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A rapid and efficient newly established method to detect *COL1A1–PDGFB* gene fusion in dermatofibrosarcoma protuberans

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ABSTRACT

The detection of fusion transcripts of the collagen type 1α1 (*COL1A1*) and platelet-derived growth factor-BB (*PDGFB*) genes by genetic analysis has recognized as a reliable and valuable molecular tool for the diagnosis of dermatofibrosarcoma protuberans (DFSP). To detect the *COL1A1–PDGFB* fusion, almost previous reports performed reverse transcription polymerase chain reaction (RT-PCR) using multiplex forward primers from *COL1A1*. However, it has possible technical difficulties with respect to the handling of multiple primers and reagents in the procedure. The objective of this study is to establish a rapid, easy, and efficient one-step method of PCR using only a single primer pair to detect the fusion transcripts of the *COL1A1* and *PDGFB* in DFSP. To validate new method, we compared the results of RT-PCR in five patients of DFSP between the previous method using multiplex primers and our established one-step RT-PCR using a single primer pair. In all cases of DFSP, the *COL1A1–PDGFB* fusion was detected by both previous method and newly established one-step PCR. Importantly, we detected a novel *COL1A1* breakpoint in exon 5. The newly developed method is valuable to rapidly identify *COL1A1–PDGFB* fusion transcripts in DFSP.

1. Introduction

Dermatofibrosarcoma protuberans (DFSP) is an uncommon tumor in the dermis extending to the subcutaneous tissue [1]. Although it has been difficult to clinically distinguish DFSP from other fibrous tumors based on histology alone, the detection of fusion transcripts of the collagen type 1α1 (*COL1A1*) and platelet-derived growth factor-BB (*PDGFB*) genes by genetic analysis has recognized as a reliable and valuable molecular tool for diagnosis [2,3]. Characteristic cytogenetic features of DFSP include either supernumerary ring chromosomes composed of sequences derived from chromosome 17 and 22, or t(17; 22)(q22; q13) translocation [4]. Chimeric gene fusion induces the overexpression of PDGF, and paracrine or autocrine activation of PDGF receptor, which may lead to the development of DFSP [5].

To detect the *COL1A1–PDGFB* fusion, using reverse transcription polymerase chain reaction (RT-PCR) in combination with fluorescence in situ hybridization (FISH) is useful [2]. In RT-PCR analysis, almost all of previous reports performed reverse transcription polymerase chain reaction (RT-PCR) using 16 forward primers from *COL1A1* and a specific reverse primer from *PDGFB* exon 2, according to Wang et al. [6] with some modifications [2,7–9]. Sixteen forward primers were designed in *COL1A1* exons 5 to 49, and considered to be sufficient to span the various breakpoints within regions coding for the α-helical domain (Fig. 1A). As we previously reported, we could detect chimeric gene using these multiplex primers [7], however, this method can be complex due to the multiple primers and reagents and, therefore, cause possible technical difficulties in relation to the handling of many clinical samples. To overcome this complicated and time consuming problem, we developed a rapid, easy, and efficient one-step method of RT-PCR which uses only a single primer pair.

2. Materials and methods

2.1. Materials

Specimens after surgical excision in five patients of DFSP were used for analysis. Informed consent was obtained from all patients.

2.2. Previous method of RT-PCR using multiplex primers to identify the fusion transcripts of the COL1A1 and PDGFB genes

RNA was extracted from frozen specimens after surgical excision. Reverse transcription was performed using a Superscript III cDNA synthesis kit (Invitrogen, CA, USA). Next, two stages of PCR was carried out. In the first stage, the *PDGFB* reverse specific primer was combined with four different sets of *COL1A1* forward primers. In respective order: Set A spanned exon 5–15; set B spanned exon

