

Case Report

Atrophic dermatofibrosarcoma protuberans with the fusion gene COL1A1-PDGFB detected by RT-PCR using only a single primer pair

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Received April 4, 2015; Accepted May 19, 2015; Epub June 1, 2015; Published June 15, 2015

Abstract: Dermatofibrosarcoma protuberans (DFSPs) is an uncommon dermal tumor of intermediate to low-grade malignancy. A few patients have clinically persistent plaques that might be atrophic, and they are difficult to be diagnosed clinically. With the development of cytogenetic and molecular biology techniques, the detection of fusion transcripts of the collagen type 1a1 (COL1A1) and platelet-derived growth factor-BB (PDGFB) genes has been recognized as a reliable and valuable molecular tool for the diagnosis of DFSPs. We reported a 24-year-old woman who had a 2 years history of atrophic DFSPs, and detected the gene fusion between COL1A1 to PDGFB by one-step method of RT-PCR using only a single primer pair. The gene fusion detected by this rapid and efficient one-step method in our patient appears to be the first report of atrophic DFSPs, and we detected a novel COL1A1 breakpoint between exon 2 and exon 3.

Keywords: Dermatofibrosarcoma protuberans, atrophic, COL1A1-PDGFB, CD34, RT-PCR

Introduction

Dermatofibrosarcoma protuberans (DFSPs) is a locally aggressive mesenchymal neoplasm [1, 2]. The lesions of this tumor usually present as indurated plaques that protrude above the surface of the skin [3]. A few patients have clinically persistent plaques that might be atrophic, and the atrophic variant is a rare form of the tumor [4]. Although it has been difficult to clinically distinguish DFSPs from other fibrous tumors just based on histology, the detection of fusion transcripts of the collagen type 1a1 (COL1A1) and platelet derived growth factor-B (PDGFB) genes by genetic analysis has been recognized as a reliable and valuable molecular tool for diagnosis [5]. To detect the COL1A1-PDGFB fusion, using reverse transcription polymerase chain reaction (RT-PCR) or fluorescence in situ hybridization is useful [6].

In RT-PCR analysis, almost all of previous reports performed RT-PCR using 16 forward primers from COL1A1 and a specific reverse primer from PDGFB exon 2 [7]. In Yoko et al [8]

study, they established a one-step method of RT-PCR which used 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.

Here we reported a 24-year-old woman who had a 2 years history of atrophic DFSPs, and detected the gene fusion between COL1A1 to PDGFB.

Case report

In Nov. 2013, a 24-year-old Chinese woman presented to our department of dermatology with a 2 years history of an asymptomatic, slowly enlarging rash located on the chest. Physical examination showed a smooth-surfaced, round-like, depressed plaque on the chest area. On palpation the rash was atrophic with no indurations (**Figure 1**).

Histopathology of this neoplasm was composed of monomorphic spindle cells that aligned hori-



Figure 1. Clinical presentation, a smooth-surfaced, round-like, depressed plaque on the chest area.

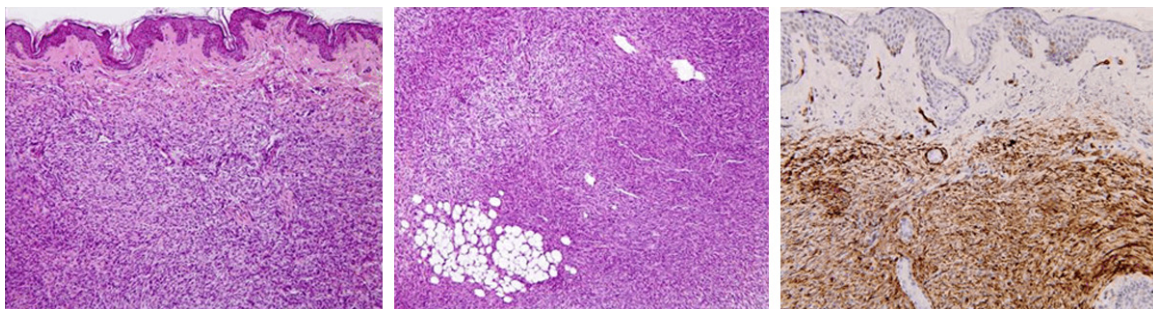


Figure 2. Photomicrograph showing normal epidermis. The dermis is diffusely infiltrated by spindle shaped cells embedded in a fibrous stroma and arranged in a storiform pattern (H.E. $\times 100$). CD34 immunostaining showing diffuse positive staining of neoplastic cells (immunoperoxidase $\times 400$).

zonally to the epidermis; the spindle cells were immunohistochemically positive for human progenitor cell antigen CD34 (**Figure 2**) and negative for factor XIIIa.

