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Detection of *COL1A1-PDGFB* fusion transcripts and PDGFB/PDGFRB mRNA expression in dermatofibrosarcoma protuberans

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Fusion of the collagen type I alpha 1 (COL1A1) gene with the platelet-derived growth factor beta chain (PDGFB) gene has been described in dermatofibrosarcoma protuberans. The abnormal fusion transcripts probably cause PDGFB and its receptor (platelet-derived growth factor receptor beta, PDGFRB) autocrine stimulation and cell proliferation, which are responsible for the development of dermatofibrosarcoma protuberans. A reverse transcription–polymerase chain reaction assay was performed to detect the *COL1A1-PDGFB* fusion transcripts in 57 samples. In addition, the *PDGFB* gene amplification and PDGFB/PDGFRB mRNA levels were quantified by a real-time PCR system for the samples in which the fusion transcripts had been successfully detected. The fusion transcripts were detected in 42 of 57 samples. Various exons of the *COL1A1* gene were fused in frame with the *PDGFB* gene; exons 7 and 25 were found to be slightly more frequently involved than the other exons. The *PDGFB* gene amplification levels varied from 0.6 to 8.3 (mean 2.4) in 42 tumor samples and from 0.4 to 3.0 (mean 1.2) in 20 adjacent normal tissue samples. In the 20 paired samples, the *PDGFB* gene amplification in the tumor was significantly higher than that in the normal tissue. The presence of PDGFB and PDGFRB mRNAs was demonstrated in 26 and 21 of 26 cases, respectively. The PDGFB and PDGFRB mRNA expression levels showed a good correlation ($r = 0.76$, $P < 0.0001$). These results indicate that the fusion protein, which is processed by the *COL1A1-PDGFB* transcripts, can serve as a functional ligand for PDGFRB.
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Keywords: dermatofibrosarcoma protuberans; *COL1A1-PDGFB* fusion transcripts; platelet-derived growth factor; platelet-derived growth factor receptor

Dermatofibrosarcoma protuberans is a dermal and subcutaneous tumor of intermediate malignancy. Tumors are slow growing and rarely metastasize, but they are prone to local recurrence after surgery. Cytogenetic features of dermatofibrosarcoma protuberans include a recurrent translocation t(17;22, q22;q13) and supernumerary ring chromosomes. Translocation 17;22 has been shown to result in a fusion of the platelet-derived growth factor beta chain (PDGFB) gene in 22q13 with the collagen type I alpha 1 (COL1A1) gene in 17q22. In all analyzed translocations, the breakpoints occur in the intron preceding exon 2 of the *PDGFB* gene, whereas the *COL1A1* part varies and includes various exons in the alpha-helical domain.[1] The fusion deletes all known elements that negatively control *PDGFB* transcription and translation, which are considered as oncogene-activating events.[2] The abnormal fusion transcripts probably cause PDGFB and its receptor (platelet-derived growth factor receptor beta, PDGFRB) autocrine stimulation and cell proliferation, which are responsible for the development of dermatofibrosarcoma protuberans. Recently, patients with unresectable dermatofibrosarcoma protuberans have been reported to be successfully

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treated with imatinib, a potent and specific inhibitor of several protein-tyrosine kinases, including PDGFRB.[3–6] Accordingly, both generations of the *COL1A1-PDGFB* fusion transcripts and their downstream PDGFB/PDGFRB signaling pathway are considered to play an important role in tumorigenesis and treatment of dermatofibrosarcoma protuberans. In this study, we detected the fusion gene transcripts and quantified the *PDGFB* gene amplification and PDGFB/PDGFRB mRNA levels by using a real-time polymerase chain reaction (PCR) system.

