the manuscript critically for important intellectual content and scientific integrity. All authors have read and approved the final manuscript.

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Availability of data and materials
Records and data pertaining to both the cases are in the patient’s secure medical records in Zhejiang Provincial People’s Hospital, People’s Hospital of Hangzhou Medical College. All searched data by literature review are included in this paper.

Declarations
Ethics approval and consent to participate
Samples were used in accordance with ethical guidelines for the use of retrospective tissue samples provided by the local ethics committee of the
Zhejiang Provincial People’s Hospital, People’s Hospital of Hangzhou Medical College.

Consent for publication
Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the consent form is available for review by the Editor of this journal.

Competing interests
The authors declare that they have no competing interests.

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