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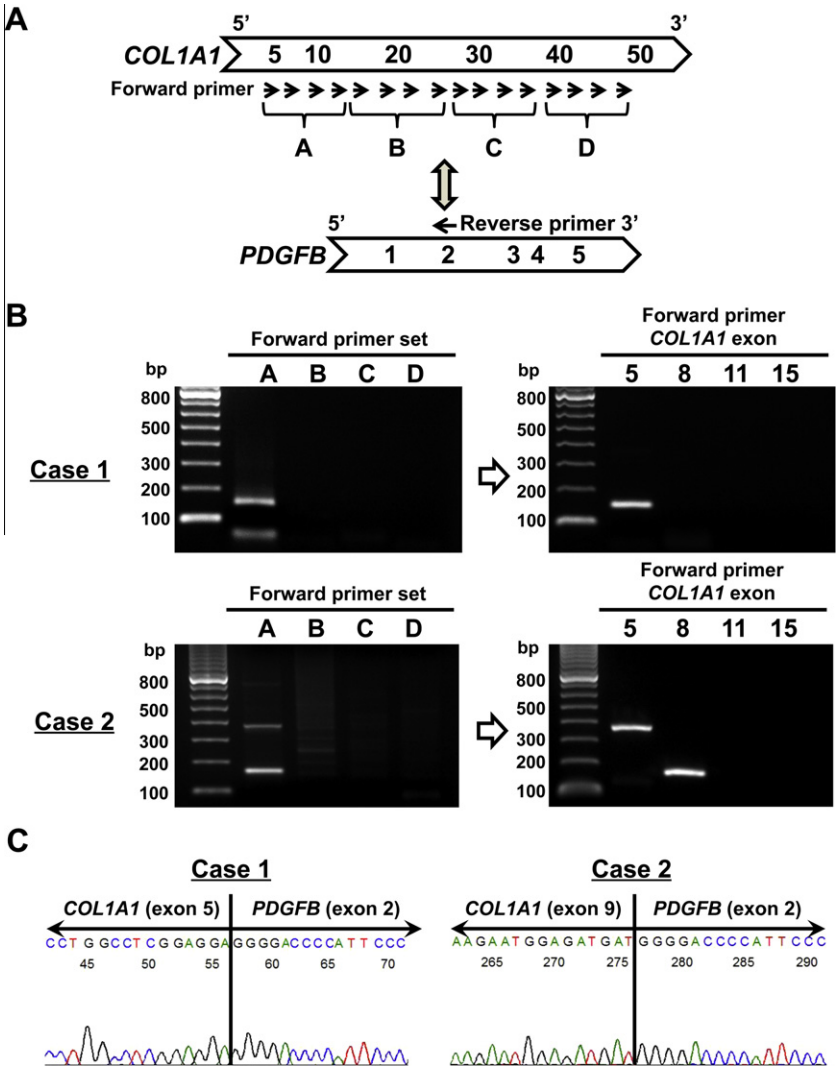

Fig. 1. Previous method of RT-PCR for detection *COL1A1–PDGFB* fusion transcripts (A) A scheme of 16 forward primers designed in *COL1A1* exons 5, 8, 11, 15, 17, 20, 23, 26, 27, 32, 35, 38, 40, 43, 46, and 49, and four different sets of *COL1A1* forward primers (set A: exon 5–15; set B: exon 17–25; set C: exon 29–38; and set D: exon 40–49). Specific reverse primer was designed in *PDGFB* exon 2. (B) Two stages of RT-PCR of COL1A1–PDGFB fusion transcripts in case 1 and case 2. In the first stage (left panel), *PDGFB* reverse specific primer was combined with four different sets of *COL1A1* forward primers, respectively (lane 1: set A (exon 5–15); lane 2: set B (exon 17–25); lane 3: set C (exon 29–38); lane 4: set D (exon 40–49)). In the second stage (right panel), PCR was performed using forward primer in *COL1A1* exons 5, 8, 11, 15, respectively (lane 1: exon 5; lane 2: exon 8; lane 3: exon 11; lane 4: exon 15). (C) Sequence analysis of the *COL1A1–PDGFB* fusion transcripts. In case 1, the *COL1A1* gene exon 5 was fused with the exon 2 in *PDGFB* gene. In case 2, the *COL1A1* gene exon 9 was fused with the exon 2 in *PDGFB* gene.

17–25; set C spanned exon 29–38; and set D spanned exon 40–49. (Fig. 1A) as previously described [8,9]. In the second stage, PCR was performed using forward primer in *COL1A1* exon 5, 8, 11, 15, respectively. The PCR products were directly sequenced using an ABI Prism 310 sequence analyzer.

2.3. Newly established one-step RT-PCR using a single primer pair to identify the fusion transcripts of the COL1A1 and PDGFB genes

We designed the forward primer in exon 2 of *COL1A1* gene, and reverse primer in exon 2 of *PDGFB* for long PCR according to the PCR application manual in Roche Applied Science (forward: 5′-GACGTGATCTGTGACGAGACCAAGAACTGCCC-3′; reverse: 5′-GCGTT GGAGATCATCAAAGGAGCGGATCGAGTGG-3′) (Fig. 2A). PCR was performed with Taq DNA polymerase (TaKaRa LA Taq, TAKARA Bio Inc. Shiga, Japan), and PCR amplification profile consisted of an initial cycle 94 °C for 1 min followed by 35 cycles of 98 °C for 10 s, and 68 °C for 10 min. Final extension was performed at 72 °C for 10 min.