Materials and methods

Tumor Samples and Detection of Fusion Gene Transcripts

For nucleic acid extraction, 57 consecutive cases of dermatofibrosarcoma protuberans registered between 1975 and 2004 were retrieved from the files of the Department of Anatomic Pathology, Graduate School of Medical Sciences, Kyushu University, Japan. Since written informed consent was not obtained, for strict privacy protection, identifying information for all samples was removed before analysis; this procedure was in accordance with the Ethical Guidelines for Human Genome/Gene Research enacted by the Japanese Government. Frozen samples stored at −80°C were preferred because such materials would enable further genetic analyses. However, frozen materials could be obtained in only five cases. In the remaining 52 cases, the analysis was restricted to DNA and RNA extracted from the paraffin-embedded tissue. Genomic DNA was purified using standard proteinase K digestion and phenol/chloroform extraction. RNA was extracted from the frozen tissue according to the standard procedures. For RNA extraction from the paraffin-embedded tissue, 1 ml xylene was added to the paraffin curls and spun in a microcentrifuge for 5 min. Xylene was removed and the first step was repeated. The tissue was then washed twice in 1 ml 100% cold ethanol and spun for 5 min at a high speed; this was followed by vacuum drying for 7 min and overnight digestion at 55°C in 0.2 ml RNA digestion buffer (20 mM Tris-HCl (pH 8.0), 20 mM EDTA, 2% SDS in diethyl pyrocarbonate (DEPC) water), and 10 μl of 100 μg/μl proteinase K. After incubation, 1.0 ml of Trizol reagent (Gibco BRL, Gaithersburg, MD, USA) was added. Subsequently, 200 μl of chloroform was added, mixed well by vortexing, and spun at a high speed for 15 min at room temperature. The top aqueous RNA layer was transferred to another fresh tube and an equal volume of isopropanol was added. The content was spun for 15 min and the supernatant was removed, followed by washing in 70% ethanol. The dry pellet was left at room temperature for 15–20 min and 10–20 μl of DEPC water was then added. The quality of RNA was determined using an OD ratio of 1.6–1.8 and was found to be acceptable.

Reverse transcription was carried out using the SuperScript III First-Strand Synthesis System (Invitrogen, Japan), with the following modifications. Of a total volume of 20 μl, 2 μg RNA or a maximum allowable volume (8 μl) of RNA when it was below the measurable level was added. The first-strand cDNA synthesis was primed using the PDGFBexon2-1 antisense primer (Table 1). To amplify the *COL1A1-PDGFB* fusion transcripts,

Table 1 Oligonucleotides used for RNA extraction and detection of the *COL1A1-PDGFB* fusion transcripts

Exon	*Primer(5′-3′)*	*Exon*	*Primer(5′-3′)*
COL1A1			
6	gctccccagctgtcttatgg	31	cggcaacgatggtgctaag
7	ctctggtcctcgtggtctcc	32	gaacgtggtgcagctggtctt
8	tcagggtgctcgaggattgc[a]	33,34	gccccattggtcctcctg
10	aacctggtcgtcctggtgag	35	cagcggccctgctggt
12	gtggtttggatggtgccaag	36	agaccgtggtgagcctggt
14	cctgcctggtgagagaggtc	37	ctggtgctaaaggcgatgct
15	ggtgctcgtggaaatgatgg[a]	38	tgctcctggagccaaaggtgc[a]
17	agggtgtgcgtggtgagc	39	ggtgctactggtttccctggt
19	gctcctggcttccctggt	40	tgctggcgagaaaggatcccctg[a]
20	cctggcagcaaaggagacac	41	gaggcttccctggtcttcct
21	aggtgaacccggacccact	42	acccccctggtgaatctggac
23	aagctggtcgtcccggtgaagc[a]	44	tggcaagagtggtgatcgtgg[a]
24	ggcagccctggtcctgat	46	tggcttctctggcctccaggg[a]
25	caggctggtgtgatgggatt	47	gtccctctggagcctctggt
27	gcaaagatggagaggctgga	48	gtctccctggccccattg
29	ccaggtgaagcaggcaaacc	49	tgccgtgacctcaagatgtg
PDGFB			
Exon 2-1	atcaaaggagcggatcgagtggtc[a]		
Exon 2-2	tggtcactcagcatctcataaagc		

[a]The same primers as previously described.[7]

single-step PCR was carried out using a set of specific COL1A1 sense primers and the PDGFBexon2-2 antisense primer (Table 1). The COL1A1 primers corresponded to the alpha-helical domain of the *COL1A1* gene (exons 6–49). Some primers were the same as described previously;[7] however, other primers were designed using primer 3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). The PCR profile consisted of 45 cycles of denaturation at 94°C for 1 min, annealing at 66°C for 45 s, and elongation at 72°C for 50 s, followed by a final extension at 72°C for 10 min. The PCR products were visualized by ethidium bromide staining on a 3% agarose gel. The DNA band was purified using a SUPREC tube (TAKARA BIO, Tokyo, Japan) and the products were reamplified for 25 cycles. The amplified product was purified by Microcon centrifugal filter devices (Millipore, Bedford, MA, USA). After purification, direct sequencing was carried out by the dideoxy chain termination method using an ABI Prism 310 sequence analyzer (Applied Biosystems, Foster City, CA, USA).

