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INVESTIGATIVE REPORT

Gene Mutation Analysis in Five Cases of Dermatofibrosarcoma Protuberans Using Formalin-fixed, Paraffin-embedded Tissues

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Fusion of the collagen type I α 1 (*COL1A1*) gene with the platelet-derived growth factor B-chain (*PDGFB*) gene has been pointed out in dermatofibrosarcoma protuberans. Various exons of the *COL1A1* gene have been shown to be involved in the fusion with exon 2 of the *PDGFB* gene. We studied the breakpoints of the *COL1A1* gene using formalin-fixed, paraffin-embedded tumour specimens from five patients with dermatofibrosarcoma protuberans (three reconfirmations and two new cases). Reverse transcriptase-PCR was performed using paraffin-embedded tissues. Nucleotide sequence analysis was carried out using the PCR products to identify the breakpoints. The *COL1A1-PDGFB* fusion transcripts were detected from the tumour specimens. Sequence analysis revealed that the ends of exons 18, 29, 38, 42 and 44 in the *COL1A1* gene were fused with the start of exon 2 in the *PDGFB*. This study identified a novel *COL1A1* breakpoint, namely, exon 44 of the *COL1A1* gene. Detection of the aberrant fusion transcript using formalin-fixed, paraffin-embedded tumour specimens is useful as a diagnostic aid for dermatofibrosarcoma protuberans in cases where fresh or frozen samples of tumour tissue are not available. *Key words: dermatofibrosarcoma protuberans; paraffin-embedded tissue;* COL1A1; PDGFB.

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Dermatofibrosarcoma protuberans (DFSP) is an uncommon neoplasm of the dermis extending to the subcutis (1). This tumour is considered to be a locally invasive neoplasm of intermediate malignancy because it grows aggressively, recurs at a high rate, but metastasizes rarely. Recent cytogenetic studies have revealed that a reciprocal translocation, t(17;22)(q22;q13), and a supernumerary ring chromosome derived from the translocation, r(17;22), are highly characteristic of DFSP (2, 3). These chromosomal re-arrangements fuse the collagen type I α 1 (*COL1A1*) and the platelet-derived growth factor B-chain (*PDGFB*) genes (4). We analysed gene mutations in five cases of DFSP (three reconfirmations and two new cases) using formalin-fixed, paraffin-embedded tissues and identified a novel breakpoint of the *COL1A1* gene.

MATERIALS AND METHODS

RNA extraction

The extraction of RNA from paraffin-embedded tumour tissues was performed according to Wang et al. (5) with minor modification. In brief, six sections, each 6 μm thick, were cut from a block of a formalin-fixed, paraffin-embedded tumour sample and collected in a sterile microtube. After the pellets underwent deparaffinization with two exchanges of xylene and three washes with 100% ethanol, the pellets were minced in 200 μl lysis buffer (20 mmol/l Tris-HCl, pH 8.0; 20 mmol/l EDTA and 2% SDS) using a hand homogenizer and then 50 μl proteinase K (20 mg/ml) were added to the samples. After incubation at 55℃ for 48 h, 1.0 ml Trizol reagent (Gibco BRL, Gaithersburg, MD, USA) was added to the sample, followed by 200 μl chloroform. After vortex mixing and centrifugation, the aqueous phase was transferred into a 1.5-ml sterile microtube and precipitated with 0.75 ml 2-propanol. The RNA pellet was resuspended in 20 μl of sterile water.

Reverse transcription-polymerase chain reaction (RT-PCR) and sequencing

To detect the presence of *COL1A1-PDGFB* fusion transcripts, RT-PCR was carried out using 16 *COL1A1* forward primers and a specific *PDGFB* reverse primer according to Wang et al. (5). Sixteen *COL1A1* forward primers were designed in the following *COL1A1* exons: exon 5, 8, 11, 15, 17, 20, 23, 26, 27, 32, 35, 38, 40, 44, 46, 49, and these primers were considered sufficient to span the various breakpoints within the region encoding the alpha-helical domain of the *COL1A1* polypeptide (exon 6 to exon 49) (5). The PCR products were directly sequenced by an Applied Biosystems 373A automated DNA sequencer to identify the breakpoints.

RESULTS

Clinical and histological features of the patients

Table I shows a summary of the clinical and histological features of five patients. Cases 1, 2 and 3 were reported elsewhere in detail (6, 7). Patient 4 (a 17-year-old Japanese woman) was seen in June 1986 for a small nodule on the anterior aspect of the left lower leg (Fig. 1a). The nodule had been present for 2 years and had slowly enlarged. A skin biopsy specimen showed a

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Table I. *The clinical features, histopathology findings, fusion genes and reverse transcription-PCR results in five patients with dermatofibrosarcoma protuberans*