The lesion was diagnosed as atrophic variant of DFSPs and was excised with conventional operation. The patient remains without evidence of recurrent after 6 months of follow-up.

Methods and results

Written informed consent was obtained from the patient and her parents. RNA was extracted from paraffin-embedded tissues after biopsy in this patient using RNA Extraction Kit (CWbio. Co. Ltd, Cat#CW0535). Reverse transcription was performed using HiFi-MMLV first chain cDNA synthesis kit (CWbio. Co. Ltd, Cat#CW0744). The target gene is fused by the COL1A1 gene on chromosome 17 to PDGFB on chromosome 22 (**Figure 3A**). According to Yoko et al [8] study, we designed the forward primer

in exon 2 of COL1A1, and reverse primer in exon 2 of PDGFB (forward: 5'-GACGTGATCTGTGACGAGACCAAGAACTGCCC-3'; reverse: 5'-GCGTTGGAGATCATCAAAGGAGCGGATCGAGTGG-3') (**Figure 3B**).

PCR amplification profile, Using 2*GoldStar Taq MasterMix (CWbio. Co. Ltd, Cat#CW-0960), 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. The amplification product was detected using forward primer in exon 2 of COL1A1 gene and reverse primer in exon 2 of PDGFB (**Figure 3C**).

We did base sequencing analysis of the forward of PCR product, and confirmed the sequence as: ACGTCGAGAGGTCGTCGATCGCTCTTTGATGATCTCCAACGCA, then compared the result of base sequencing to the mRNA of PDGFB by BLAST in NCBI: almost matching (**Figure 4**).

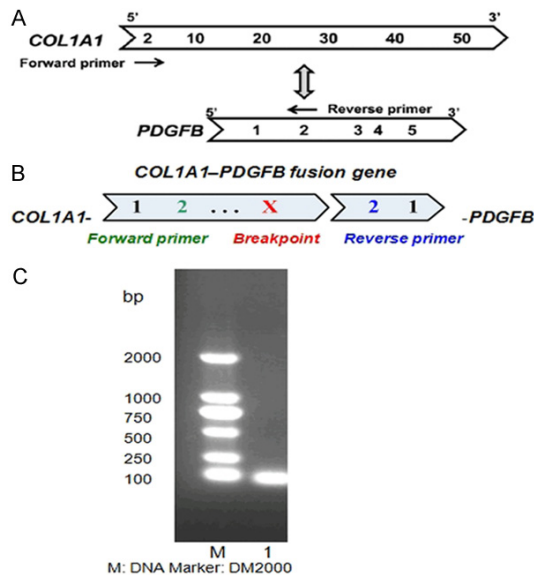


Figure 3. One step RT-PCR method using gl primer pair. A. A scheme of the forward primer in exon 2 of COL1A1 gene, and reverse primer in exon 2 of GF for long PCR. B. COL1A1DGB fusion gene. C. RT-PCR using forward primer in exon 2 of COL1A1 gene, and reverse primer in exon 2 of PDGFB in cases 1. In the cases of atrophic DFSP, amplification product was detected (lane1: 96 bp).

We did base sequencing for backward of the PCR product, and confirmed the sequence as: TCGTCAAGCGTGAGTTCTTGGTCTCGTCACAGAT-CACGTC, then, compared the result of base sequencing to the mRNA of COL1A1: almost matching. In our case, approximately 90 bp of PCR product was detected, indicating that the breakpoint in COL1A1 gene was located at around 90 bp down-stream from the forward primer in exon 2, which is between exon 2 and exon 3 (Figure 5).