Evaluation of the PDGFB Gene Amplification Level

Real-time quantitative PCR was performed on genomic DNA extracted from 42 cases in which the *COL1A1-PDGFB* fusion transcripts had been successfully detected. In 20 cases, adjacent normal tissue samples were also evaluated. An SYBR green assay was used to evaluate *PDGFB* gene amplification in comparison with a reference gene and a normal tissue. We selected the albumin gene located at 4q11-q13 as the reference gene because no genetic alteration has been detected in this chromosomal region, as determined by CGH studies.[8–10] PCR was performed using the ABI Prism 7700 Sequence Detection System (Applied Biosystems) according to the manufacturer's protocol. The *PDGFB* gene copy numbers were calculated from the standard curve constructed from normal DNA amplification. The sequences of the primers are summarized in Table 2. The primers for amplification of the albumin gene were the same as described previously.[11]

Evaluation of PDGFB and PDGFRB mRNA Expression

Quantitation of mRNA was performed using the TaqMan assay. Reverse transcription procedure was carried out in almost the same manner as described above, whereas the first-strand cDNA synthesis was primed using a random hexamer. The quality of the extracted RNA was assessed by RT-PCR using primers for PGK transcripts (189 bp).[12] Twenty-six cases that yielded a visible PCR product on a 2% agarose gel were selected for further analysis. The primers and TaqMan probe of PDGFB, PDGFRB, and GAPDH as an endogenous control were selected from predesigned TaqMan gene expression assays (assay ID: PDGFB, Hs00234042_m1; PDGFRB, Hs00182163_m1; GAPDH, Hs99999905_m1). PCR was performed using the ABI Prism 7700 Sequence Detection System. To construct the standard curve for the quantitation of PDGFB and PDGFRB mRNA levels, we evaluated five representative osteosarcoma cell lines (MG-63, U-2/OS, SaOS-2, OST, and MNNG/HOS) because several studies have demonstrated the expression of PDGF and PDGFR in these cell lines.[13,14] The standard curve was then constructed using 10-fold serially diluted total RNA extracted from the MNNG/HOS cell line, which showed the highest expression level (data not shown). The data obtained were standardized using the GAPDH data.

Statistical Analysis

A paired *t*-test was used to compare the *PDGFB* gene amplification level of the tumor tissue and that of the adjacent normal tissue samples. Linear regression and Pearson's correlation coefficient were used to determine correlation between the two parametric variables acquired from the quantitative PCR analyses. A *P*-value of less than 0.05 was considered statistically significant.

Results

Fusion Gene Transcripts and Clinicopathological Findings

The distribution of clinicopathological characteristics is outlined in Table 3. The *COL1A1-PDGFB* fusion transcripts were detected in 42 (74%) of 57 samples. Five samples were obtained from recurrent tumors, whereas the remaining samples were obtained from primary tumors. Of the 42 samples, 22 were ordinary dermatofibrosarcoma protuberans and 20 were dermatofibrosarcoma protuberans-associated histological variants (10 dermatofibrosarcoma protuberans with fibrosarcomatous component

Table 2 Oligonucleotides used for evaluation of the *PDGFB* gene amplification

	Primer (5′-3′)		*Primer (5′-3′)*
PDGFB exon 5 F	acggcctgtgacccgaag	PDGFB intron 5 R	ctgagcctggaaaggtggtt
Albumin F	tgaaacatacgttcccaaagagttt[a]	Albumin R	ctctccttctcagaaagtgtgcatat[a]

[a]The same primers as previously described.[11]

Table 3 Clinicopathological and molecular analysis data of dermatofibrosarcoma protuberans (DFSP)

