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CASE REPORT

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A case of dermatofibrosarcoma protuberans of the vulva with a *COL1A1/PDGFB* fusion identical to a case of giant cell fibroblastoma

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Abstract Dermatofibrosarcoma protuberans (DFSP) is a highly recurrent low-grade soft tissue sarcoma, which is usually located on the trunk. Presentation in the vulva is rare, with only 13 cases being reported to date, none of which have been investigated at the cytogenetic or molecular level. Specific cytogenetic abnormalities, involving chromosomes 17 and 22, are characteristic features of DFSP and giant cell fibroblastoma (GCF), a tumor closely related to DFSP. These chromosomal rearrangements result in the fusion of the *COL1A1* and *PDGFB* genes in both lesions and show wide variation in the position of the fusion point in *COL1A1*. Here, we describe a case of DFSP of the vulva with a typical monotonous storiform pattern, with no foci of multinucleated giant cells. Cytogenetic analysis showed a 47,XX,+r karyotype in 50% of the cells, and molecular investigation disclosed the presence of a transcript fusing *COL1A1* exon 37 to *PDGFB* exon 2. This is the first case of DFSP showing such a fusion point, which is intriguingly identical to that found in a GCF case, indicating that the *COL1A1/PDGFB* fusion point position does not seem to affect tumor morphology. This finding further underlines the very close relationship between these two morphologically distinct entities.

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Introduction

Dermatofibrosarcoma protuberans (DFSP), also known as tumor of Darier and Ferrand [8], is a highly recurrent low-grade soft tissue sarcoma, which typically arises in the deep dermis and subcutis. Metastases are rare, occurring in less then 0.3–0.5% of cases [15]. DFSP is slightly more frequent in males and is usually diagnosed during mid-adult life, although cases have been reported in infants [13] and neonates [11]. Favored locations include the trunk, mainly in the shoulder region, and the proximal lower limbs [16]; occurrence in the vulva is rare [10]. DFSP is one of numerous soft tissue tumors in which the histogenesis remains controversial [5]. Proposals include a histiocytic origin due to the storiform pattern of cells, dermal interstitial cell origin due to CD34 staining, and a myofibroblastic/fibroblastic origin due to ultrastructural similarities [9], focal myoblastic differentiation, and progression to fibrosarcoma [2, 6, 17]. The latter proposal has been supported recently by the discovery of the involvement of a collagen gene in DFSP pathogenesis [26]. Upon cytogenetic analysis, DFSP displays the presence of supernumerary ring chromosomes, derived from the translocation t(17;22), or more seldomly the translocation t(17;22)(q22;q13) [21, 22, 23]. Both rings and translocations contain the same molecular rearrangement an in-frame fusion of the *COL1A1* gene on chromosome 17 and the *PDGFB* proto-oncogene on chromosome 22 [26]. Here, we describe the morphological, cytogenetic, and molecular features of a new case of DFSP located in the vulva.

Clinical data

In March 1997, a 39-year-old Caucasian woman presented with a non-tender firm subcutaneous mass in the vulva. The patient first

noted the nodule about 1 year before she was admitted to the hospital. The mass had increased steadily in size during the intervening months. Physical examination showed a multinodular subcutaneous mass, covered by normal skin, centered in the vulva, extending to the perineal raphe. The margins were indistinct; the mass was not fixed to deep structures. The patient underwent a wide local excision and has no sign of recurrence at this time of writing.

Materials and methods

Pathology

The gross appearance was that of a multinodular mass, 6 cm in diameter, extending to the subcutaneous fat with finger-like projections. Cross sections showed the tumor to be lobular, gray–yellow, and hard. Multiple specimens were obtained for histology, immunohistochemistry, and cell cultures for use in cytogenetic and molecular analysis.

Histology and immunohistochemistry

Sections 4-µm thick were cut from formalin-fixed, paraffin-embedded material and stained with hematoxylin and eosin. Sections from paraffin blocks were also examined immunohistochemically by means of the avidin–biotin–peroxidase technique using antibodies to the following antigens: CD34 (Dako, Glostrup, Denmark, monoclonal QBEnd/10, dilution 1:100), smooth muscle actin (Dako, monoclonal IA4, dilution 1:16.000), muscle actin (Dako, Carpinteria Calif., monoclonal HHF-35, dilution 1:50), and desmin (Dako, monoclonal D33, dilution 1:300). Positive and negative controls were used throughout.