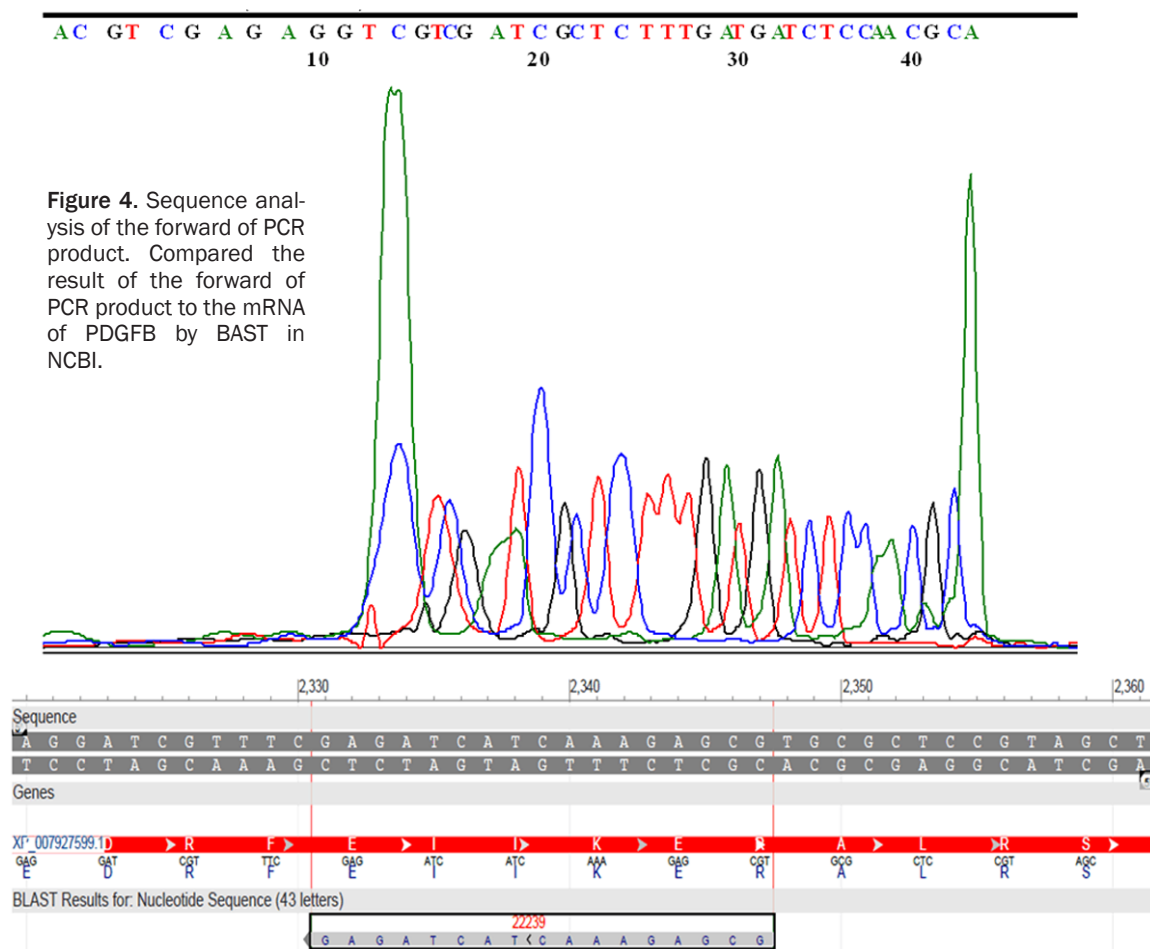
Discussion

DFSPs is an uncommon aggressive mesenchymal neoplasm with an intermediate grade and a high rate of local recurrence and a low risk of distant metastasis [1, 9]. The incidence is no difference between Men and women. The mean age at disease onset was 34.4 years with 50% aged between 20 and 40 years [2]. Involved sites were most often on the trunk and the head and neck [10, 11], and poor prognosis [12, 13]. A common clinical feature is a single firm nodule or multiple papules with erythematous or skin-colored base that sometimes remain unchanged for several years [9, 14]. Some patients have clinically persistent pla-

ques that might be atrophic. The atrophic variant of DFSPs may be confused with some common skin diseases with atrophic appearance [4]. These atrophic plaques are believed to represent early lesions in the course of DFSPs, and have rarely been reported and characterized in detail [15]. 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 and negative for factor XIIIa [9, 16]. Immunostains are helpful to differentiate DFSPs from other fibrohistiocytic neoplasms [17].

