ORIGINAL ARTICLE

Transformed dermatofibrosarcoma protuberans: real time polymerase chain reaction detection of COL1A1–PDGFB fusion transcripts in sarcomatous areas

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Background: Recent cytogenetic studies have shown that reciprocal translocation t(17;22)(q22;q13) and a supernumerary ring chromosome derived from the translocation r(17;22) are highly characteristic of dermatofibrosarcoma protuberans (DFSP). The chromosomal rearrangements fuse the collagen type I α 1 (COL1A1) and the platelet-derived growth factor B-chain (PDGFB) genes. The COL1A1–PDGFB fusion transcript has been shown not only in conventional DFSP but also in a small series of DFSP with fibrosarcomatous areas (DFSP-FS) using reverse transcriptase-based conventional polymerase chain reaction. Nothing is known about the status of the COL1A1–PDGFB chimaeric gene in the pleomorphic areas of DFSP-PleoSarc (formerly known as DFSP-malignant fibrous sarcoma).

Aims: To show the COL1A1–PDGFB fusion transcript in transformed malignant fibrous histiocytoma.

Method: A real-time polymerase chain reaction assay for the COL1A1–PDGFB fusion transcript in a series of DFSP containing sarcoma was conducted to determine whether the chimaeric gene could be identified in both components of DFSP-FS and DFSP-PleoSarc. Eight cases were analysed.

Results: In seven cases, transcriptable RNA was detected, and in these cases, translocations were found between COL1A1 and PDGFB genes involving exons 27, 32, 34, 40 and 47 of the COL1A1 gene and exon 2 of the PDGFB gene.

Conclusions: From a diagnostic aspect, this assay can be particularly useful in confirming the diagnosis of sarcomatous DFSP. On the other hand, the COL1A1–PDGFB fusion gene was shown in three cases of DFSP containing pleomorphic sarcoma, which supports the theory of the common histogenesis.

Dermatofibrosarcoma protuberans (DFSP) is a fibrohistiocytic tumour of intermediate malignancy. DFSP is morphologically heterogeneous; several rare variants have been described. Most of the variants of DFSP are not associated with marked differences in clinical behaviour.^{1–2} A small number of cases of DFSP contain areas of fibrosarcoma (DFSP-FS) or, more rarely, pleomorphic high-grade sarcoma (DFSP-PleoSarc; formerly known as malignant fibrous histiocytoma). Sarcomatous change in DFSP represents a form of tumour progression, and is associated with a prognosis worse than ordinary DFSP.^{3–14}

Recent cytogenetic studies have shown that reciprocal translocation t(17;22)(q22;q13) and a supernumerary ring chromosome derived from the translocation r(17;22) are highly characteristic of DFSP. The chromosomal rearrangements fuse the collagen type I α 1 (COL1A1) and the platelet-derived growth factor B-chain (PDGFB) genes.^{15–17}

The fusion causes deregulation of the PDGF gene by removing upstream sequences from exon 2 and placing then under the direct control of the COL1A1 gene. This gene rearrangement results in uncontrolled production of the growth factor, which seems to be the most important genetic event in the pathogenesis of DFSP.^{18–23} Detection of the COL1A1–PDGFB chimaeric mRNA by a reverse transcription-polymerase chain reaction (RT-PCR) assay has also proved to be a useful diagnostic marker for DFSP.^{24–27}

Moreover, the COL1A1–PDGFB fusion transcript has been shown not only in conventional DFSP but also in a small series of DFSP-FS using reverse transcription-based conventional polymerase chain reaction.^{28–30} Nothing is known about the status of the COL1A1–PDGFB chimaeric gene in the pleomorphic areas of DFSP-PleoSarc.

By contrast, real-time PCR plays an increasingly important part in clinical testing, because it is objective, rapid, cost effective and can be performed on small tissue samples. Advantages of real-time methods include the elimination of the need for post-PCR processing and confirmation of PCR products. As real-time PCR is sensitive and uses small fragments of cDNA for amplification, it is also well suited for use with formalin-fixed, paraffin-wax-embedded material.

We therefore conducted a real-time PCR assay for the COL1A1–PDGFB fusion transcript in a series of DFSP containing sarcoma, to determine whether the chimaeric gene could be identified in both components of DFSP-FS and DFSP containing high-grade sarcoma.

MATERIALS AND METHODS

Tumour samples

Eight cases of DFSP containing sarcoma were retrieved from the routine histological files of the Department of Pathology, Medical and Health Science Center, University of Debrecen, Debrecen, Hungary. Formalin-fixed, paraffin-wax-embedded material was available for evaluation. The clinical and histopathological features of the cases have been described in our previous work (table 1).¹⁴

Five cases of conventional DFSP were included as a control group.