Case	*Age*	*Sex*	*Location*	*Breakpoint of COL1A1*	*Histology*	*PDGFB gene amplification (tumor)*	*PDGFB gene amplification (normal)*	*mRNA level of PDGFB*	*mRNA level of PDGFRB*
1	27	F	Breast	Exon 6	DFSP	1.0	0.4	N/A	N/A
2	48	M	Clavicular region	Exon 7	Myxoid DFSP	1.2	N/A	N/A	N/A
3	13	F	Groin	Exon 7	DFSP	1.7	N/A	26877	948
4	31	F	Abdominal wall	Exon 7	DFSP	1.8	1.4	4261	39
5	N/A	N/A	N/A	Exon 7	DFSP	3.3	N/A	N/A	N/A
6	39	M	Chest wall	Exon 7	DFSP with FS[b]	2.1	1.3	N/A	N/A
7[a]	81	F	Groin	Exon 13	DFSP	2.1	1.3	1013	31
8	28	M	Abdominal wall	Exon 16	DFSP	1.8	1.3	7	U/L
9[a]	66	M	Groin	Exon 16	Myxoid DFSP	2.3	N/A	N/A	N/A
10	36	M	Chest wall	Exon 16	DFSP	1.4	N/A	114012	9612
11[a]	82	M	Abdominal wall	Exon 17	DFSP with FS	4.3	N/A	275	3
12	26	M	Abdominal wall	Exon 25	DFSP with FS	3.1	1.8	61513	2413
13	39	M	Chest wall	Exon 25	DFSP	2.1	1.5	5583	336
14	45	M	Chest wall	Exon 25	DFSP	1.9	1.6	N/A	N/A
15	61	F	Abdominal wall	Exon 25	DFSP	4.1	1.2	86451	1708
16	47	F	Chest wall	Exon 25	DFSP with FS	2.3	N/A	N/A	N/A
17	50	F	Chest wall	Exon 25	DFSP	1.7	N/A	N/A	N/A
18	52	M	Abdominal wall	Exon 25	DFSP	0.8	N/A	14406	98
19	55	F	Abdominal wall	Exon 26	DFSP with FS	5.0	N/A	N/A	N/A
20	41	M	Groin	Exon 32	DFSP	3.5	1.0	19239	838
21	31	F	Thigh	Exon 32	DFSP	2.8	1.4	N/A	N/A
22	64	F	Abdominal wall	Exon 32	DFSP	2.1	N/A	120	U/L
23	39	F	Back	Exon 34	Myxoid DFSP	3.6	N/A	379484	7605
24	41	M	Chest wall	Exon 35	DFSP	0.6	N/A	19007	U/L
25	N/A	N/A	N/A	Exon 35	DFSP	2.0	N/A	30321	997
26	43	F	Shoulder	Exon 37	DFSP with FS	2.6	N/A	41949	419
27[a]	28	F	Back	Exon 38	Bednar	5.0	3.0	N/A	N/A
28	64	M	Scapular region	Exon 40	SAF[c]	2.1	N/A	728	U/L
29	64	F	Groin	Exon 42	DFSP	2.8	0.6	31834	3050
30	26	F	Abdominal wall	Exon 42	DFSP with FS	1.8	N/A	N/A	N/A
31	52	F	Chest wall	Exon 42	SAF	2.2	N/A	N/A	N/A
32	31	F	Head	Exon 43	Bednar	0.6	1.0	99892	2308
33	31	M	Chest wall	Exon 44	DFSP	2.6	1.1	83134	2369
34	23	M	Groin	Exon 44	DFSP	1.5	N/A	16175	880
35	51	F	Chest wall	Exon 44	DFSP	0.9	1.0	10063	U/L
36	39	F	Back	Exon 46	DFSP	2.3	N/A	302	10
37	35	M	Back	Exon 46	SAF	8.3	N/A	N/A	N/A
38	49	M	Groin	Exon 47	SAF	2.3	N/A	N/A	N/A
39	27	M	Groin	Exon 47	DFSP with FS	0.6	1.1	28669	2294
40[a]	30	M	Cheek	Exon 47	Bednar	2.5	1.1	703	109
41	36	M	Shoulder	Exon 48	DFSP with FS	1.0	0.8	3631	8
42	26	F	Vulva	Exon 48	DFSP	1.7	0.8	N/A	N/A

N/A, not available; U/L, undetectable level.