Cytogenetics and fluorescence in situ hybridization

For cytogenetic analysis, tumor tissue from the histologically examined specular region was processed as previously described [29]. For conventional cytogenetic analysis, chromosome metaphases were G-banded using Wright's stain [31] and 30 metaphases analyzed. Fluorescence in situ hybridization (FISH) was performed using biotinylated whole chromosome painting (WCP) DNA libraries specific for chromosomes 17 and 22 (Cambio, UK), and a chromosome 22 alpha-satellite probe (D22Z1) (Oncor, Gaithersburg, Md.), according to the recommendations of the supplier. Cosmid N10C3 [26] was used as a FISH probe as described [22].

Table 1 Primers used in reverse-transcription polymerase chain reaction

Name	Sequence(5'-3')
P1	TCAAAGGAGCGGATCGAGT
P2	GCGCTGCACCTCCACACA
C1	CCGACGGCTCAGAGTCAC
C2	CCCCAGCTGTCTTATGGCTATG
C3	CTGGTCCCAAGGGTAACAG
C4	GGTTTCCCTGGCGCAGAT
C5	AGGGCCTAAGGGTGACAGA
C6	GGCCCTGCTGGTCCCACT
C7	GGACCCCCTGGTGAATCT
C8	ACAAGGGTGAGACAGGCG
Col.amp2	TCCTCCGGTTTGATTTCTC

Reverse-trasncription polymerase chain reaction

Total RNA was extracted from tumor cell suspensions using acidic guanidinium thiocyanate–phenol–chloroform extraction. Reverse transcription (RT) of total RNA was performed using a P2 primer specific for *PDGFB* exon 4. For all RT polymerase chain reaction (PCR) primer sequences see Table 1. Positive controls included using the previously characterized tumor RNA, TNM2 [19], in the above reaction and using Col.amp2, a *COL1A1* primer, in RT synthesis using test RNA. Multiplex PCR amplification of the resulting cDNA was performed using P2, combined with four external *COL1A1* primers – C1, C3, C5, and C7. An aliquot of this reaction was subjected to four separate PCR reactions, each containing a nested *COL1A1* primer, C2, C4, C6, or C8, in combination with P1, a *PDGFB* exon-2-specific primer. PCR of RT controls also used the above method, except for the *COL1A1* RT amplification, which used C1 and Col.amp2 primers. Resulting bands were cut from low-melting-point agarose gels, re-amplified and sequenced using PCR primers.

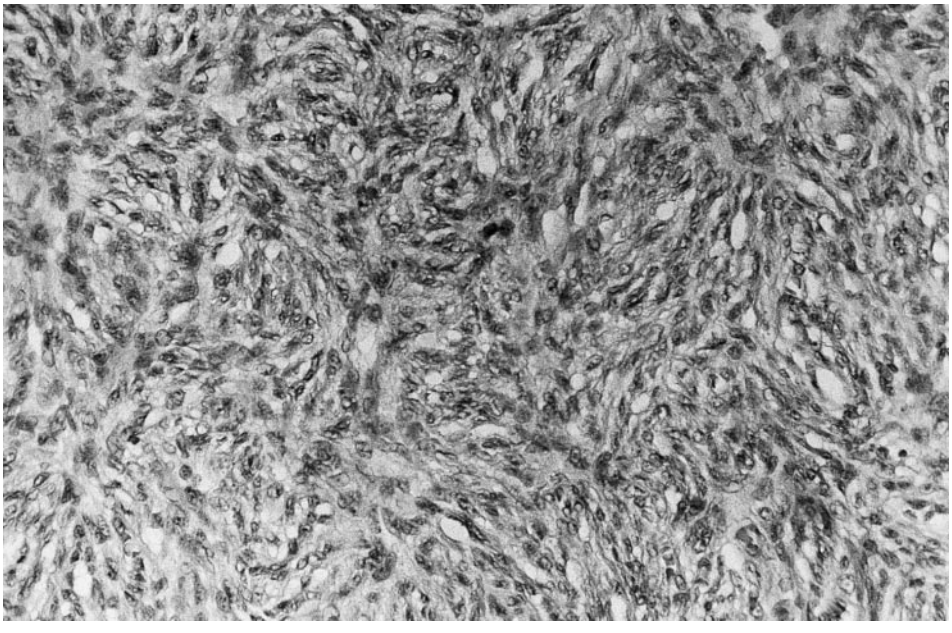

Fig. 1 Histologic pattern of the dermatofibrosarcoma protuberans (DFSP): note the high cellularity and the storiform pattern with scattered small blood vessels. Hematoxylin and eosin stain, original magnification 250×

