Platelet-derived growth factor in Dupuytren's disease

This study investigated whether platelet-derived growth factor, a potent inducer of cell proliferation, was identifiable in association with myofibroblasts in Dupuytren's disease. Myofibroblasts in the hypercellular disease stages showed a strong reaction to platelet-derived growth factor antibody using light and electron microscopic immunochemical labels. Platelet-derived growth factor may play a role as a cellular signal for myofibroblast proliferation in the formation of the pathognomonic nodule in Dupuytren's disease. (J HAND SURG 1992;17A:317-23.)

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Dupuytren's disease is characterized by progressive, irreversible flexion of one or more digits. The work of Gabbiani and Majno was the first to define the myofibroblast as the dominant cell type associated with the formation of the pathognomonic nodule of the palmar fascia. Subsequently, the reports of many investigators have also shown increased amounts of type III collagen, fibronectin, and hexosamine glycosaminoglycan in affected palmar fascia. However, to date, the cause and pathogenesis of Dupuytren's disease are unknown.

It has long been recognized that, on a cell biological basis, Dupuytren's disease progresses through three stages. These stages have been defined by Luck. The first is the proliferative stage, in which cell density begins to increase in the palmar fascia. The second stage is the involutional stage, in which cells proliferate in extreme numbers in the formation of the pathognomonic nodule. The third stage, termed the residual stage, is characterized by the inexplicable disappearance of cells with only contracted cords remaining. This cell response has been defined as a proliferation.
Fig. 1. Photomicrograph of a nodule in the proliferative-involutional stage of Dupuytren's disease. Myofibroblasts appear as darkly stained cells (closed arrow) throughout the photograph. Blood vessels (BV) also appear darkly stained (open arrows) as endothelium has ATPase activity. (ATPase stain; original magnification × 120.)

Fig. 2. Photomicrograph of a nodule in the residual stage of Dupuytren's disease. Spindle-shaped fibroblasts (F) are shown oriented with the long axis of the collagen bundles. Blood vessels (BV) appear at the lower left. (Hematoxylin and eosin stain; original magnification × 120.)

Further, the myofibroblast has been shown by many investigators to be a contractile cell. It has also been shown that myofibroblasts are physically connected to each other by hemidesmosomes and to surrounding collagen by fibronectin anchoring strands. Thus, during the hypercellular stage of the disease, in which myofibroblasts proliferate, it is postulated that the collagen tissue fabric is shortened in a progressive manner by the contractile forces of myofibroblasts acting on each other, as well as, collagen fibrils as they are synthesized. In this regard, Dupuytren's disease is biologically similar to wound repair, and myofibroblasts are commonly identified in wound scars. In recent years, the cell biology of wound healing has been examined with special emphasis on the contribution of growth factors. Since
Dupuytren's disease is biologically similar to wound repair, growth factors may also play a role in the pathobiology of this disease.

This study was undertaken to investigate whether platelet-derived growth factor (PDGF) is identifiable in association with fibroblasts and/or myofibroblasts in the palmar fascia of Dupuytren's disease.

**Materials and methods**

Samples of palmar fascia were obtained at surgical release from 28 patients who had fasciectomy for treatment of Dupuytren's disease. These included 16 right hands and 12 left hands. Twenty-six patients were men, mean age 63 years and two patients were women, mean age 45 years. Control palmar fascia was obtained from...
18 patients who had hand surgery for carpal tunnel release. These included nine right and nine left hands. Of the controls, 12 patients were women, mean age 45 years and six patients were men, mean age 55 years. This study was approved by our Institutional Committee on research involving human subjects. Informed consent was obtained from each patient before obtaining tissue samples.

All samples were placed in glycerol embedding compound and frozen, protected by isopentane, using liquid nitrogen. Transverse sections of tissue samples were cut on a cryostat at −20°C at 10 μm. Special emphasis was placed on obtaining serial 10 μm sections on separate slides so that the same cells could be stained using different histological, histochemical or immunocytochemical techniques.

