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Detection of *COL1A1-PDGFB* Fusion Transcripts in Dermatofibrosarcoma Protuberans

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Background: Dermatofibrosarcoma protuberans (DFSP) is an uncommon infiltrative tumor of the dermis and subcutaneous tissue. Recent cytogenetic studies have demonstrated a chromosomal translocation of the collagen type I alpha 1 (*COL1A1*) gene on chromosome 17 to the platelet-derived growth factor B-chain (*PDGFB*) gene on chromosome 22. Various exons of the *COL1A1* gene have been reported to be involved in the fusion with exon 2 of the *PDGFB* gene. **Method:** The *COL1A1-PDGFB* fusion transcript was detected by reverse transcriptase-polymerase chain reaction (RT-PCR) using frozen tissue from 4 DFSP patients. Nucleotide sequence analyses were carried out using the PCR products to identify the breakpoints. **Results:** *COL1A1-PDGFB* fusion transcripts were detected in all tumor specimens. Sequence analyses revealed that the end of exon 25, 45, 32, or 11 in the *COL1A1* gene was fused with the start of exon 2 in the *PDGFB* gene. **Conclusion:** Detection of this aberrant fusion transcript can be useful as a diagnostic method for DFSP. (Kitakanto Med J 2009；59：259～263)

Key Words: dermatofibrosarcoma protuberans, *COL1A1-PDGFB* fusion transcripts, reverse transcriptase-polymerase chain reaction

Introduction

Dermatofibrosarcoma protuberans (DFSP) is a dermal and subcutaneous tumor of intermediate malignancy. DFSP tumors grow slowly and rarely metastasize; however, they often recur after excision. Histopathologically, DFSP is composed of $CD34^+$ spindle-shaped tumor cells, which occur in a storiform pattern. The cytogenetic features of DFSP are characterized by either supernumerary ring chromosomes composed of sequences derived from chromosomes 17 and 22, or, more rarely, by translocations, t (17；22) (q22；q13).[1-3] These chromosomal rearrangements lead to the formation of a specific chimeric gene via fusion of the collagen type I alpha 1 (*COL1A1*) gene and the platelet-derived growth factor B-chain (*PDGFB*) gene. We examined the COL1A1-PDGFB fusion transcript in frozen tumor tissues by using the reverse transcriptase-polymerase chain reaction (RT-PCR).

Materials and Methods

Tumor samples

Four patients with DFSP were surgically treated between January 2007 and March 2008 in the Department of Dermatology, Gunma University Graduate School of Medicine, Japan. We examined the *COL1A1-PDGFB* fusion transcripts using frozen specimens after surgical excision or skin biopsy. Informed consent was obtained from all patients.

Mutation Analysis

Total RNA was extracted from frozen tissue sections using an RNeasy Mini Kit (Qiagen, Hilden, Germany), and reverse transcription was performed using a Superscript Ⅲ cDNA synthesis kit (Invitrogen Corp., Carlsbad, CA, USA). In order to detect the presence of *COL1A1-PDGFB* fusion transcripts, PCR was carried out using 16 *COL1A1* forward primers and a specific *PDGFB* reverse primer as described previously.[4] The 16 *COL1A1* forward primers were designed from the following *COL1A1* exons: exon 5, 8, 11, 15,

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Table. The clinical features, immunohistochemical findings and fusion genes results in four cases with DFSP

case	age/sex	site	size(mm)	CD34	*COL1A1-PDGFB*
1	30/F	arm	10	positive	*COL1A1* exon 25-*PDGFB* exon 2
2	30/M	back	20×20	positive	*COL1A1* exon 45-*PDGFB* exon 2
3	35/M	abdominal wall	60×45	positive	*COL1A1* exon 32-*PDGFB* exon 2
4	49/M	supraclavicle	150×55	positive	*COL1A1* exon 11-*PDGFB* exon 2