3. Results

To validate new method, we compared the results of RT-PCR between the previous method and our newly established one-step PCR. RNA was extracted from frozen specimens of DFSP in five patients, and then reverse transcription was performed. The clinical and molecular features of the five cases with DFSP are summarized in Table 1. Next, two stages of PCR was carried out. In the first stage, the *PDGFB* reverse specific primer was combined with four different sets of *COL1A1* forward primers (Set A–D) (Fig. 1A). The PCR product was amplified with the forward primers set A in case 1, and case 2, indicating that the breakpoint in *COL1A1* gene was located upstream of exon 15 (Fig. 1B: left panel). In the second stage, PCR was performed using forward primer in *COL1A1* exon 5, 8, 11, 15, respectively (Fig. 1B: right panel). In case 1, PCR product was obtained by amplification with the forward *COL1A1* primer in exon 5, but not in exon 8, 11, 15 (Fig. 1B), suggesting that breakpoint of *COL1A1* was downstream of exon 5 primer, and upstream of exon 8 primer. In case 2, PCR product was detected using *COL1A1*

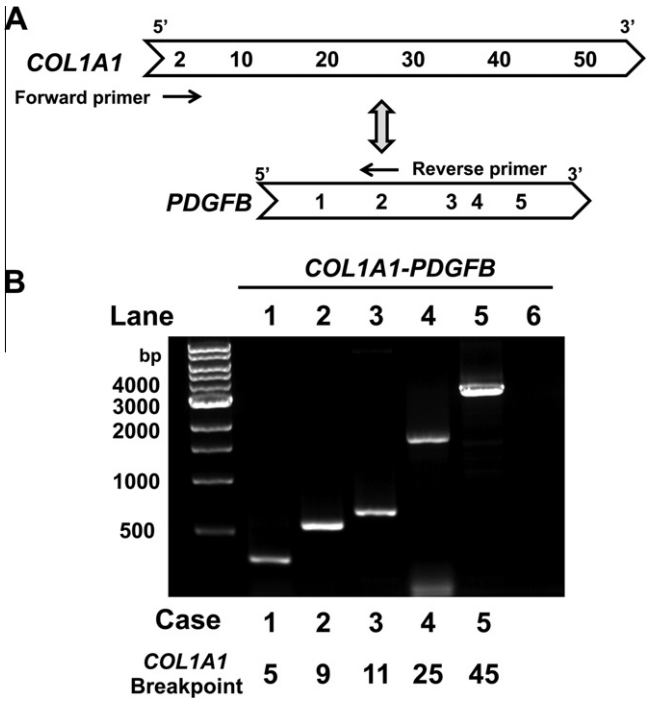

Fig. 2. Newly established RT-PCR method using single primer pair (A) A scheme of the forward primer in exon 2 of *COL1A1* gene, and reverse primer in exon 2 of *PDGFB* for long PCR. (B) RT-PCR using forward primer in exon 2 of *COL1A1* gene, and reverse primer in exon 2 of *PDGFB* in cases 1–5. In all cases of DFSP, amplification product was detected (lane 1: case 1 (333 bp); lane 2: case 2 (558 bp); lane 3: case 3 (666 bp); lane 4; case 4 (1629 bp); lane 5; case 5 (3231 bp)). No amplification products were detected in normal human skin (lane 6).

Table 1
Molecular and clinical features of 5 cases of DFSP.

Case	Age (y)/ Sex	Location	Size (cm)	*COL1A1* exon breakpoint
1	39/F	Abdomen	1 × 1	5
2	51/M	Abdomen	5.8 × 5.5 × 3.5	9
3	49/M	Supraclavicular area	150 × 55	11
4	30/F	Arm	10	25
5	30/M	Back	20 × 20	45

primer in exons 5 and 8 (Fig. 1B), suggesting that breakpoint of *COL1A1* was downstream of exon 8 primer, and upstream of exon 11 primer.

Sequence analysis of PCR product confirmed that *COL1A1* gene exon 5 was fused with exon 2 in *PDGFB* genes (case 1: Fig. 1C), and that *COL1A1* gene exon 9 was fused in case 2 (Fig. 1C). In addition, we identified that the breakpoints of *COL1A1* gene were located in exon 11 (case 3), 25 (case 4), and 45 (case 5) by the previous method (Table 1).