[a]Recurrent tumors.

[b]Dermatofibrosarcoma protuberans with fibrosarcomatous component.

[c]Superficial adult fibrosarcoma.

(Figure 1a), four superficial adult fibrosarcoma (SAF, Figure 1b), three myxoid dermatofibrosarcoma protuberans (Figure 1c), and three Bednar tumor (Figure 1d)). Sequence analysis of the PCR products revealed that various exons of the *COL1A1* gene were fused in frame with the *PDGFB* gene (Table 3; Figure 2). Exons 7 and 25 were found to be slightly more frequently involved than the other exons. Bednar tumor and SAF showed a tendency to have distal breakpoints (exons 38, 40, 42, 43, 46, and 47); however, the distribution was not statistically significant. Among these genetically confirmed cases, the age, sex, and tumor location of two patients were unknown. The age of the patients when the primary tumor occurred varied from 13 to 64 years, and the mean age was 40.3 years. These tumors were located on the trunk in 36 patients and on the extremities and head in two patients each.

PDGFB Gene Amplification Level

The amplification level (copy number of *PDGFB*/copy number of *albumin*) varied from 0.6. to 8.3 (mean 2.4) in 42 tumor samples, and from 0.4 to 3.0 (mean 1.2) in 20 adjacent normal tissue samples (Table 3). In the 20 paired samples, the *PDGFB* gene amplification level in the tumor samples was