Abbreviations: COL1A1, collagen type I α 1; DFSP, dermatofibrosarcoma protuberans; DFSP-FS, dermatofibrosarcoma-fibrosarcomatous; DFSP-PleoSarc, dermatofibrosarcoma protuberans-PleoSarc; PDGFB, platelet-derived growth factor B-chain; RT-PCR, reverse transcription-polymerase chain reaction

Table 1 Histopathological diagnosis of our cases

Case number	Diagnosis
1	DFSP-FS
2	DFSP-FS
3	DFSP-PleoSarc
4	DFSP-FS
5	DFSP-FS
6	DFSP-PleoSarc
7	DFSP-FS
8	DFSP-PleoSarc

DFSP-FS, dermatofibrosarcoma protuberans containing fibrosarcoma;
DFSP-PleoSarc, dermatofibrosarcoma protuberans containing high grade
pleomorphic sarcoma.

Tissue preparation and microdissection

Microdissection was performed on sections in the same way as described previously by Wang *et al.*²⁸ Using RNA-free conditions, formalin-fixed, paraffin-wax-embedded samples were cut into 10 µm thick sections on a microtome with a disposable blade. Sections were collected on clean and sterile treated glass. The samples were deparaffinised in two changes of xylene for 10 min, rehydrated in 100% ethanol, 90% ethanol and 70% ethanol for 5 min each, stained lightly with haematoxylin and eosin for identification, rinsed in RNase-free water for 30 s and finally immersed in 100% ethanol for 1 min. The sarcomatous areas were cut off from the sections using sterile fine needles.

Extraction of total RNA

Deparaffinised, microdissected sections were placed in a sterile tube, resuspended in a proteinase K-containing buffer and incubated at 60°C for 16 h until the tissue was completely solubilised. RNA was purified with EZ1 Biorobot with EZ1 RNA Tissue Mini Kit (Qiagen, Hilden, Germany).

Probe and primer design

Primers and probe sequences were chosen (table 2) with the software Primer Express V. 3.0 (Applied Biosystems, Foster City, California, USA) on the basis of the length between the exons within the region encoding the α-helical domain (exons 5–49). To eliminate the need for multiple probe sequences, we used one probe sequence complementary to the 5' end of the PDGFB gene. Primers and probes were purchased from IDT (Coralville, Iowa, USA).

One-step real-time PCR

PCR amplification was performed in duplicate using a 96-well plate (Applied Biosystems), with a 50 µl final reaction mixture containing 10 µl extracted RNA, 25 µl 2× Quantitect Probe PRT-PCR Mix, 0.5 µl Quantitect RT Mix, 0.7–0.7 µl from forward and reverse primers and 0.3 µl probe, and additionally 17.8 µl RNA-free water (Quantitect Probe RT PCR Kit, Qiagen). The integrity of RNA was evaluated by running a parallel PCR for the ubiquitously expressed 36B4 gene using the following primer and probe sequences: 36B4-forward 5'-AGA TGC AGC AGA TCC GCA T-3', 36B4-reverse 5'-ATA TGA GGC AGC AGT TTC TCC AG-3', and 36B4-probe 5'-FAM-AGG CTG TGG TGC TGA TGG GCA AGA A-TAMRA-3'.

The reaction started with reverse transcription for 30 min at 50°C, followed by denaturation at 95°C for 15 min. After the initial step, 40 cycles of amplification were performed, with extension for 30 s at 76°C, denaturation at 94°C for 15 s and annealing/detection at 56°C for 30 s. The data were analysed by Sequence Detection Software 7300 1.3.1 (Applied Biosystems).

Table 2 Sequences of the collagen type Iα and 1platelet-derived growth factor B-chain primers and the probe