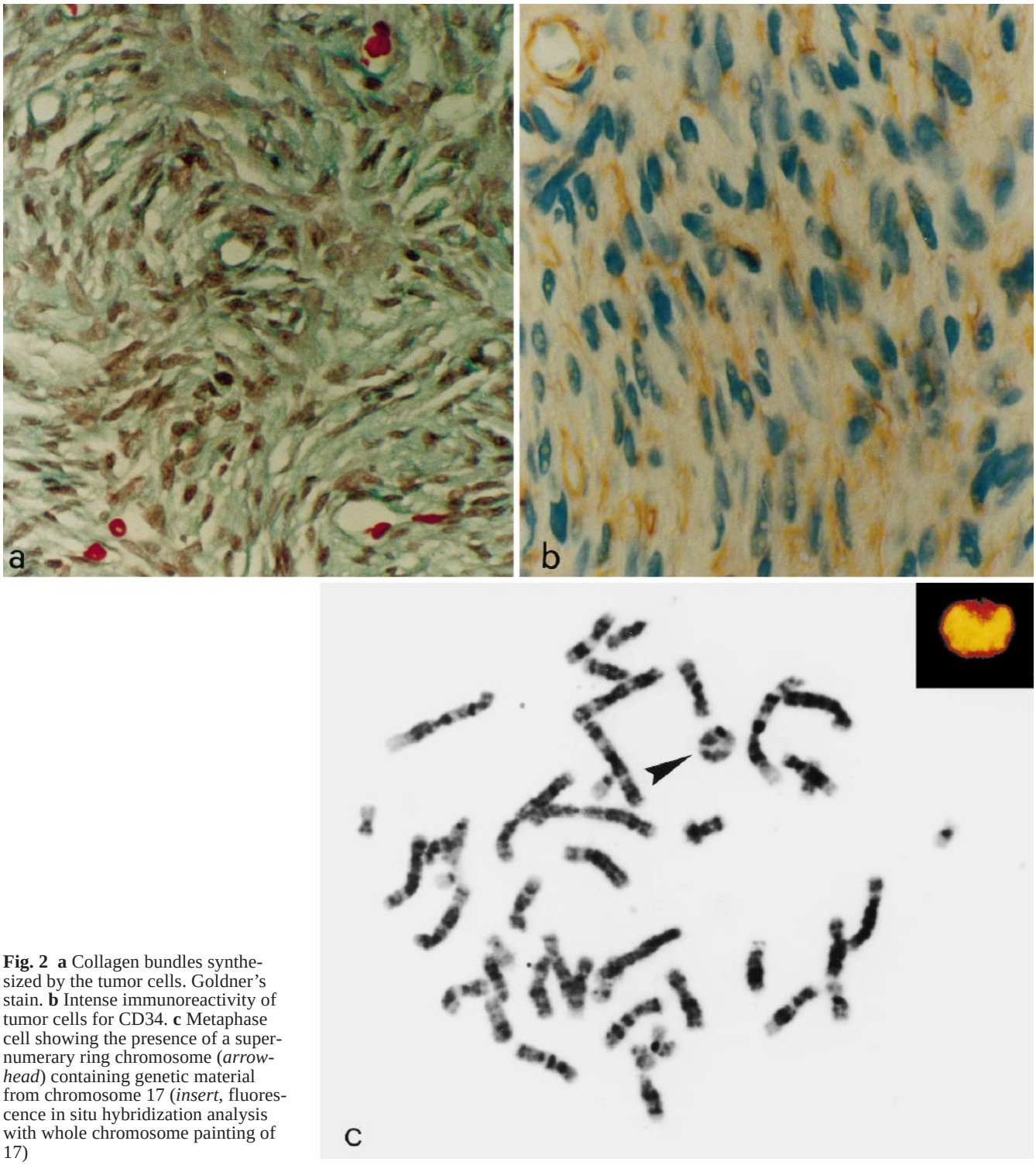

Fig. 2 **a** Collagen bundles synthesized by the tumor cells. Goldner's stain. **b** Intense immunoreactivity of tumor cells for CD34. **c** Metaphase cell showing the presence of a supernumerary ring chromosome (*arrowhead*) containing genetic material from chromosome 17 (*insert*, fluorescence in situ hybridization analysis with whole chromosome painting of 17)

Pathology and laboratory findings

Pathological findings

Histological analysis showed a monotonous storiform growth pattern, with permeative and diffuse margins and extensive infiltration of subcutaneous fat. The storiform pattern appeared redundant and frequently centered around small vessels (Fig. 1). Collagen strands were present but uncommon (Fig. 2a). Necrosis was absent. Tumor cells were monomorphic, with scanty cytoplasm and large elongated nuclei containing fine and dusty chromatin. Nucleoli were apparent though small. The mitotic rate was five mitoses per ten high power field.