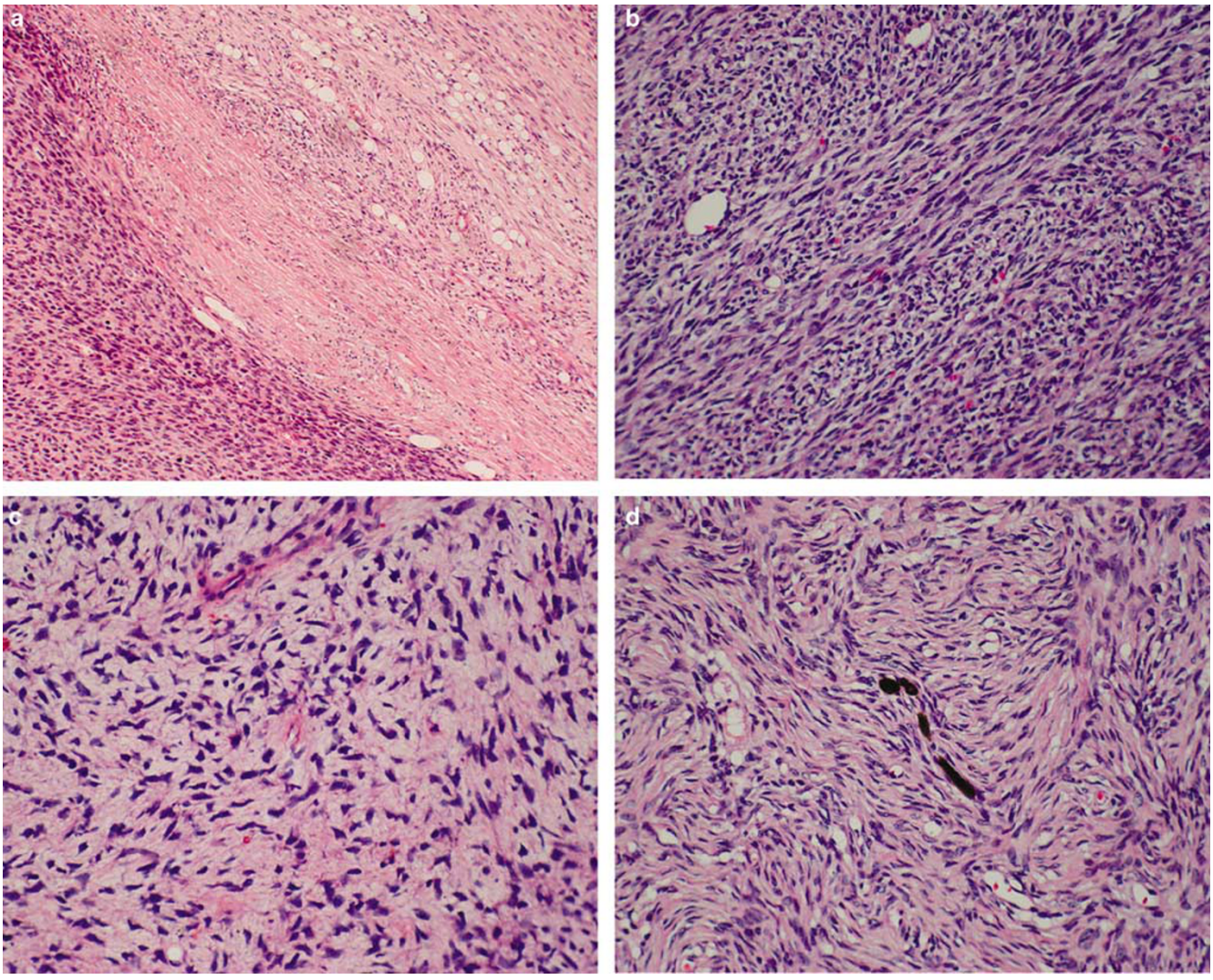

Figure 1 (**a–d**) Dermatofibrosarcoma protuberans-associated histological variants. (**a**) Dermatofibrosarcoma protuberans with a fibrosarcomatous component (case 16). This case shows an abrupt transition from ordinary dermatofibrosarcoma protuberans to the fibrosarcomatous component. (**b**) Superficial adult fibrosarcoma (case 38). (**c**) Myxoid dermatofibrosarcoma protuberans (case 23). (**d**) Bednar tumor (case 27).

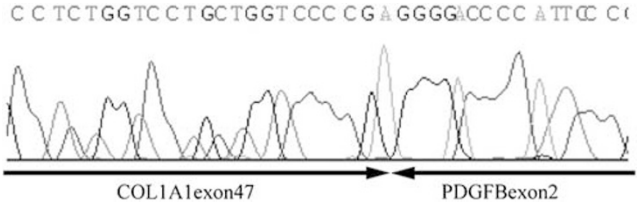

Figure 2 The *COL1A1-PDGFB* fusion transcript (case 39). The end of exon 47 in the *COL1A1* gene was fused with the start of exon 2 in the *PDGFB* gene.

significantly higher than that in the normal tissue samples (paired *t*-test, $P=0.0002$; Figure 3). Of the 42 tumor samples analyzed, three showed a deletion (level of amplification <0.7); in two of these three samples, the amplification levels were lower than that of the paired normal tissue. A correlation was not detected between the gene amplification level and clinicopathological parameters.

PDGFB and PDGFRB mRNA Expression

The analysis of mRNA expression was performed in 26 cases with adequate RNA quality. The presence of PDGFB and PDGFRB mRNAs was demonstrated in 26 (100%) and 21 (81%) of 26 cases, respectively. The expression level (PDGFB or PDGFRB mRNA value/GAPDH mRNA value) varied from 120 to 379 484 (mean 41525) for PDGFB expression, and from 3 to 9612 (mean 1718) for detectable PDGFRB expression. The detectable expression levels of both mRNAs showed a good correlation ($r=0.76$, $P<0.0001$; Figure 4); however, no correlation was observed between the PDGFB mRNA level and the *PDGFB* gene amplification level ($r=0.28$).

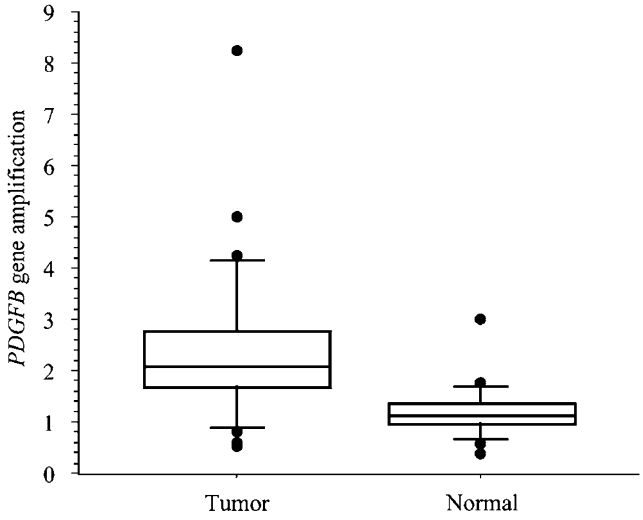

Figure 3 Box and whisker plot of the *PDGFB* gene amplification of the tumor samples and the adjacent normal tissue samples ($n=20$). A statistically significant difference was observed between the two groups (paired *t*-test, $P=0.0002$).