Primer	Nucleotide sequence
COL1A1 (forward)	
Exon 5	5'-AGATGGCATCCCTGGACAGC
Exon 7	5'-TTCGACGTTGGCCCTGTC
Exon 8	5'-TGGCGAGCCTGGAGCTTC
Exon 9	5'-CCCTGGAAAGAATGGAGATGAT
Exon 10	5'-AAGCTGGAAAACCTGGTCGTC
Exon 11	5'-CCTGGAATGAAGGGACACAGA
Exon 12	5'-ATGGTGCCAAGGGAGATGCT
Exon 13	5'-TGGCAGCCCTGGTGA AAA
Exon 14	5'-CTGCCTGGTGAGAGAGGTCG
Exon 15	5'-GAAATGATGGTGCTACTGGTCT
Exon 16	5'-CCTGGTGCTGTTGGTGCTAA
Exon 19	5'-TGGCTGCCTGGTGAGAG
Exon 20	5'-GGAGACACTGGTGCTAAGGGAGAG
Exon 21	5'-CTGGAGAGGAAGGAAAGCGA
Exon 22	5'-GACCTGGTAGCCGTGGTTTC
Exon 23	5'-TGAAGCTGGTCTGCCTGGT
Exon 24	5'-TCCTGATGGCAAACTGGC
Exon 26	5'-AAGCTGGAGAGCGAGGTGTT
Exon 27	5'-ACCCCTGGCCCTGCT
Exon 28	5'-TGGCGAGAGAGGTGAACAAG
Exon 29	5'-GGTCCTCCAGGTGAAGCAGG
Exon 30	5'-TGGTGAGAGAGGTGCGCCCT
Exon 31	5'-GGTTCCCTGGCGAGCGT
Exon 32	5'-GGGAATGCCTGGTGAACGT
Exon 33	5'-AAGATGGCGTCCGTGGTCT
Exon 34	5'-GCCCTGCTGTTCCCACTG
Exon 35	5'-GGAGACCTGGTGAGCCTG
Exon 37	5'-GGTAATGTTGGTGCTCCTGGAG
Exon 38	5'-TTCCCTGGTGCTGCTGG
Exon 39	5'-CCTGGTGCTGATGGTCTCTG
Exon 40	5'-GGCTTCCCTGGTCTTCTG
Exon 41	5'-CCCTGGTGAATCTGGACGTGA
Exon 42	5'-CCCCTGGACGAGACGGTT
Exon 44	5'-TCCTGTGCGCCCTGTGG
Exon 45	5'-AGGGTCACCGTGGCTTCTC
Exon 46	5'-CTGGTGAAACAAGGTCCCTCTG
Exon 47	5'-ACTGGTGATGCTGCTCTGTT
Exon 48	5'-CCACTCTGACTGGAAGAGTGGG
Exon 49	5'-CTCAAGATGTGCCACTCTGACT
PDGFB (reverse)	5'-GCGTTGGAGATCATCAAGGA
probe	5'-FAM- CCGAGGAGCTTTATGAGATGCTGAGTGACC- TAMRA-3'

COL1A1, collagen type Iα1; PDGFB, platelet-derived growth factor B-chain.

RESULTS

All cases except case 1 contained amplifiable RNA as determined by successful amplification of the 36B4 gene. All cases from the control group contained amplifiable RNA.

Table 3 shows the real-time PCR results. PCR showed that transformed DFSPs show translocations between exons 27, 32, 34, 40 and 47 of the COL1A1 gene and exon 2 of the PDGFB gene in the sarcomatous area of DFSP.

Table 3 Translocations between COL1A1 and PDGFB genes in sarcomatous dermatofibrosarcoma protuberans

Case number	Translocation
1	No transcriptable RNA
2	Exon 32 COL1A1-exon 2 PDGFB
3	Exon 34 COL1A1-exon 2 PDGFB
4	Exon 40 COL1A1-exon 2 PDGFB
5	Exon 27 COL1A1-exon 2 PDGFB
6	Exon 32 COL1A1-exon 2 PDGFB
7	Exon 47 COL1A1-exon 2 PDGFB
8	Exon 40 COL1A1-exon 2 PDGFB

Selected detector: all
Well(s): E12
Document: case 6 (absolute quantification)

■ FAM ■ ROX
Well: E12
Document: case 6 (absolute quantification)

Figure 1 Amplification plot of real-time reverse transcriptase-polymerase chain reaction assay for the exon 32 collagen type I α 1-exon 2 platelet-derived growth factor B-chain fusion transcript in case 6.
m, run; FAM, ROX, fluorescent cycles

The cases from the control group showed translocations between exons 29, 32, 34, 38 and 42 of the COL1A1 gene and exon 2 of the PDGFB gene.

DISCUSSION

DFSP is a locally aggressive fibrohistiocytic neoplasm of intermediate malignancy. DFSP has a potential for local recurrence and a low risk of distant metastasis.

Rarely, DFSP may contain areas of fibrosarcoma, with sweeping bundles of spindle cells instead of tumour cells arranged in storiform pattern. Mitotic activity is usually higher, and nuclear chromatin is coarser than that found in cells composing the usual DFSP.

Rarely, DFSP contains focal areas in which the cells are large and pleomorphic. In these areas, the mitotic activity is usually increased, and abnormal mitotic figures are not unusual. We label these tumours as DFSP-PleoSarc (formerly known as malignant fibrosis sarcoma).