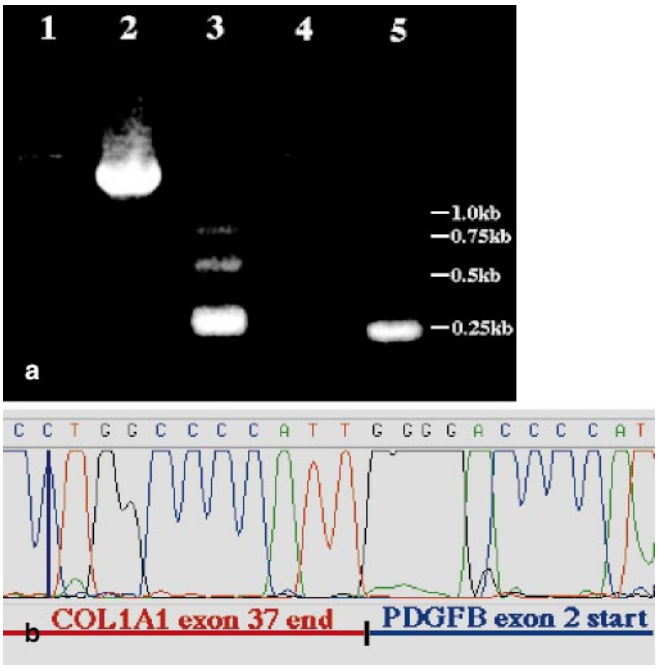

Fig. 3 a Reverse-transcription polymerase chain reaction (RT-PCR) products in low-melting-point agarose gel. *Lanes 1 to 4* contain second-round PCR reactions which used primers C2, C4, C6, and C8, respectively, in combination with primer P1. *Lane 5* contains the internal RT-PCR positive control which used primers C1 and Col.amp2. Bands were cut, re-amplified and sequenced. The 1.2-kb and 260-bp bands in *lanes 2 and 3* re-amplified in third-round PCR, while weaker unspecific bands failed to re-amplify. **b** Sequence chromatogram of the 260-bp RT-PCR product shown in **a**. Note the in-frame fusion of *COL1A1* exon 37 to *PDGFB* exon 2. Both the 1.2-kb and 260-bp bands contain the fusion point. The sequence of the 260-bp product is identical to that of a 260-bp *COL1A1/PDGFB* RT-PCR product found in a giant cell fibroblastoma case K2471 [19] (accession no. Y15913)

Immunohistochemical analyses showed diffuse and intense positivity of tumor cells for CD34 (Fig. 2b), and focal positivity for SMA and HHF-35. Desmin and S100 protein were constantly negative. Neither angiectoid spaces nor giant cells were detected at the periphery of the neoplasm.

Cytogenetic and molecular findings

Conventional cytogenetic analysis performed on G-banded metaphases showed the presence of an extra ring chromosome in 50% of the examined metaphases (Fig. 2c). FISH analysis using the 17 WCP DNA probe showed hybridization signals on both of the normal chromosomes and on the ring chromosome (Fig. 1c, insert). The 22 WCP DNA probe revealed the presence of two whole painted chromosome 22 and no FISH signal on the ring chromosome. The alpha-satellite probe specific for chromosome 22 centromere (D22Z1) hybridized to the centromeres of chromosomes 22 but no FISH signal was visible on the ring. Only one destained slide was available for FISH analysis using cosmid N10C3, which contains the whole *PDGFB* gene: while weakly positive signals were seen in some metaphases, they were not of sufficient strength to confirm the presence of the *PDGFB* gene on the ring chromosome. The final karyotype was: 47,XX,+r [15].ish r(17)(wcp17+, wcp22–,D22Z1–)/46XX [15]. However, RT-PCR detected the presence of a *COL1A1/PDGFB* chimeric gene and demonstrated a transcript fusing *COL1A1* exon 37 to *PDGFB* exon 2 (Fig. 3 and Fig. 4).

