Distribution of ED-A and ED-B Containing Fibronectin Isoforms in Dupuytren's Disease

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Different fibronectin (FN) isoforms arise via alternate splicing of a single gene transcript in a cell- and tissue-specific manner. Antibodies were used to evaluate the presence and distribution of FN and its isoforms in Dupuytren's diseased and normal palmar fascia. Immunolocalization studies show extracellular FN fibrils, including FN isoforms containing extra domains A (A-FN) and B (B-FN), in proliferative and involutional stage Dupuytren's diseased tissue. However, B-FN appears less abundant and more restricted in its distribution as compared to A-FN or total FN. Total FN and A-FN are significantly reduced in residual tissue, while B-FN is not present. A-FN and B-FN are not present in normal palmar fascia, while total FN staining is slight and restricted to the loose connective tissue surrounding the large, parallel bundles of collagen fibers. The presence of A-FN and B-FN in Dupuytren's diseased palmar fascia represents a disease-induced appearance of these FN isoforms and further evidence of an association between Dupuytren's disease and wound healing. (J Hand Surg 1994;19A: 428–434.)

Dupuytren's contracture is a disease that results in shortening of the palmar fascia, leading to flexion contracture of one or more digits.[1] Previous studies have demonstrated that the disease progresses through three histologically distinct phases: proliferative, involutional, and residual.[2–4] During the proliferative phase, a local fibroplasia in the fascia results in the formation of a nodular lesion. The fibroblasts align themselves with the lines of stress in the tissue during the involutional phase. The residual stage is characterized by the disappearance of the nodule, leaving a relatively acellular, scar-like tissue. Previous work by Brickley-Parsons and coworkers[5] demonstrated that biochemical changes that occur during active stages of Dupuytren's disease are similar to changes observed in connective tissue wound repair. Those biochemical changes include rapid synthesis and turnover of collagen, which result in more abundant type III collagen accumulation in the involved tissue than is commonly seen in normal tissue. These results suggest that the shortening of the palmar fascia involves active remodeling of the extracellular matrix and its replacement with a new shortened extracellular matrix.[5]

Another biochemical change observed in the palmar fascia of Dupuytren's disease is the appearance of fibronectin (FN).[6–8] The FNs are a family of glycoproteins with numerous isoforms that arise by differential splicing of a single gene transcript.[9–11] Previous studies have demonstrated that sequence

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variations occur in three areas of the FN monomer.[10,11] Extra domains A and B of human FN may be either included or deleted, while the IIICS region of human FN can vary in length when present. The function of the A-FN and B-FN isoforms is unclear. It has been shown previously that extra domain A and extra domain B exons are expressed only in cellular FN,[12–14] suggesting a possible role for these isoforms in interactions with cells and the pericellular matrix. In addition, A-FN and B-FN isoform expression appears to be regulated in a cell- and tissue-specific manner during development and aging, and they tend to be highly restricted in their distribution in adult tissues.[15–18] It has been demonstrated previously that A-FN and B-FN are reexpressed during remodeling of granulation tissue in wound healing.[19] These results suggest a potentially unique role for A-FN and B-FN during tissue remodeling. The purpose of this study is to evaluate the distribution of A-FN and B-FN in Dupuytren's diseased palmar fascia.

Materials and Methods

Tissue

Palmar fascia from 20 patients with Dupuytren's disease was obtained at the time of operation. The tissue was transported to the laboratory in sterile, ice-cold, balanced saline solution. Surrounding fat and apparently normal fascia were carefully dissected away from the nodular tissue. The nodule was cut into 3 mm^3 pieces and prepared for histologic and immunocytochemical staining. Normal palmar fascia was obtained from seven patients undergoing carpal tunnel release. The unfixed tissue was infiltrated with 30% sucrose, mounted in OCT compound (Lab-Tek Products, Miles Laboratories, Naperville, Ill), and frozen rapidly by immersion in liquid nitrogen. Cryostat sections (7–10 μm) were thaw-mounted onto glass slides and allowed to air-dry at room temperature. Representative sections were stained with hematoxylin and eosin and staged as described by Luck.[2] Sections either were used immediately for immunocytochemical staining or stored at −20°C.