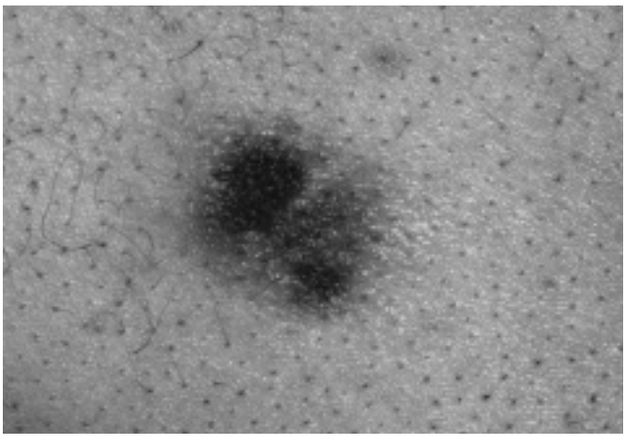

Fig. 1 (a). Clinical feature of Case 2: An atrophic reddish plaque measuring 20×20mm on the back.

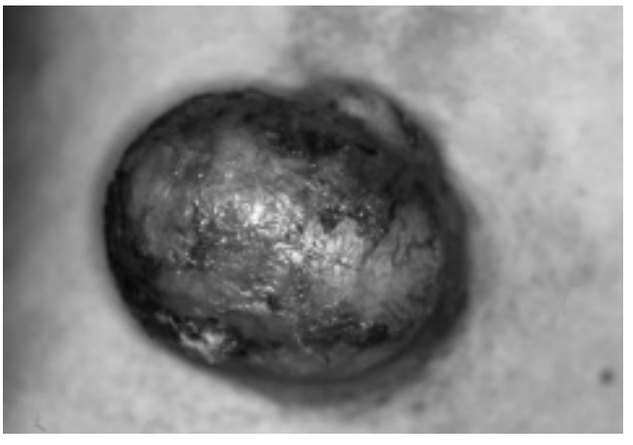

Fig. 1 (b). Clinical feature of Case 3: Solitary elastic hard tumor on the abdominal wall.

17, 20, 23, 26, 27, 32, 35, 38, 40, 44, 46, and 49. These primers were considered sufficient to span the various breakpoints within the region encoding the alpha-helical domain of the *COL1A1* polypeptide (exon 6 through exon 49).[4] The PCR products were directly sequenced using an ABI Prism 310 sequence analyzer.

Results

Clinical and histological features of the patients

Table summarizes the clinical and histological features of the 4 patients.

A 30-year-old Japanese woman (Case 1) was referred to our department with a 2-year history of a tumor on her right upper arm. The tumor had been surgically excised by an orthopedist and histologically diagnosed as a DFSP by a pathologist. We further excised the operated area with a 3-cm margin, including the underlying the fascia. Histopathological findings revealed a storiform pattern composed of monomorphous spindle cells in the reticular dermis extending into the subcutaneous fat. Cytological atypia of the tumor cells was mild and mitotic activity was low. Immunohistochemical staining of a paraffin-embedded tissue section revealed that the tumor cells expressed the CD34 antigen. No local recurrence or distant metastasis has been found during the follow-up period.

A 30-year-old Japanese man (Case 2) visited our department in March 2007 with a 20-year history of a red-brown atrophic plaque on the upper back. The

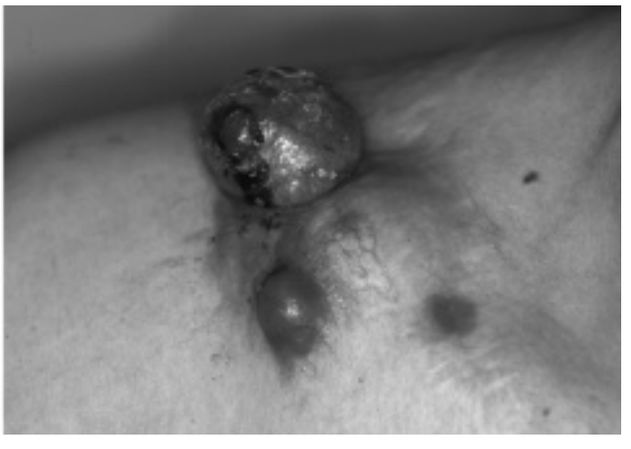

Fig. 1 (c). Clinical feature of Case 4: A cluster of multiple elastic hard reddish-brown nodules on the supraclavicle.