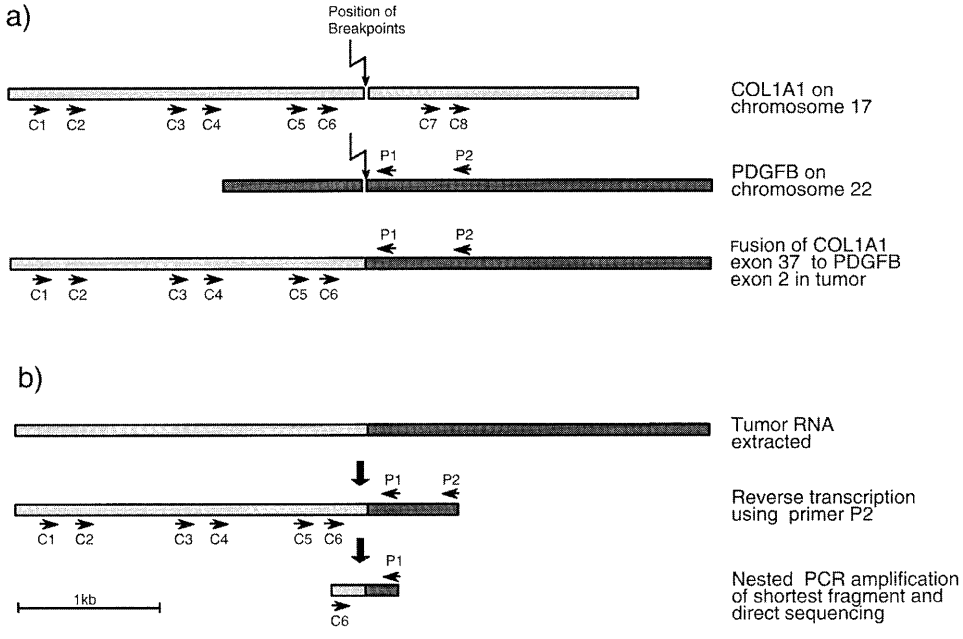

Fig. 4 a Position of breakpoint in chimeric transcript relative to normal *PDGFB* and *COL1A1* transcripts (to scale). *Crooked arrows* indicate breakpoint positions; *horizontal arrows* indicate position and direction of primers. **b** RT-PCR; Reverse transcription was primed using primer P2 while nested polymerase chain reaction used all primers. The 260-bp fragment obtained was sequenced using primers C6 and P1, revealing the *COL1A1* exon 37 to *PDGFB* exon 2 fusion

Discussion

DFSP preferentially originates in the trunk and lower limbs. The present lesion was located in the vulva, a rather rare site where, to date, only 13 cases have been reported [1, 2, 3, 4, 10, 12, 14, 20, 27, 28]. The histological picture was characterized by a typical DFSP storiform pattern. Due to the tendency of DFSP to infiltrate the surrounding tissue via microscopic projections, a very wide excision was required, with surgical margins of 3 cm being recommended [24]. To avoid unnecessary wide surgical margins, misdiagnosis with more innocuous lesions that may mimic DFSP should be excluded, such as the cellular variant of benign fibrous histiocytoma [5, 30]. To this aim, the possibility of confirming the diagnosis using other ancillary analyses, such as cytogenetic and molecular studies, seems particularly useful. In the present case, conventional cytogenetic analysis confirmed the intraoperative histological diagnosis, corroborating the surgeons' choice of reaching margins as wide as possible, although considering the necessity to preserve surrounding structures, such as the clitoris.

Cytogenetic analysis of this case ascertained the presence of a ring chromosome, in which sequences from chromosomes 17 and 22 were expected to be present [21]. FISH analysis demonstrated a positive signal for only WCP 17 but not WCP 22. However, DFSP chromosomal rearrangements are often cryptic, and rings can contain quantities of chromosome 22 material that are negative upon WCP [18]. Accordingly, we confirmed that the absence of chromosome 22 material on the ring was only apparent, since we detected a chimeric *COL1A1/PDGFB* transcript using RT-PCR. This specific gene fusion has been found in every DFSP case studied by RT-PCR to date and has only been detected in one other type of tumor, that of giant cell fibroblastoma (GCF) [19]. GCF is cytogenetically and immunohistochemically related to DFSP. In fact, cases of GCF may recur as DFSP and vice versa, cells of both tumors show CD34 immunoreactivity, and are characterized by rearrangements involving chromosomes 17 and 22 [7, 25]. At the molecular level, there is wide variation in the position of the fusion point in the *COL1A1* gene in DFSP and GCF [19]. Intriguingly, the present case is the first DFSP case displaying a *COL1A1* exon 37 to *PDGFB* exon 2 fusion, which is identical to a previously described fusion in a GCF case (case K2471 [19]). However, the present case showed a monotonous storiform pattern with no foci of multinucleated giant cells, nor peripheral areas reminiscent of GCF and, therefore, is clearly a DFSP case. This unique finding further supports the close relationship between these two lesions. It also demonstrates that even an identical *COL1A1/PDGFB* fusion point position does not seem to affect the tumor morphology, which is the clinicopathological distinction between the neoplasms.

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