Monoclonal Antibodies

The IST-4 antibody interacts with an epitope located within the first four type III homology repeats and therefore recognizes all human FN isoforms.[20] The IST-9 antibody recognizes an epitope within the ED-A sequence,[21,22] and BC-1 is specific for ED-B containing FN.[23,24] The ED-A and ED-B sequences are present only in cellular FN. Undiluted culture supernatants were used in these immunofluorescence studies.

Immunofluorescence Staining

Cryosections of Dupuytren's diseased palmar fascia or normal palmar fascia were washed in phosphate-buffered saline (PBS), pH 7.4, for 5 minutes. Sections were then incubated with the appropriate primary antibody for 1 hour at room temperature and washed three times in PBS for 5 min/wash. Sections were incubated for 30 minutes with the appropriate secondary antibody (goat antimouse IgG conjugated to rhodamine diluted 1:100 in PBS) and again washed three times in PBS for 5 min/wash. Sections were mounted in a 1:9 solution of PBS and glycerol and coverslipped. Purified FN was used in preabsorption control experiments to confirm monoclonal antibody specificity. To control for nonspecific staining, the primary antibody was replaced with PBS, followed by the fluorochrome-conjugated goat antimouse IgG. All slides were examined using an Olympus Vanox microscope equipped with epifluorescence optics. Photographs were taken with Kodak TRI-X Pan film push-processed to 1000 ASA.

Results

Fibronectin Distribution in Dupuytren's Diseased Palmar Fascia

Proliferative areas of the sections displayed abundant immunoreactivity with the IST-4 antibody, revealing the presence of total FN in the pericellular matrix (Fig. 1A). Similar results were obtained with monoclonal antibody IST-9, showing that A-FN was also widely expressed in proliferative tissue (Fig. 1B). BC-1 staining demonstrated that B-FN shows a related, though more limited, distribution in the proliferative areas of Dupuytren's diseased tissue (Fig. 1C). With all three antibodies, the staining pattern was finely fibrillar with little apparent organization. Hematoxylin and eosin staining demonstrates that this tissue is highly cellular, with only small amounts of extracellular matrix present. The cells in this proliferative tissue lacked any apparent orientation (Fig. 1D).

Dupuytren's diseased palmar fascia that was in the involutional stage of the disease also showed immunoreactivity to FN antibodies. Involutional tissue stained with the IST-4 antibody (total FN) demonstrates the presence of abundant, organized, and aligned FN fibrils (Fig. 2A). The same tissue showed a similar, but somewhat more restricted staining pattern for A-FN when stained with the