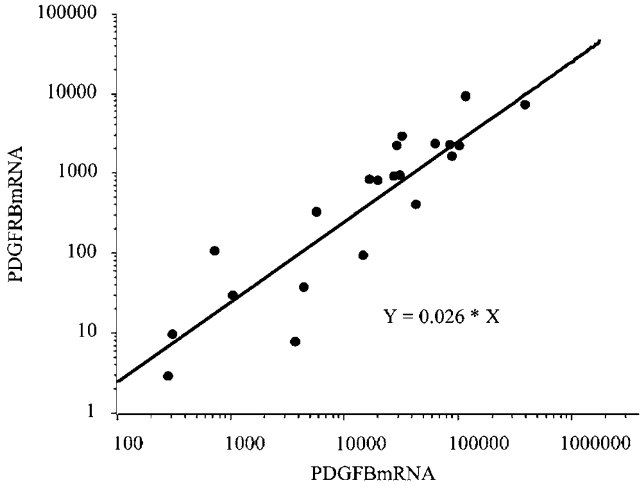

Figure 4 Relationship between the PDGFB and PDGFRB mRNA expression levels ($n=21$; $r=0.76$, $P<0.0001$, logarithmic scale).

Discussion

Karyotypic analyses of dermatofibrosarcoma protuberans have shown recurrent aberrations of chromosomes 17 and 22 and have led to the molecular definition of a characteristic rearrangement that fuses various exons of the *COL1A1* gene to exon 2 of the *PDGFB* gene. The *COL1A1-PDGFB* fusion transcripts have also been detected in the cells of tumors closely related to dermatofibrosarcoma protuberans, giant cell fibroblastoma,[2,15] Bednar tumor,[7] granular cell variant of dermatofibrosarcoma protuberans,[16] and SAF.[17] It has been reported that in cases with a fibrosarcomatous area in ordinary dermatofibrosarcoma protuberans or with fibrosarcoma in the recurrent or metastatic lesion, the tumor shows an aggressive clinical course.[18–23] By using a microdissection technique, Wang *et al*[24] detected identical *COL1A1-PDGFB* fusion transcripts in the two cellular components of dermatofibrosarcoma protuberans with fibrosarcomatous areas. In the present study, we detected *COL1A1-PDGFB* fusion transcripts in 42 of 57 cases that included 10 dermatofibrosarcoma protuberans with fibrosarcomatous areas, four SAF, three myxoid dermatofibrosarcoma protuberans, and three Bednar tumor. To our knowledge, this is the largest series of cases in which the *COL1A1-PDGFB* fusion transcripts were detected. 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; in the present study, exons 7 and 25 were found to be slightly more frequently involved than the other exons. Bednar tumor and SAF showed a tendency to have distal breakpoints; however, the number of cases with these histologic subtypes was too small.

The *COL1A1* gene encodes the major component of type I collagen, which is produced primarily by fibroblasts. The PDGFB protein is a potent mitogen for cells expressing functional PDGF receptors, typically cells of mesenchymal origin. The *COL1A1-PDGFB* fusion transcript is translated to form a precursor fusion protein that is processed into a mature and fully functional PDGFB protein—a ligand for PDGFRB.[25,26] Consequently, the abnormal fusion transcripts probably cause autocrine stimulation and cell proliferation, which are responsible for the development of dermatofibrosarcoma protuberans. To evaluate the importance of the autocrine signaling of PDGFB in dermatofibrosarcoma protuberans, in the present study, we quantified the *PDGFB* copy numbers and mRNA expression by using a real-time PCR system. We showed that the *PDGFB* gene amplification level in the tumor samples was significantly higher than that in the normal tissue samples. Our results were confirmed by similar results obtained in some CGH-based studies, which showed the t(17;22) translocation is usually associated with increased copy numbers of the *COL1A1* and *PDGFB* genes.[8–10,27] Interestingly, of the 42 tumor samples analyzed, three showed a deletion; in two of these three samples, the amplification levels were lower than that of the paired normal tissue. These cases were suggested to have unbalanced translocations instead of the supernumerary ring chromosome. Although all available samples showed PDGFB mRNA expression, the expression level widely varied and was not related to the *COL1A1* gene breakpoint. Gene amplification often results in an increased expression from the amplified locus (gene dosage effect). However, the present study showed no correlation between the gene amplification and mRNA expression levels of PDGFB. Two possible reasons were suggested to explain this discrepancy. The gene amplification level of the *PDGFB* gene in dermatofibrosarcoma protuberans is relatively low when