	Case 1 (Ref. 6)	Case 2 (Ref. 7)	Case 3 (Ref. 7)	Case 4 (Fig. 1a)	Case 5 (Fig. 1b)
Age/sex	41/M	39/F	18/M	17/F	52/M
Site	Lower back	Buttock	Thigh	Lower leg	Chest
Preoperative duration	25 years	15 years	8 years	2 years	5 years
Clinical feature	Multinodular plaque	Multinodular plaque	Dome-shaped nodule	Small nodule	Multiple nodules
Tumour size	58×38 mm	35×15 mm	47×35 mm	8×8 mm	66×41 mm
Histopathology					
Storiform pattern	Present	Present	Present	Present	Present
Herringbone pattern	Absent	Absent	Present	Absent	Absent
Cytological atypia	Mild	Mild	Moderate	Mild	Mild
Fusion gene	*COL1A1* (exon 18)	*COL1A1* (exon 42)	*COL1A1* (exon 29)	*COL1A1* (exon 38)	*COL1A1* (exon 44)
RT-PCR result					
COL1A1 primer	Exon 17	Exon 40	Exon 27	Exon 38	Exon 44
PDGFB primer	Exon 2	Exon 2	Exon 2	Exon 2	Exon 2
Amplified product	173 bp	319 bp	220 bp	106 bp	91 bp

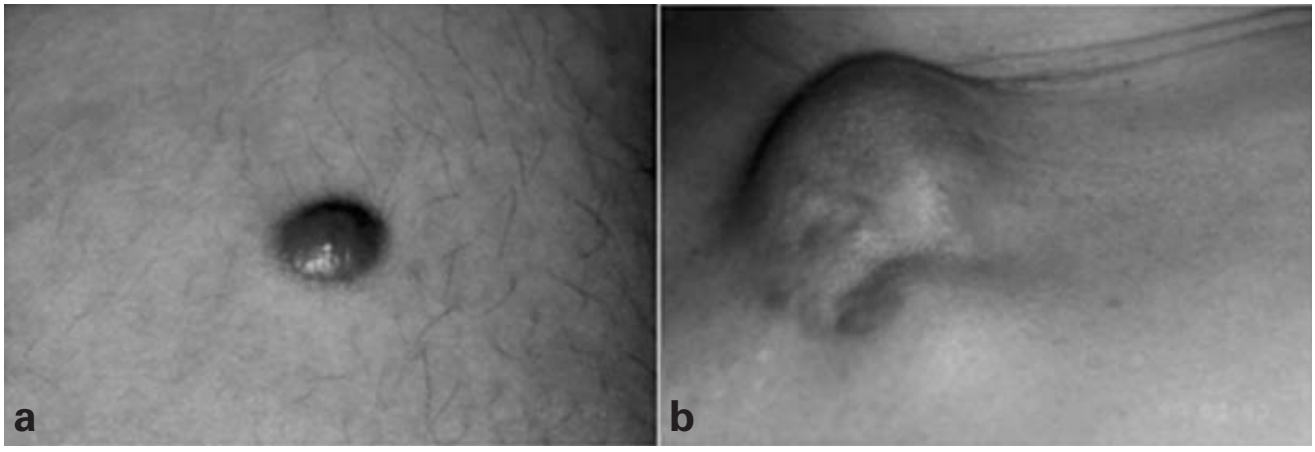

Fig. 1. Clinical features. (a) Case 4: a small reddish-purple nodule measuring 8×8 mm on the lower leg. (b) Case 5: a cluster of multiple elastic hard reddish-brown nodules measuring 66×41 mm on the upper chest.

tumoral proliferation of spindle-shaped cells with a storiform arrangement (result not shown but summarized in Table I). Some of the cells penetrated into the subcutaneous fat. Cytological atypia of tumour cells was mild and mitotic activity was low. We diagnosed this case as DFSP and the lesion was excised with 2-cm margins above the fascia and sutured.

Patient 5 (a 52-year-old Japanese man) was seen in December 1987 for a tumour on the upper chest. The lesion was a cluster of multiple elastic hard reddish-brown nodules (Fig. 1b). The nodules had been present for 5 years and had slowly enlarged and elevated. A skin biopsy specimen disclosed a dense, tumoral proliferation of spindle-shaped cells with a storiform arrangement (Table I). Cytological atypia of tumour cells was mild. We diagnosed this case as DFSP and the lesion was excised with 5-cm margins above the fascia (including part of the muscle) and the skin was grafted.

RT-PCR and sequencing

Table I also shows the RT-PCR results. PCR revealed that 173-, 319-, 220-, 106- and 91-bp DNA products were obtained by the amplification with *COL1A1* primers exon 17, 40, 27, 38 and 44, and *PDGFB* primer exon 2 in cases 1–5, respectively (data not shown). Nucleotide sequence analysis disclosed that the ends of exon 18, 42, 29, 38 and 44 in the *COL1A1* gene were fused with the start of exon 2 in the *PDGFB* gene in cases 1–5, respectively (Fig. 2, for cases 4 and 5).