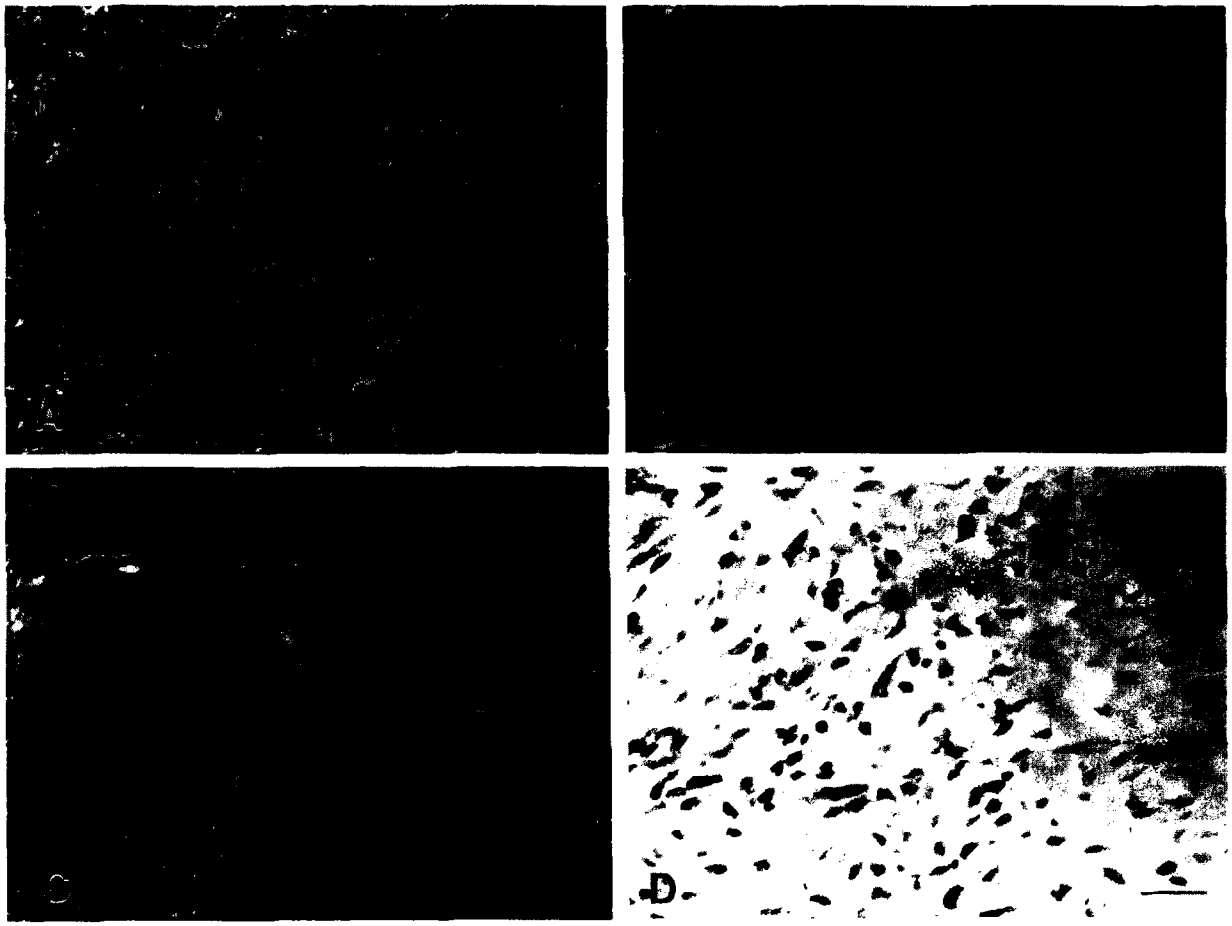

Figure 1. Serial sections showing indirect immunofluorescence staining of proliferative-stage Dupuytren's diseased palmbar fascia. (A) monoclonal antibody IST-4 (total FN), (B) monoclonal antibody IST-9 (A-FN), (C) monoclonal antibody BC-1 (B-FN), (D) hematoxylin and eosin (bar = 25 μm).

IST-9 monoclonal antibody (Fig. 2B). Involutional areas of the tissue showed a particularly reduced expression of B-FN (Fig. 2C). The organized fibrillar staining with all three FN antibodies was aligned parallel with the direction of the cells in involutional tissue (Fig. 2D).

Residual areas of Dupuytren's diseased tissue exhibited a much reduced staining pattern as compared with the nodular areas. Residual tissue stained with IST-4 showed moderate to little positive staining for total FN depending on the cell density. Areas with increased cell density stained lightly, with a fibrillar and patchy pattern (data not shown), while areas composed of mostly extracellular matrix showed little to no positive staining (Fig. 3A). Residual tissue stained with IST-9 demonstrated a similar pattern of staining but a notable decrease in staining intensity (Fig. 3B). In contrast, B-FN staining of residual tissue was always negative (Fig. 3C). Hematoxylin and eosin staining of this residual tissue revealed abundant, disorganized extracellular matrix with few fibroblasts present (Fig. 3D).