tumor was 20×20mm in size, depressed below the level of the surrounding skin, and had a smooth and regular surface with induration on palpation (Fig. 1a). Histological examinations of biopsied specimens established the diagnosis of DFSP. The plaque was surgically removed with a 3-cm margin beneath the fascia, and a split-thickness skin graft was performed to cover the defect. A histological examination of the excised tumor revealed a proliferation of spindle cells (mostly slender) in the dermis. The spindle-shaped cells exhibited a dense, poorly circumscribed, tumoral proliferation with a storiform arrangement. Immunohistochemical staining demonstrated that most of the tumor

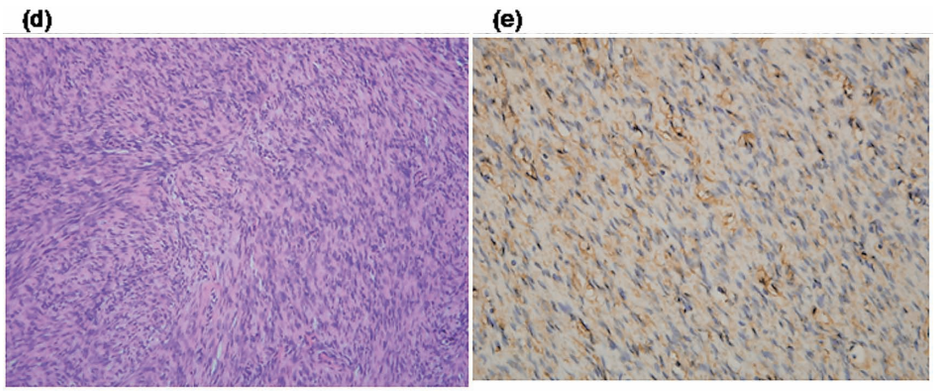

Fig. 1 (d). Histological aspect of case 4 : Uniform proliferation of spindle cells arranged in a monotonous storiform pattern.
(e). Immunohistochemical aspect of case 4 : Immunohistochemical stain for CD34 showed dense staining in spindle-shaped cells.

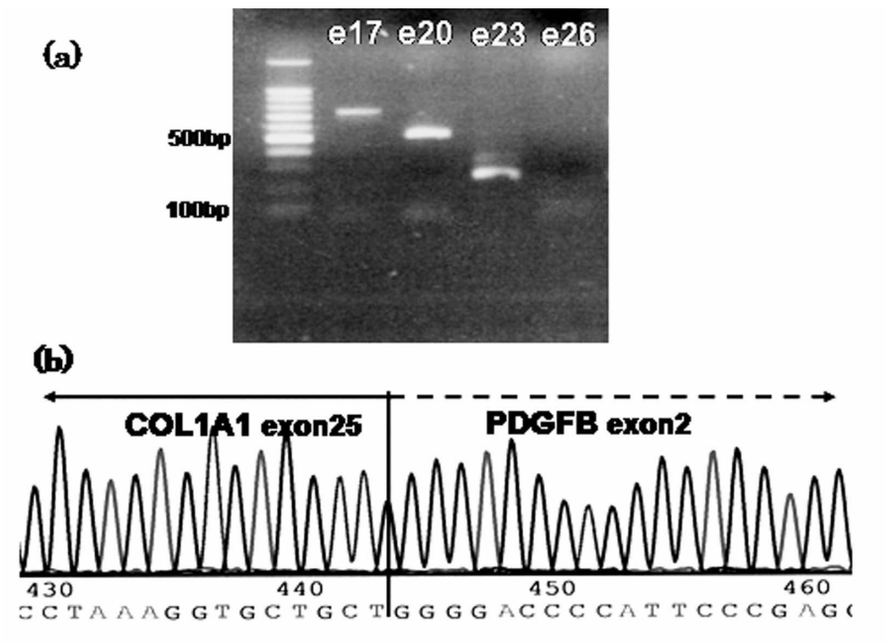

Fig 2. Expression of the *COL1A1-PDGFB* fusion transcript of case 1.
(a) Agarose gel stained by ethidium bromide showed RT-PCR products from the frozen tissue of case 1.
(b) Nucleotide sequence of the *COL1A1-PDGFB* fusion transcripts showed fusion of *COL1A1* exon 25 with PDGFB exon 2.

cells were CD34 positive. No local recurrence or distant metastasis has been found during the follow-up period.