Next, we performed a newly established one-step PCR using a single primer pair. We designed the forward primer in exon 2 of *COL1A1* gene, and reverse primer in exon 2 of *PDGFB* for long PCR (Fig. 2A). In all cases of DFSP, amplification product was detected using forward primer in exon 2 of *COL1A1* gene and reverse primer in exon 2 of *PDGFB* (Fig. 2B). In case 2 (lane 2), approximately 560 bp of amplification product was detected, indicating that the breakpoint in *COL1A1* gene was located at around 560 bp downstream from forward primer, which is within exon 9. This result was consistent with the result by original method in Fig. 1. The size of amplification product in all other cases of DFSP was also consistent with the breakpoint in *COL1A1* gene detected by original method in Fig. 1 (Fig. 2B, lanes 1, 3–5). These results indicate that our newly established method of PCR is reliable, rapid and efficient for detection of COL1A1–PDGFB fusion transcripts.

4. Discussion

DFSP is a dermal tumor of intermediate malignancy which has 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, and tumor cells are positive for CD34. Several clinical and histological variants are described, including pigmented Bednar tumor, fibrosarcoma-like DFSP, and giant cell fibroblastoma-like DFSP [10]. Since DFSP often exhibits diverse patterns in clinical and histological findings, diagnosis is not always easy. Recent cytogenic and molecular studies have shown that the *COL1A1–PDGFB* fusion is specific to DFSP. Therefore, detection of the *COL1A1–PDGFB* fusion provides a powerful complementary tool for the diagnosis of DFSP. In addition, detection of the *COL1A1–PDGFB* fusion is helpful for deciding whether to use PDGF inhibitors for treatment of DFSP.

To detect the fusion, using RT-PCR and FISH analysis is useful [2]. Giacchero et al. suggested that the determination of the COL1A1 break point position within the fusion gene dose not provide any medically valuable information, and therefore FISH analysis seems to be a more appropriate method than multiplex RT-PCR for routine detection of fusion [11]. However, they also indicated that the RT-PCR and sequencing analysis should be reserved for validation of unexpected negative FISH results and for research prospective purposes.

In RT-PCR analysis, almost all of previous reports performed RT-PCR using a large number of forward primers from *COL1A1* to cover all *COL1A1* gene breakpoints [2,6–9]. In this study, we established the one-step method of PCR which uses only a single primer pair to detect the *COL1A1–PDGFB* fusion. This technique solves the problems in previous method of PCR using 16 forward primers, including complicated and time consuming problem.

Mahajan et al. also reported a case of DFSP identified the *COL1A1–PDGFB* fusion by RT-PCR using a single primer [12]. They designed the forward primer COL1A1FC2 which is specific to the collagen triple helix repeat F01391 within the *COL1A1* gene, and reverse primer in exon 2 of *PDGFB*. This forward primer COL1A1FC2 binds *COL1A1* gene at 21 sites within the total gene transcript, indicating that forward primer binds to multiple sites in *COL1A1* gene. Our established method also uses a single primer pair. However, forward primer binds to specific site in exon 2 of *COL1A1* gene, and single PCR product is amplified by our established PCR method (see Fig. 2B), and the location of breakpoint can be predicted from size of PCR product. In contrast, various lengths of PCR products might be amplified using the PCR method by Mahajan et al., because forward primer binds to multiple sites within *COL1A1* gene. In addition, it is not possible to predict the location of breakpoints within *COL1A1* before sequencing from the result of PCR Mahajan et al. proposed. These findings suggest that our established PCR method has different theory, and might be more simple compared with that proposed by Mahajan et al.

Giacchero et al. summarized 172 cases of DFSP with *COL1A1–PDGFB* fusion including previously published cases [11]. They confirmed the very high variability of the *COL1A1* break point position which is localized in 38 different *COL1A1* exons. To the best of our knowledge including the result of Giacchero et al. and other previously published results, there has been no report of the *COL1A1* breakpoint in exon 5. Thus, this is the first report of DFSP with COL1A1 (exon 5)-PDGFB (exon 2) fusion transcripts (case 1: Figs.1B, C, and 2B).

In conclusion, we identified a novel *COL1A1* breakpoint in exon 5, and the newly developed method of RT-PCR using a single-pair primer is valuable to rapidly identify *COL1A1–PDGFB* fusion transcripts in DFSP.

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