Staining controls were performed on Dupuytren's diseased tissue. To test for nonspecific staining, the primary monoclonal antibodies were replaced with PBS, followed by secondary antibody staining using the appropriate concentration of fluorochrome-conjugated goat antimouse IgG. Specificity was evaluated using preabsorbed antibody as the primary antibody, followed by the appropriate fluorochrome-conjugated goat antimouse IgG. No fluorescent staining was detected in the control. (not illustrated).

Fibronectin Distribution in Normal Palmar Fascia

Normal palmar fascia from seven patients showed very little, if any, positive immunoreactivity to the total FN antibody (IST-4) used in this study (Fig. 4). All positive staining was restricted to the loose connective tissue that surrounds the large, parallel bundles of collagen. Sections of normal palmar fascia were also evaluated for the presence of A-FN and B-FN and were found to be negative.

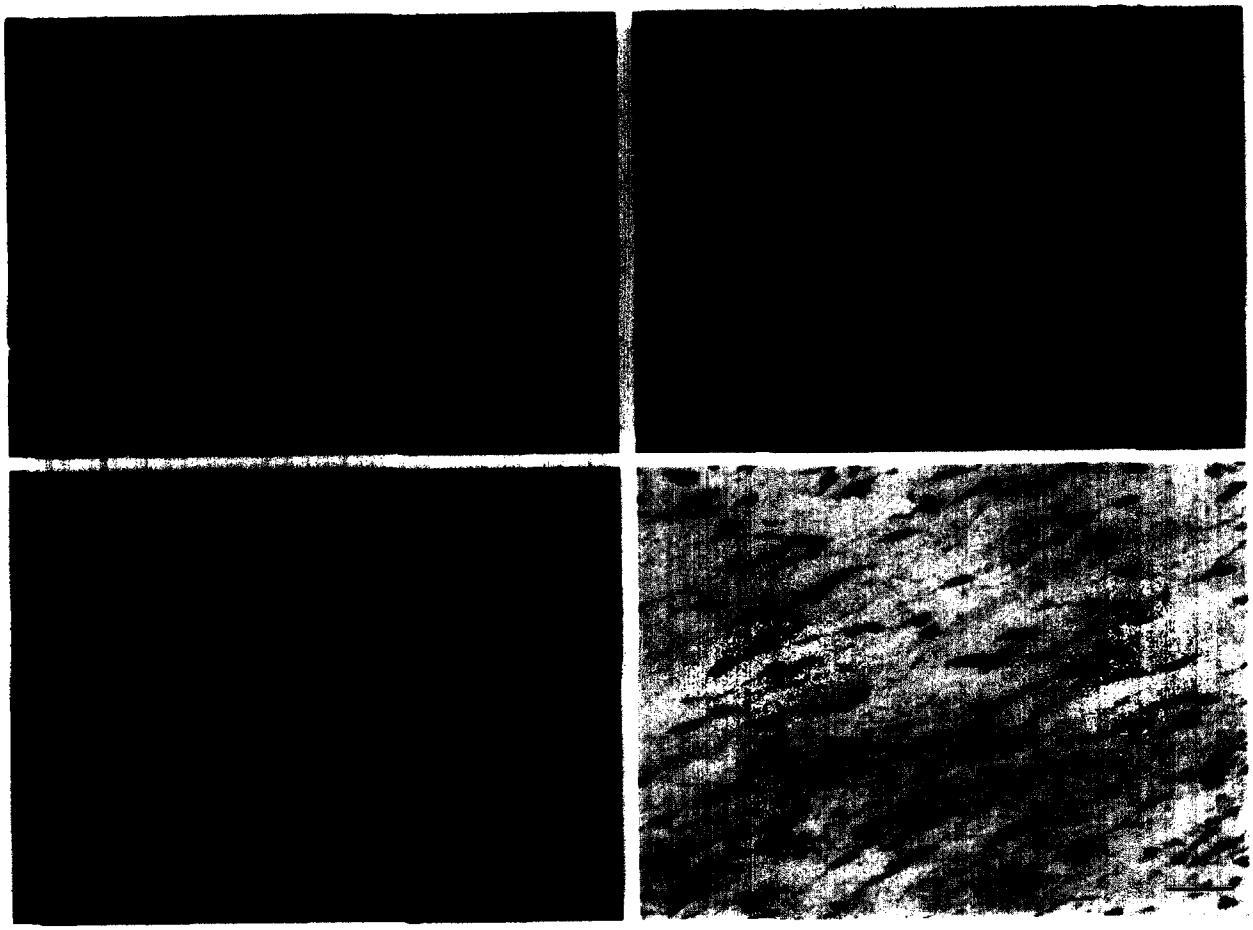

Figure 2. Serial sections showing indirect immunofluorescence staining of involutional-stage Dupuytren's diseased palmar fascia. (A) monoclonal antibody IST-4 (total FN), (B) monoclonal antibody IST-9 (A-FN), (C) monoclonal antibody BC-1 (B-FN), (D) hematoxylin and eosin (bar = 25 μm).

Discussion

To determine the patterns of FN isoform expression in Dupuytren's disease, we used indirect immunofluorescence utilizing domain-specific monoclonal antibodies directed at total, ED-A, and ED-B containing FNs. Palmar fascia from both normal and Dupuytren's diseased patients were evaluated. We found little to no FN present in normal palmar fascia. When present in normal fascia, FN appears to be