Tumours with malignant fibrous histiocytoma, diffuse marked cellular polymorphism and nuclear atypia are definitively relegated to the dermal pleomorphic sarcoma category if they involve the subcutis, or to the atypical fibroxanthoma category if they are limited to the dermis.

Unfortunately, the number of atypical and pleomorphic cells and the degree of atypia required to distinguish DFSP from pleomorphic sarcoma has never been quantified; tumour cells may show mild pleomorphism and atypia in one or two microscopic fields and the tumour still be classified as DFSP. If atypia and pleomorphism is more severe or more extensive, we include the tumour in the pleomorphic sarcoma category or DFSP-PleoSarc.³¹

Recent cytogenetic and molecular studies have shown that fusion of the COL1A1 gene with the PDGFB gene is characteristic of DFSP. The COL1A1 gene encodes the major component of type I collagen, and PDGFB is a potent mitogen for a number of cell types.^{15–17} The location of breakpoints within COL1A1 varies greatly, but is always limited to the region encoding the α -helical domain. The exons of this COL1A1 gene segment end at the last base of the codon. The PDGFB segment of the chimaeric 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 act as an active promoter for PDGFB. The translocation removes negative regulatory segments at the 5' end of the PDGFB gene that enhances protein production.^{18–23} Wang *et al.*^{24–28} showed that the COL1A1–PDGFB transcripts are preserved in the fibrosarcoma areas of most DFSP-FS using conventional PCRs; they suggested that the COL1A1–PDGFB chimaeric gene is still involved in the fibrosarcomatous transformation of DFSP and that the deregulated PDGFB continues to act as a growth factor in the process of tumour progression.

Although the COL1A1–PDGFB fusion transcript has been studied in DFSP, giant cell fibroblastoma (juvenile form of DFSP), DFSP containing fibrosarcoma and superficial fibrosarcoma, it has not been investigated in the DFSP-PleoSarc variant.³²

In this study, we have shown that the COL1A1–PDGFB fusion transcripts were detectable not only in the ordinary DFSP component of transformed DFSPs but also in DFSP-FS. We found DFSP-specific translocation not only in fibrosarcomatous areas but also in pleomorphic sarcomatous areas. Exons 32, 34 and 40 of the COL1A1 gene were found to be involved in DFSP-PleoSarc. This finding indicates that the COL1A1–PDGFB fusion gene can be involved in the pathogenesis of high-grade transformation of DFSP.

Take-home messages

- Real-time polymerase chain reaction-based gene analysis may be used in the diagnosis of dermatofibrosarcoma protuberans (DFSP) and transformed DFSP cases.
- The collagen type I α 1-platelet-derived growth factor B-chain fusion transcript can be shown in DFSP containing pleomorphic sarcoma, which supports the theory of the common histogenesis of the two components.

We also investigated five cases of DFSP containing fibrosarcoma. We found no amplifiable cDNA in one case (case 1) probably because of the age of the specimen. Four cases showed the characteristic translocation between various exons (exons 27, 32, 40 and 47) of the COL1A1 and PDGFB genes.

Analysing our seven cases with transcriptable RNA, we could not show specific, sarcomatous change-related translocation, but from a diagnostic standpoint, this assay can be particularly useful in confirming the diagnosis of sarcomatous DFSP.

By contrast, previous reports described a basic PCR with reverse transcription and sequence analysis, which enables a multistep method of detecting the specific translocation.^{24–27} In theory, real-time PCR combines the objectivity of fluorescence detection with the simplicity of original PCR, and results obtained using fluorescence-based PCR reagents are accepted as the standard of detection of genetic changes. Real-time PCR makes post-PCR manipulations of the PCR products unnecessary, which reduces the analysis time and lowers the risk of PCR contamination.

The sensitivity of conventional PCR and real-time PCR differs. Real-time PCR uses a smaller amount of transcriptable RNA than basic PCRs. This is important, because RNA isolated from paraffin-wax-embedded tissue blocks is of poor quality as extensive degradation of RNA can occur before completion of the formalin fixation process. Moreover, formalin fixation causes cross linkage between nucleic acids and proteins, making subsequent RNA extraction and reverse transcription problematic. In addition, real-time PCR can be useful in the quantitation of gene expression, but in formalin-fixed, paraffin-wax-embedded tissues, it has serious limitation because of the degradation of RNA.³³

In conclusion, we have shown that real-time PCR-based gene analysis may be used in the diagnosis of DFSP and transformed DFSP cases. We have shown the COL1A1–PDGFB fusion transcript in DFSP containing fibrosarcoma or pleomorphic sarcoma, which supports the theory of the common histogenesis of the two components.

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