compared with the amplification observed in other tumors such as amplification of the *MYCN* gene in neuroblastoma or that of the *ERBB2* gene in breast carcinoma; these tumors frequently contain 20–50 copies of the amplified gene per cell. Consequently, the gene dosage effect in dermatofibrosarcoma protuberans was considered to be relatively limited. Another reason is the existence of other mechanisms that regulate PDGFB mRNA expression in addition to gene amplification. As mentioned above, the *PDGFB* segment of the fusion transcript starts with exon 2, placing the *PDGFB* gene under the control of the *COL1A1* promoter, and removing all known elements that negatively control *PDGFB* transcription and translation. It is known that the *COL1A1* promoter activity is sensitive to DNA methylation and the *COL1A1* gene is methylated in some human cancer cells.[28] Such epigenetic change could affect the transcription of the *COL1A1-PDGFB* fusion gene.

The mRNA expression levels of PDGFB and PDGFRB showed a good correlation. This indicates that the fusion protein, which is processed by the *COL1A1-PDGFB* transcripts, functions as a ligand for PDGFRB. The expression level varied, and in our PCR system, the expression level was below the detectable level in five of 26 available cases. There was no correlation between the PDGFRB expression level and histologic subtype of dermatofibrosarcoma protuberans. As mentioned earlier, dermatofibrosarcoma protuberans with fibrosarcomatous areas shows more aggressive clinical course than ordinary dermatofibrosarcoma protuberans. Histologically, dermatofibrosarcoma protuberans with fibrosarcomatous areas shows higher proliferative activity (higher mitotic rate or MIB-1 labeling index) than ordinary dermatofibrosarcoma protuberans.[23,29,30] Since no correlation was observed between the mRNA expression level of PDGFB/PDGFRB and the histologic subtype, the fibrosarcomatous transformation of dermatofibrosarcoma protuberans is thought to be caused by mechanisms such as the *TP53* gene mutation[29,31] or microsatellite instability[31] rather than the generation of the fusion gene and the activation of its downstream PDGFRB signaling pathway.

The optimal treatment option for dermatofibrosarcoma protuberans is complete surgical resection with wide margins. Recently, a limited number of clinical reports have suggested that imatinib, a potent and specific inhibitor of several protein-tyrosine kinases, including PDGFRB, can induce regression in patients with unresectable, recurrent, or metastatic dermatofibrosarcoma protuberans.[3–6] Maki *et al*[3] and McArthur *et al*[5] demonstrated that cases with no evidence of t(17;22) showed poor response to imatinib. However, some cases that carried a definite t(17;22) also showed a relatively poor response. Our study did not include patients treated with imatinib; therefore, further studies are needed to clarify the relationship between the clinical response to imatinib and PDGFRB expression level.

In summary, we detected the *COL1A1-PDGFB* fusion transcripts by using tumor specimens from 42 patients with dermatofibrosarcoma protuberans and dermatofibrosarcoma protuberans-related tumors. In addition, we investigated gene copy numbers of the *PDGFB* gene and the expression level of PDGFB/PDGFRB mRNA by using a quantitative real-time PCR system. The gene copy numbers of the tumor samples were significantly increased than those of the adjacent normal tissue samples. PDGFB mRNA expression was detected in all analyzed samples and a correlation was observed between the PDGFB and PDGFRB mRNA expression. These results indicate that the fusion protein, which is processed by the *COL1A1-PDGFB* transcripts, functions as a ligand for PDGFRB. This study demonstrated that the generation of the *COL1A1-PDGFB* fusion transcripts and the activation of the downstream PDGFB/PDGFRB signaling pathway were involved in tumorigenesis of dermatofibrosarcoma protuberans.

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