The cytoskeleton and extracellular matrix of the Dupuytren’s disease “myofibroblast” : An immunofluorescence study of a nonmuscle cell type

The cytoskeleton and the extracellular matrix of myofibroblasts in nodules from Dupuytren’s diseased palmar fascia were examined by indirect immunofluorescence. Primary antibodies used as probes of these tissue compartments were directed against (1) smooth muscle myosin, (2) nonmuscle myosin—components of the cytoplasmic contractile apparatus in smooth muscle and nonmuscle cells, respectively, (3) laminin, and (4) fibronectin—extracellular glycoproteins mediating cell-matrix attachment in smooth muscle and nonmuscle fibroblastic cells, respectively. The Dupuytren’s nodular cells stained for nonmuscle myosin and fibronectin but not for smooth muscle myosin or laminin; this indicated that, at the level of biochemical differentiation, these cells are a nonmuscle type. Staining for fibronectin between nodular cells was dramatically increased over that seen between fibroblasts of the normal palmar fascia. Because of the nonmuscle nature of the distinctive contractile cell type of the Dupuytren’s nodule, we suggest that the term myofibroblast should be considered a misnomer when applied to this pathogenic cell type. (J HAND SURG 11A:365-71, 1986.)


Dupuytren’s disease is characterized by progressive, irreversible flexion of one or more digits as the result of contraction of the palmar fascia. Significant changes that occur in the palmar fascia during the progress of the disease are the appearance of large cellular areas that are identified as the pathognomonic nodule and the occurrence of adjacent contracted bands or cords. The highly cellular nodules develop during the early stages of the disease, while the cords, which consist principally of collagen fibers, form later during the progressive flexion of the digits.

Contracture of the palmar fascia is generated by a process that is not understood, although there has been extensive investigation of this problem. Ultrastructural studies of the nodule at active contracture have identified a cell type that appears to have the characteristics of both fibroblasts and smooth muscle cells. These cells, in addition to containing numerous mitochondria and large amounts of rough endoplasmic reticulum typical of fibroblasts, contain large bundles of actin microfilaments, which are similar in appearance to the myofibrils in smooth muscle. Furthermore, an interrupted, amorphous extracellular layer, which resembles the basement membrane found around smooth muscle cells, has been identified at the surface of these cells. Because the morphologic characteristics of these cells resemble both fibroblasts and smooth muscle cells, Gabbiani and Majno have termed them “myofibroblasts.” Morphologic findings and distribution of myofibroblasts in the Dupuytren’s nodule suggest that they are responsible for generating the force that results in contracture of the fascia and subsequent digital flexion. This cell type has also been postulated to be the active agent in the granulation tissue of actively contracting wounds and in hypertrophic scars. Extensive morphologic studies have not determined...
whether the myofibroblast more closely resembles the fibroblast or the smooth muscle cell. Therefore, we examined the cytoskeleton and extracellular matrix of myofibroblasts in Dupuytren’s nodules with immunologic probes for fibroblastic proteins and proteins associated with smooth muscle cell types. Two aspects of the myofibroblast were studied: (1) the type of myosin in the cytoskeleton and (2) the macromolecules in the extracellular matrix. These two cellular components were chosen because of their likely involvement in force generation and transmission.

Myosin is a cytoskeletal protein that interacts with actin to generate contractile force in both muscle and nonmuscle cells. Antibodies that have been shown in previous immunocytochemical studies to distinguish between myosin in smooth muscle cells and that in nonmuscle cells were used to determine that the myofibroblast in Dupuytren’s nodules contains only nonmuscle myosin.

Smooth muscle cells are surrounded by a highly organized extracellular layer, called a basement membrane, that serves to attach each cell to its neighbors and to transmit cellular shape changes, such as contraction across the tissue fabric. This structure contains the glycoprotein laminin, which binds the cell to the surrounding type IV collagenous layer. In contrast, fibroblasts lack a basement membrane and, therefore, laminin. A different glycoprotein, fibronectin, attaches fibroblasts to their surrounding stromal (types I and III) collagen. Antibodies specific for either laminin or fibronectin were employed to examine the extracellular matrix of myofibroblasts in Dupuytren’s nodules. By this method we determined that these cells are surrounded by a matrix that is rich in fibronectin but lacks laminin. Therefore, both our cytoskeletal and extracellular matrix immunologic probes provide evidence that the myofibroblasts in Dupuytren’s nodules generate and transmit contractile forces by mechanisms different from those used by smooth muscle cells.