The standard treatment of the DFSPs is local excision with wide margins [18]. Mohs surgery allows complete examination of the margins while sparing the maximum amount of healthy tissue [19]. DFSPs with positive margins following conventional surgery should undergo repeat excision because in the majority of such cases residual tumor is detected on histological evaluation [20]. percutaneous cryoablation may be a relatively safe and efficient technique for the treatment of local recurrence of DFSPs [21]. Imatinib mesylate, targeting platelet-derived growth factor receptor beta (PDGF-BB), has clinical potential in DFSPs. It has been demonstrated that imatinib mesylate inhibits the tyrosine kinase activity of PDGF-BB and causes apoptosis of the tumor cells [22]. Dhir et al [23] described the first case of unresectable pancreatic metastases from DFSPs treated with neoadjuvant Imatinib and subsequently R0 metastectomy.

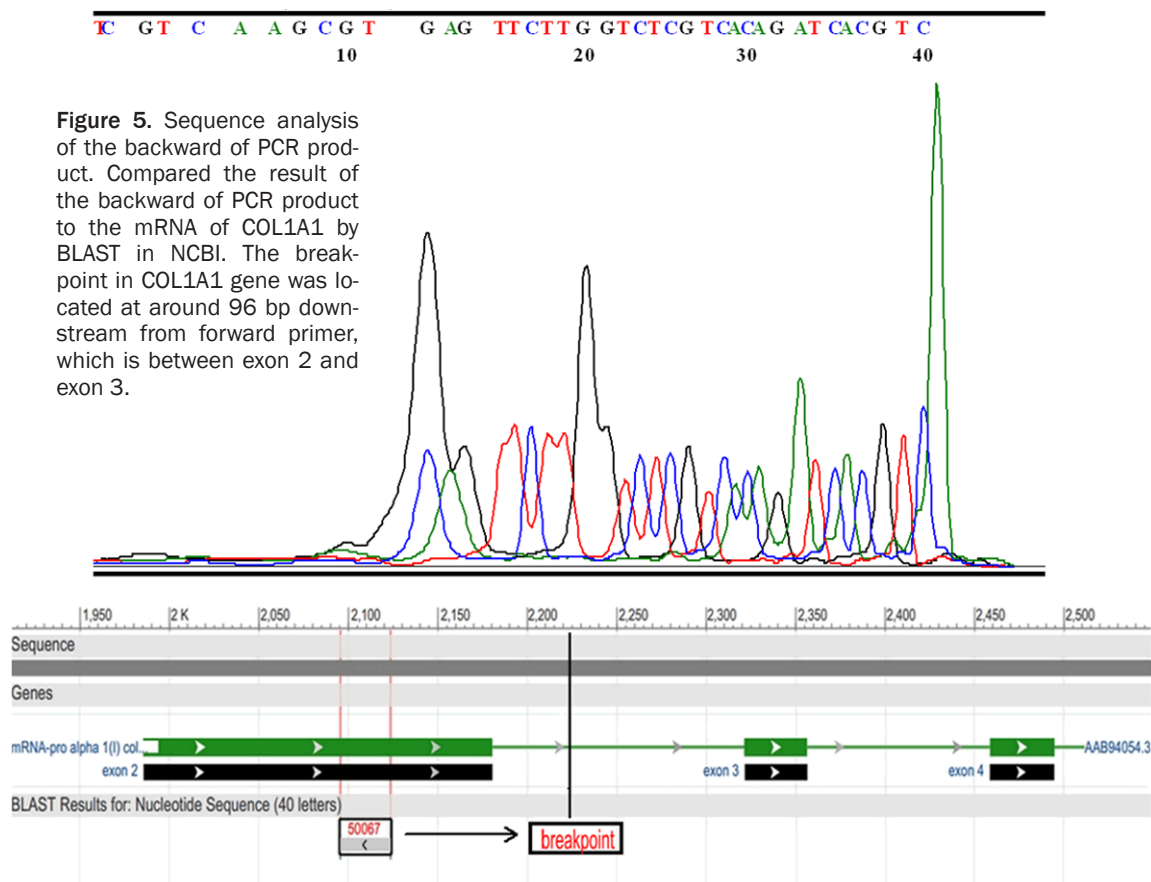
Recent cytogenic and molecular studies have shown that the COL1A1-PDGFB fusion is specific to DFSPs [6, 24]. Therefore, detection of the COL1A1-PDGFB fusion provides a powerful complementary tool for the diagnosis of DFSP. Nearly all DFSPs carry a t(17;22) (q22;q13) chromosomal translocation, which fuses the COL1A1 gene on chromosome 17 to PDGFB on chromosome 22, and result in a chimeric COL1A1-PDGFB protein, few DFSPs has negative gene change [25]. The translocation can be identified by fluorescence in situ hybridization, or RT-PCR, and confirmed by sequencing of the COL1A1/PDGFB. In the fusion gene, PDGFB is placed under the direct control of the COL1A1 gene, leading to an unscheduled production of PDGFB, a potent mitogen. Overproduction of PDGFB constitutively activates