restricted to the loose connective tissue that surrounds fascicles of parallel collagen bundles. In contrast, there appears to be a significant increase in FN accumulation, including A-FN and B-FN, during the proliferative stage of Dupuytren's disease. Fibronectin levels remain high during the involutional or contractile phase of the disease. As the disease progresses from the involutional to the residual stage, there is a gradual decrease in FN levels. The B-FN isoform shows reduction in distribution earlier in the progression of the disease than A-FN. These results suggest that there is a transient increase in the synthesis and accumulation of A-FN an B-FN isoforms during the active stages of Dupuytren's disease and a programed loss of these isoforms with loss of cellular activity.

These results are consistent with previous studies evaluating the distribution of cellular FN isoforms in wound healing and other morphogenetically active tissues, such as seen in the embryo.[15-19] Cellular isoforms of FN are actively synthesized during the early stages of wound healing, with a gradual decline in the cellular isoforms later in the wound healing process.[19] In addition, there is apparently less accumulation of B-FN than A-FN in wound tissue,[19] such as we observed in Dupuytren's disease. Likewise, A-FN and B-FN are prevalent during times of active proliferation and cell migration in both the chick and human embryo, but they are spliced out when embryogenesis and organogenesis are complete.[15,17,18] The presence of A-FN and B-FN isoforms in Dupuytren's disease reflects a reappearance of an embryonic pattern of FN splicing. Alterations in the splicing of FN mRNA during