A 35-year-old Japanese man (Case 3) presented with a 10-year history of an enlarging tumor on the lower abdominal wall. The tumor was erythematous and elastic hard, measuring 60×45mm (Fig. 1b). Histological examination confirmed a proliferation of $CD34^{+}$ spindle-shaped cells in a storiform arrangement. The tumor was surgically removed with a 3-cm margin beneath the fascia, and a split-thickness skin graft was performed to cover the defect.

A 49-year-old Japanese man (Case 4) visited our department in May 2007 complaining of an enlarging tumor that had been present for 10 years. Physical examination revealed a cluster of multiple red-brown firm nodules on the right supraclavicular region (Fig. 1c). After establishing the histological diagnosis of DFSP, we performed surgical resection with a 3-cm margin above the muscle, followed by a split-thickness skin graft. Histological examinations revealed long straight fascicles of spindle-shaped cells creating a storiform pattern (Fig. 1d). The tumor cells were CD34 positive (Fig. 1e). No local recurrence or distant metastasis has been found during the follow up period.

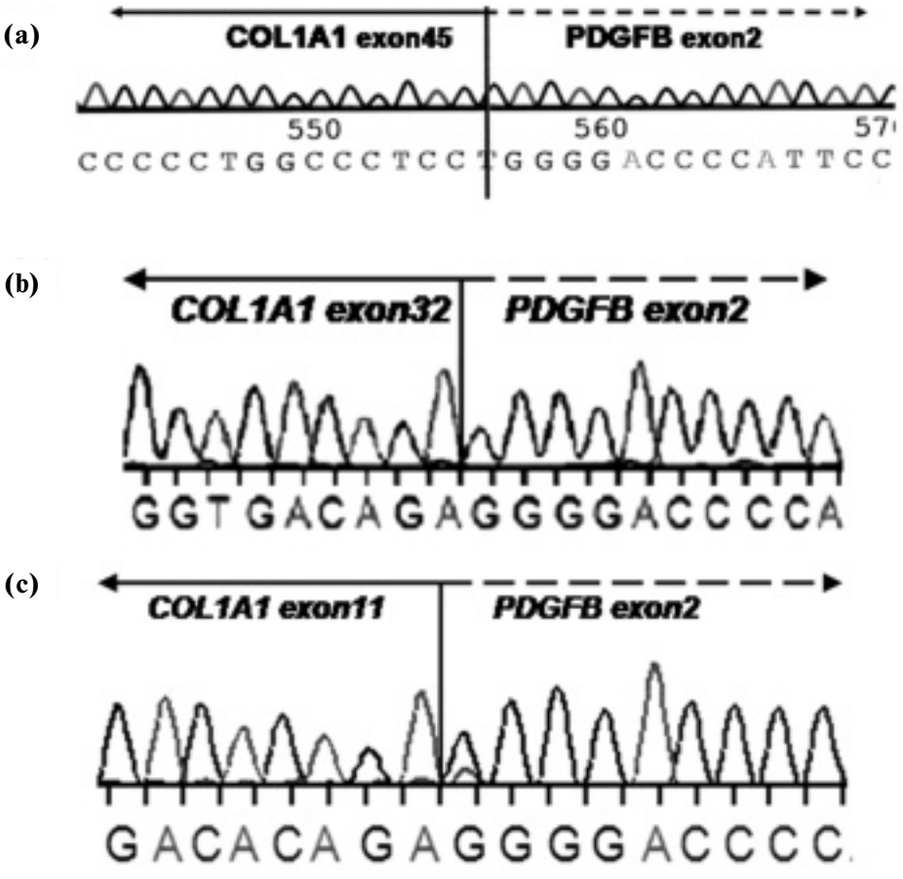

Fig 3. Nucleotide sequence of the *COL1A1-PDGFB* fusion transcripts.
(a) Case 2: exon 45 in the *COL1A1* gene was fused with exon 2 in the *PDGFB*
(b) Case 3: exon 32 in the *COL1A1* gene was fused with exon 2 in the *PDGFB*.
(c) Case 4: exon 11 in the *COL1A1* gene was fused with exon 2 in the *PDGFB*.