the kinase activity of PDGF receptors through autocrine stimulation, which may lead to the development of DFSPs [5]. As other authors, it is believed that the different types of fusion between COL1A1 and PDGFB are not related with the clinical or histological features [26]. Therefore, detection of the COL1A1-PDGFB fusion gene can be a useful tool for differential diagnosis of DFSPs with other entities as well as in cases of unusual anatomic location, peculiar clinicopathological presentation, or poorly informative immunohistochemical staining (CD34 negative).

In RT-PCR analysis, almost all of previous reports performed RTPCR using a large number of forward primers from COL1A1 to cover all COL1A1 gene breakpoints and a specific reverse primer from PDGFB exon 2 [7, 24]. However, it has possible technical difficulties with respect to the handling of multiple primers and reagents in the procedure. In Yoko's study [8], they established a one-step method of PCR

which uses only a single primer pair to detect the COL1A1-PDGFB fusion. To validate the new method, they compared the results of RT-PCR in five patients of DFSPs between the previous method using multiplex primers and their established one-step RT-PCR using a single primer pair. In all cases of DFSPs, the COL1A1-PDGFB fusion was detected by both previous method and newly established one-step PCR. Therefore, the newly developed method was valuable to rapidly identify COL1A1-PDGFB fusion transcripts in DFSP. and it can find out the breakpoint of COL1A1 gene, just as our case, it can indicate that the breakpoint in COL1A1 gene was located at around 90 bp down-stream from forward primer. This technique solves the problems in previous method of PCR using 16 forward primers, including complicated and time consuming problem. What's more, it appears to be the first report of the fusion gene with the new breakpoint detected by the one-step RT-PCR in our patient with atrophic DFSPs



Because of the one-step RT-PCR technology, the time is obviously shortened to detect the fusion gene COL1A1-PDGFB, the clinical prospects of genetic diagnosis is improved in DFSPs. As everyone knows, the first changes of tumorigenesis is in genetic level, and the second changes is in cellular level, at last, the clinical manifestation, as mass, are appeared. Currently the main technology of tumor diagnose is the histopathology, combined with immunohistochemical when it is necessary. This technology detected the changes of cell and tissue, obviously it is later than genetic changes. There may theoretically appear the coincidence as follow, a cell in the edge of neoplasm has no significantly characteristic changes of tumor cells, but its gene may be already carcinogenesis, in this condition, the histopathologic examination may make misdiagnosis, and the genetic testing is more advantageous. If we use the one-step RT-PCR technique to detect the edge of tumor in Mohs surgery, it may be more rapid, objective, accurate than the frozen section pathological test.

Conclusion

We diagnosed a case of atrophic DFSPs with the fusion gene COL1A1-PDGFB detected by one-step RT-PCR using only a single primer pair. Importantly. The fusion gene with the new breakpoint detected by the RT-PCR method in our patient appears to be the first report in atrophic DFSPs.

Acknowledgements

This study was supported by the China National Key Technology Support Program (NO. 2007-BAI20B047). Written informed consent was obtained from the patient for publication of this case and any accompanying images.

Disclosure of conflict of interest

None.

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