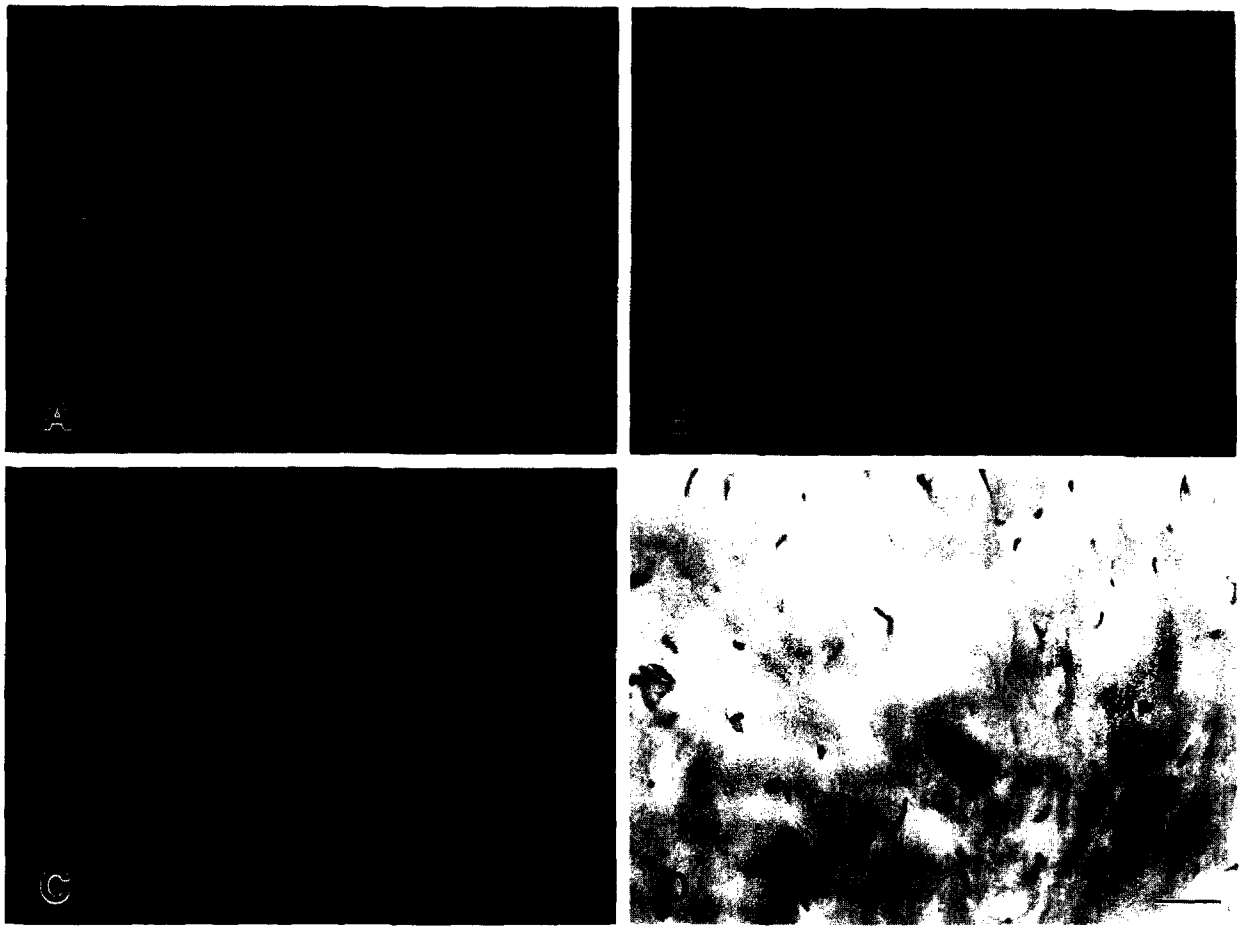

Figure 3. Serial sections showing indirect immunofluorescence staining of residual-stage Dupuytren's diseased palmar fascia. (A) monoclonal antibody IST-4 (total FN), (B) monoclonal antibody IST-9 (A-FN), (C) monoclonal antibody BC-1 (B-FN), (D) hematoxylin and eosin (bar = 25 μm).