DISCUSSION

Recent studies have revealed that fusion of *COL1A1* gene with *PDGFB* gene is highly characteristic of DFSP. *COL1A1* gene encodes the major component of type I collagen, which is produced primarily by fibroblasts. *PDGFB* is a potent mitogen for a number of cell types

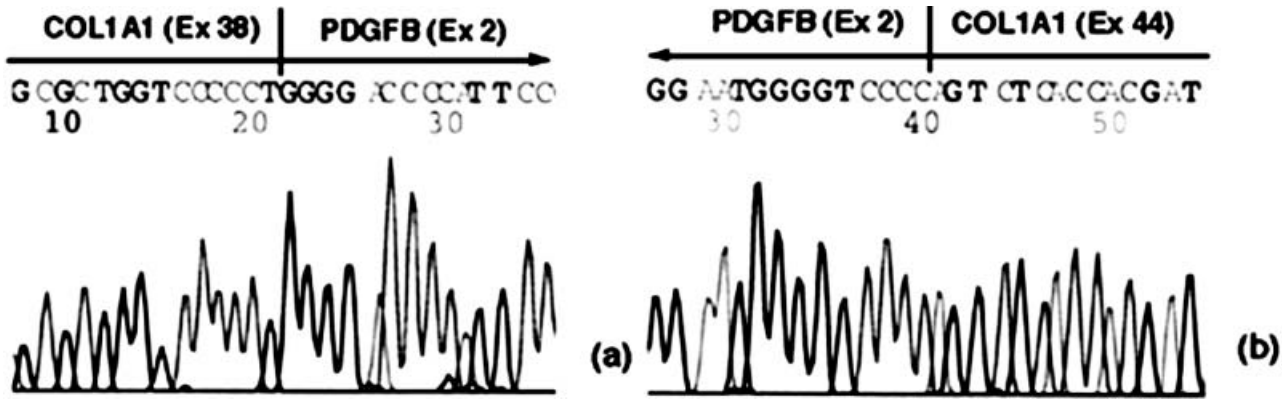

Fig. 2. Nucleotide sequence of the *COL1A1-PDGFB* fusion transcripts. (a) Case 4: exon 38 in the *COL1A1* gene was fused with exon 2 in the *PDGFB* gene (coding sequence). (b) Case 5: exon 44 in the COL1A1 gene was fused with exon 2 in the PDGFB gene (non-coding sequence).

(8). The location of breakpoints within *COL1A1* varies greatly, but is always limited to the region encoding the alpha-helical domain (9). The exons of *COL1A1* in this region end at the last base of a codon (4, 9). The *PDGFB* segment of the chimeric transcript always starts with exon 2. The resulting *COL1A1-PDGFB* fusion is in-frame, because exon 2 of *PDGFB* starts at the first base of codon 22. The *COL1A1* part of the fusion gene serves to provide an active promoter and signal peptide for PDGFB (10). Production of the abnormal fusion transcripts in the fibroblast, the suspected cell of origin of DFSP, probably causes autocrine stimulation and cell proliferation which is responsible for the development of DFSP (9, 11). Various exons in the alpha-helical domain of the *COL1A1* gene have been shown to be involved in the fusion with exon 2 of the *PDGFB* gene (4–7, 9, 11, 12). However, to the best of our knowledge, there has been no report of DFSP with *COL1A1* (exon 44)-*PDGFB* (exon 2) fusion transcript. This study identified a novel *COL1A1* breakpoint, namely, exon 44 of the *COL1A1* gene.

DFSP is an uncommon cutaneous neoplasm of intermediate malignancy. Its clinical and histological diagnosis is not difficult in typical cases, but sometimes it must be distinguished from other cutaneous tumours such as dermatofibroma, neurofibroma, neurilemmoma and malignant fibrous histiocytoma. Because the *COL1A1-PDGFB* fusion transcripts have been observed in only DFSP and DFSP-related tumours such as superficially located adult fibrosarcoma (13), detection of the aberrant fusion transcript seems to be useful at differential diagnosis. Most of the previous molecular approaches to DFSP were based on frozen tissue specimens or cultured tumour cells. Because fresh or frozen samples of tumour tissue are not always available, techniques in extraction of RNA from formalin-fixed, paraffin-embedded tissues have recently been improved (14–17). Wang et al. conducted an RT-PCR assay for the *COL1A1-PDGFB* fusion transcripts in DFSP to assess its feasibility in detecting these transcripts using archival formalin-fixed, paraffin-embedded tumour specimens (5). They detected them in 10 of 12 paraffin-embedded DFSP tumour tissues, showing that this molecular assay is useful as a diagnostic aid for DFSP. Previously, we reported three cases of DFSP and detected the fusion transcripts by RT-PCR using cultured tumour cells (6, 7). In this study, we first tried to detect the *COL1A1-PDGFB* fusion transcripts from the paraffin-embedded tumour tissues in cases in which we had already detected the transcripts from the cultured tumour cells (cases 1, 2 and 3). We did detect the same fusion transcripts in these cases in the cultured tumour cells and paraffin-embedded tumour tissues. The consistent results in different samples from the same tumours suggest that the RT-PCR assay is reliable. In addition, we attempted to detect the fusion transcripts using the archival paraffin-embedded tumour specimens from two patients with DFSP who visited our clinic in 1986 and 1987, and were also able to detect them by RT-PCR in these cases. It is sometimes difficult to diagnose DFSP only by clinical features and histopathology when fresh or frozen samples of tumour tissue are not available. Therefore, this assay using paraffin-embedded tumour tissues is very useful as a diagnostic aid for DFSP.

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