Serial sections were stained using hematoxylin and eosin for standard histological analysis. On the next serial section, myofibroblasts were identified, and myofibroblast cell density was quantitated, using enzyme histochemistry for calcium adenosine triphosphatase (ATPase) at pH 7.2 according to our previously published method.6 Myofibroblasts were identified by light microscopy based on a positive ATPase stain. Fibroblasts are not stained by this technique. Thus, routine histochemistry for calcium adenosine triphosphatase is not useful for distinguishing between fibroblasts and myofibroblasts. Both fibroblasts and myofibroblasts were quantitated as a mean number by cell counts per 10 constant magnification light microscopic fields using a computer assisted image analyzer (Donsanto Corporation, Cambridge, Mass.).

The next 10 μm serial sections were cut in a cryostat at −20°C for PDGF immunocytochemistry for both light and ultrastructural examination. Sections for light microscopy were mounted on glass slides. Sections for ultrastructure were placed in 0.15% glutaraldehyde fixative for 12 hours at 4°C. All sections were incubated in a specific human anti-rabbit platelet-derived growth factor (PDGF) antibody (Antibodies Inc., Davis, Calif.) diluted 1:10 in phosphate buffered saline (PBS) solution containing 0.3% Triton-X, overnight at 4°C in a humid chamber. Sections to be examined on the light level were further incubated in a rabbit secondary IgG antibody and this solution was used instead as a primary antibody incubation. On negative control samples, secondary IgG incubations using fluorescein isothiocyanate or immunogold silver intensified labeling were carried out.

Sections for light microscopy were examined and photographed at constant magnification with use of a Leitz Dialux microscope. Brightfield microscopy was used for hematoxylin, eosin, and ATPase stained sections. Fluorescence microscopy was used for PDGF immunocytochemistry. Sections for ultrastructural examination were dehydrated in an ascending ethanol series, cleared in propylene oxide, and embedded in Polybed 812 resin using a flat embedding mold 1 mm in thickness (Polysciences, Inc., Warrington, Pa.). Thin sections for electron microscopy were cut at 500Å using a Sorvall MT-2B ultramicrotome (Dupont-Sorvall, Wilmington, Del.) and a diamond knife. Thin sections were examined and photographed, both stained by uranyl acetate and lead citrate as well as unstained, using a Hitachi 12 electron microscope.

Results

Histology/histochemistry. Tissue samples from all patients with Dupuytren’s disease were staged, according to the classification of Luck,7 on the basis of the density of myofibroblasts per tissue field. Ten constant magnification light microscopic fields per patient were quantitated and averaged as a mean ± the standard deviation. Specimens from 13 patients with Dupuytren’s disease were in the hypercellular, myofibroblastic, proliferative-involutional disease stage (Fig. 1). Myofibroblasts were identified based on a positive staining by ATPase and had a mean cell density of 384 ± 22.3 per 10^5 μm^2. The remaining 15 tissue samples from patients in the Dupuytren’s disease were in the hypocellular, residual disease stage. These samples contained spindle-shaped fibroblasts that were not stained by ATPase and were identified by hematoxylin and eosin staining. Fibroblasts in these samples had a mean density of 45 ± 5.8 per 10^5 μm^2 (Fig. 2).

Fascial samples from all control patients contained spindle-shaped fibroblasts that were not stained by
ATPase but by hematoxylin and eosin. Fibroblasts in control samples had a mean cell density of $31 \pm 9$ per $10^5 \mu g^2$ (Fig. 3).

Immunocytochemistry. Light microscopic immunocytochemistry using a fluorescent label showed that myofibroblasts in the hypercellular, proliferative-involutional stage of Dupuytren's disease (13 patients) had a strongly positive reaction for PDGF (Fig. 4).