RT-PCR and sequencing

Figure 2 shows the results of the RT-PCR (a) and sequencing (b) of the *COL1A1-PDGFB* fusion transcript in case 1. PCR products were obtained by amplification with the *COL1A1* exon 23 primer and the *PDGFB* exon 2 primer. Nucleotide sequence analysis of the PCR product revealed that the end of exon 25 of the *COL1A1* gene was fused with the start of exon 2 of the *PDGFB* gene.

The results of RT-PCR analyses for the other cases are shown in Fig. 3 (a, b, c). Sequence analysis of the PCR products revealed that the end of the *COL1A1* gene (exon 45 in case 2, exon 32 in case 3, and exon 11 in case 4) was fused with the start of exon 2 in the respective *PDGFB* genes.

Discussion

DFSP is a locally aggressive fibrohistiocytic neoplasm of intermediate malignancy with a high incidence of local recurrence and a low risk of distant metastasis. Histologically, it is characterized by a dense and uniform proliferation of spindle-shaped cells, typically arranged in a storiform pattern. In most cases, the tumor cells are CD34 positive. However, diagnosis based on histological and immunohistochemical findings is not always easy, because DFSP often exhibits diverse patterns in both analyses. Recent cytogenetic and molecular studies have shown that fusion of the *COL1A1* gene with the *PDGFB* gene is specific to DFSP. The *COL1A1* gene encodes type I collagen, and *PDGFB* is a potent mitogen for a number of cell types.[1–3] Detection of the *COL1A1-PDGFB* fusion gene therefore provides a powerful complementary tool for the diagnosis of DFSP. The location of breakpoints within *COL1A1* varies greatly, but is always limited to the region encoding the alpha-helical domain.[5] The exons of the *COL1A1* gene segment end at the last base of a codon. The *PDGFB* segment of the chimeric transcript always starts with exon 2. The *COL1A1-PDGFB* fusion is in frame because exon 2 of the *PDGFB* gene starts at the first base of codon 22. Thus, the fused gene of *COL1A1* serves as an active promoter for *PDGFB*. The translocation removes negative regulatory elements at the 5′ end of the *PDGFB* gene and this potentiates protein production.

Various exons (7, 8, 10, 11, 18, 19, 22, 23, 24, 25, 26, 27, 29, 31, 32, 33/34, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, and 47) of the *COL1A1* gene have been shown to be involved in the fusion with the *PDGFB* gene.[4-9] In the present study, we determined that in our 4 patients the end of exons 25, 45, 32, or 11 of the *COL1A1* gene was fused with the start of exon 2 in the *PDGFB* gene. We identified no relationships between the different *COL1A1-PDGFB* fusion gene products and the clinical features noted in our patients ; however, since the number of cases was limited, we cannot rule out the possibility of such relationships.

Since extensive degradation of RNA can occur during the formalin fixation process, it is difficult to detect fusion transcripts when we use RNA extracted from paraffin embedded specimens. In previous studies, RNA extracted from formalin-fixed paraffin-embedded tissue was sometimes unavailable for subsequent RT-PCR analysis.[4,9-11] Therefore, we decided to use frozen tissue for RNA extraction, which enabled us to detect the fusion gene in all the cases of DFSP examined. This emphasizes the importance of keeping frozen tissue when encountering suspected DFSP tumors with atypical clinical or histological features. The detection of the *COL1A1-PDGFB* fusion gene provides us with strong and reliable evidence for establishing the diagnosis of DFSP.

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