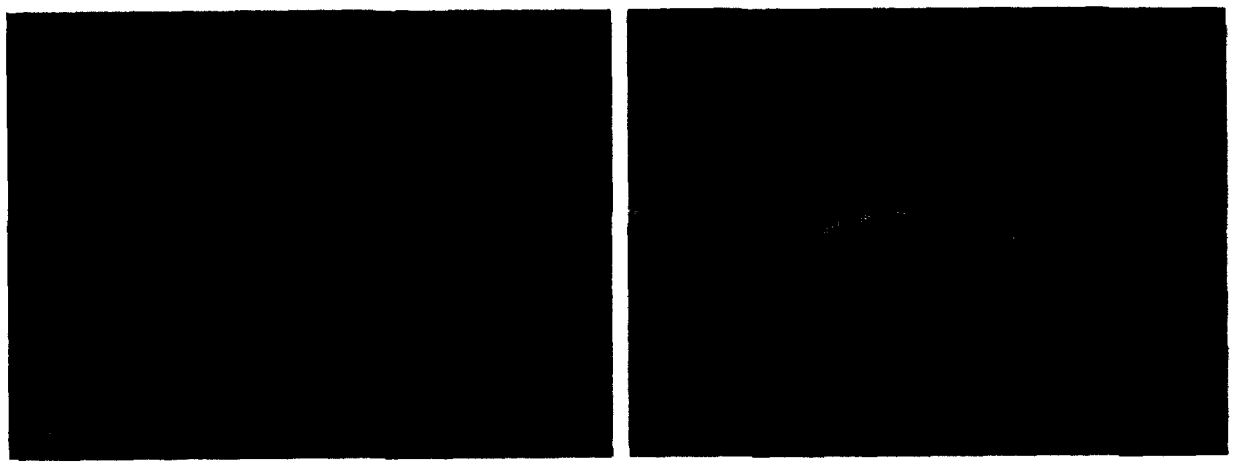

Figure 4. Indirect immunofluorescence staining of normal palmar fascia with anti-FN monoclonal antibody IST-4 (total FN). (A) Cross section, (B) longitudinal section (bar = 25 μm).

Dupuytren's disease leads to the accumulation of different isoforms of FN, which may be important in the progression of the disease.

The mechanisms responsible for the observed alterations in the pattern of splicing of FN mRNA in Dupuytren's disease are unknown. However, transforming growth factor-β (TGF-β) has been demonstrated to increase the synthesis of FN and its receptor,[25,26] as well as increase the expression and accumulation of A-FN and B-FN isoforms in cultured fibroblasts.[27,28] In addition, TGF-β, when injected subcutaneously, will produce a cellular response similar to the formation of granulation tissue seen during wound healing.[29] TGF-β may play a similar role in Dupuytren's disease by promoting increased expression of the cellular isoforms of FN. Recently, other growth factors have been localized to Dupuytren's diseased tissue, including basic fibroblast growth factor and platelet-derived growth factor.[30–32] TGF-β and these other growth factors may be involved in the molecular and cellular mechanisms of Dupuytren's disease.

Reappearance of "embryonic" isoforms of FN parallels biochemical changes in collagen expression seen in the active phases of Dupuytren's disease. The collagen present in proliferative Dupuytren's tissue is similar to collagens present in embryos and newly synthesized collagen in healing wounds. Dupuytren's diseased fascia contains abundant type III collagen, with elevated hydroxylysine and increased reducible cross-links.[5] It has been proposed that Dupuytren's disease behaves morphologically and biochemically much like wound healing.[5] The extremities of the involved fascia are progressively drawn closer together in conjunction with a rapid turnover and synthesis of new matrix, including type III collagen and FN. The progressive remodeling of the extracellular matrix results in a shorter tissue.

Fibronectins have been proposed to play important roles in cellular processes such as migration, differentiation, and adhesion.[9,33,34] All of these processes are important in the remodeling that occurs during contraction of the palmar fascia in Dupuytren's disease. Therefore, FN may play an important role in the remodeling process observed in Dupuytren's contracture. The transient expression of A-FN and B-FN in Dupuytren's tissue suggests a unique functional role for these cell- and tissue-regulated FN isoforms in Dupuytren's disease. The extra domains may provide specific sites that facilitate cell functions required during the remodeling process.

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