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**Fig. 5.** Electronmicrograph of a myofibroblast (M) from a nodule in the proliferative-involutional stage of Dupuytren's disease. A strongly positive reaction for PDGF is shown by the multiple dark particles located in association with the myofibroblast cell membrane. (PDGF immunocytochemistry-immunogold-silver intensified label; original magnification $\times 40,000$.)

**Fig. 6.** Electronmicrograph of a fibroblast (F) from the palmar fascia of a control patient. A weakly positive reaction for PDGF is shown by the dark particles associated with the fibroblast cell membrane. Collagen fibrils (C) are also shown. (PDGF immunocytochemistry-immunogold-silver intensified label; $\times 40,000$.)
Electron microscopy confirmed this reaction. With use of the immunogold-silver intensified labeling technique, it was apparent that the PDGF immunoreactivity was localized in association with the cell membrane of myofibroblasts in the form of a dense particle lining (Fig. 5).

Samples from the 15 patients with Dupuytren's disease in the hypocellular, residual disease stage and samples from control patients showed that fibroblasts had a weak immunocytochemical reaction for PDGF. On the light level this was seen as a barely visible fluorescent reaction in association with fibroblasts. Ultrastructurally, the immunogold-silver intensified labeling revealed only a few reaction particles associated with fibroblast cell membrane (Fig. 6).

Statistical analysis

In all 28 Dupuytren's samples, cell counts of ATPase positive myofibroblasts were correlated to cell counts of myofibroblasts showing a positive reaction for PDGF on serial sections from the same patient. There was a significant correlation ($r = 0.69, p < 0.01$).

In the samples from 13 patients with Dupuytren's disease in the hypercellular, proliferative-involutional disease stage, the correlation of myofibroblasts showing positive PDGF immunoreactivity was highly significant ($r = 0.73, p < 0.01$).

There was no correlation between cell counts of fibroblasts and PDGF in the residual stage of Dupuytren's disease or in the control samples.

Discussion

This study has shown that PDGF is associated with myofibroblasts in the hypercellular, proliferative-involutional stages of Dupuytren's disease. Our immunocytochemical results suggest that PDGF is bound to a cell membrane surface receptor on myofibroblasts. This finding is in agreement with other studies indicating that the PDGF receptor occurs on cell membranes.18, 19

The slight immunocytochemical reaction for PDGF on the cell membranes of fibroblasts in samples from control patients and patients in the residual, end-stage of Dupuytren's disease is consistent with other reports17–19 that recognize that PDGF binds to its cell surface membrane receptor on fibroblasts and regulates normal growth in these cells.

With regard to abnormal cell growth, there is a wide variety of cellular responses to PDGF characterized by numerous intracellular reactions. These reactions include an increase in the rate of protein synthesis,20 which is known to be increased in Dupuytren's disease.5, 6 Platelet-derived growth factor also promotes reorganization of cytoskeletal actin filaments,21 which is a characteristic of myofibroblasts.1 This growth factor also stimulates increased synthesis and release of arachidonic acid.21 By a complex cascade, arachidonic acid may be converted to prostaglandins.22 In a previous study we have demonstrated that levels of both prostaglandin E2 and prostaglandin F2α are increased in affected palmar fascia of Dupuytren's disease.23, 24 Platelet derived growth factor is a polypeptide carried in the alpha granules of human platelets,18 and Dupuytren's nodules are known to be highly vascular.

Finally, it should be noted that PDGF is known as a competence factor and is known to act in synergistic association with other growth factors, such as epidermal growth factor, to cause cell proliferation by progression into the S phase of DNA synthesis from a state of G0/G1 growth arrest.18 Our current investigations are directed at the identification of other growth factors that may also have a role in myofibroblast proliferation in Dupuytren's disease. However, it appears that PDGF may have a role in the formation of the palmar nodule by stimulating proliferation of myofibroblasts.

In the future, identification of inhibitors to PDGF may be clinically advantageous in prevention of the formation of the palmar nodule.

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REFERENCES