Materials and methods

Tissue. Palmar fascia from 16 patients with Dupuytren’s contracture was removed and transported to the laboratory in a balanced salt solution. Nodular tissue was dissected from surrounding cord and apparently normal fascia. (A nodule was defined as a hard fusiform thickening in the palmar fascia.) The nodule was cut into 2 to 3 mm² pieces. Normal palmar fascia was obtained from six patients having hand surgery for other reasons, in which removal of small quantities of the palmar fascia was necessary for surgical exposure. These tissue specimens were treated in a similar fash-
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Immunofluorescence staining. Cryosections were washed briefly in phosphate-buffered saline (PBS). Sections to be stained with anti-cytoskeletal antibodies were extracted with 0.2% triton X-100 in PBS for 2 minutes and washed with PBS. Sections to be stained with anti-extracellular matrix antibodies were incubated with normal goat serum in PBS for 30 minutes. Sections were then incubated for 30 minutes with appropriate dilutions of primary antibody. After they were washed in PBS, the appropriate secondary antibody (goat anti-rabbit IgG-rhodamine or goat anti-mouse IgA + IgG + IgM-rhodamine [Cappel Laboratories]) was applied for 30 minutes. Sections were washed in PBS and mounted in a 2:8 solution of PBS and glycerine. Routine controls for staining specificity were carried out in parallel sections. These control treatments consistently resulted in no detectable positive staining. Immunofluorescence staining was examined by incident-light fluorescence on a Leitz Orthoplan microscope.

Results

Sections of Dupuytren's nodules that were stained with hematoxylin and eosin show this tissue to be highly cellular, with a small amount of extracellular matrix.

Fig. 1. Light micrographs of cryosections stained with hematoxylin and eosin. A, Dupuytren's nodular tissue is highly cellular. Occasional blood vessels (arrow) can be seen in the nodule. (Magnification ×850.) B, Normal palmar fascia contains only a few elongated fibroblasts surrounded by collagen fibrils. (Magnification ×850.)

Fig. 2. Fluorescent micrographs of cryosections of Dupuytren's nodular tissue (A) or normal palmar fascia (B) stained with anti-actin antibody. A, Bright staining for actin is seen in the cytoplasm of the cells in the stroma (arrow) showing the presence of a large population of myofibroblasts (Myo). (Magnification ×850.) B, The fibroblasts (Fb) in the normal palmar fascia do not stain for actin. Vascular smooth muscle cells around blood vessels stain intensely for actin (arrows). (Magnification ×850.)
Fig. 3. Fluorescent micrographs of cryosections of Dupuytren's nodular tissue stained with either anti-nonmuscle myosin antibody (A) or anti-smooth muscle myosin antibody (B). A, The myofibroblasts (Myo) in the stroma of the nodule stain heavily for nonmuscle myosin. Vascular smooth muscle (VSM) does not stain with anti-nonmuscle myosin antibody, while vascular endothelium (En) does stain. (Magnification ×850.) B, Myofibroblasts (Myo) present in the stroma of the Dupuytren's nodule do not stain with anti-smooth muscle myosin antibody. As expected, vascular smooth muscle (VSM) stains intensely with this antibody. (Magnification ×850.)

Fig. 4. Fluorescent micrographs of cryosections of Dupuytren's nodular tissue (A) or normal palmar fascia (B) stained with anti-laminin antibody. A, The basement membranes surrounding blood vessels and vascular smooth muscle stain brightly for laminin (arrow). No staining for laminin is observed around myofibroblasts (Myo) present in the stroma of the nodule. (Magnification ×850.) B, As expected, there is no staining for laminin around fibroblasts in the normal palmar fascia (Fb). The basement membrane around vascular smooth muscle cells in arterioles in the normal palmar fascia stain intensely (arrow). (Magnification ×850.)

separating the cells (Fig. 1, a). An occasional small vessel can be observed running through the nodule. In contrast, normal palmar fascia is composed mainly of extracellular matrix (Fig. 1, b). A few elongated fibroblasts are dispersed throughout the tissue and oriented parallel to each other and the surrounding collagen fibers. These cryosections are representative of the ones that were stained by indirect immunofluorescence with the various antibodies.

**Actin immunofluorescence staining.** Dupuytren's nodules were examined by indirect immunofluorescence for the presence of myofibroblasts, whose cytoplasm stains intensely with anti-actin antibody (Fig. 2, a). Electron microscopic observation confirmed the presence of myofibroblasts in nodules identified by immunofluorescence as containing this cell type (not illustrated). In contrast to Dupuytren's myofibroblasts, fibroblasts in the normal palmar fascia do not stain with anti-actin antibody (Fig. 2, b). The inability of anti-actin antibody to stain normal fibroblasts...
is not due to the absence of actin in these cells but is most likely due to their lack of large bundles of actin microfilaments.\textsuperscript{5, 22, 23} The only other cell type to stain with anti-actin antibody in both the Dupuytren’s and normal tissues is the vascular smooth muscle cell (Fig. 2, b).

Biopsy results showed that only seven out of sixteen nodules contained myofibroblasts by actin immunofluorescence. In the remaining nine nodules few, if any, of the nodular cells stained with anti-actin antibody. These results are in agreement with other studies that have reported extensive variability in the numbers of myofibroblasts in Dupuytren’s nodules.\textsuperscript{4, 24} Only biopsy specimens that exhibited high concentrations of myofibroblasts were further analyzed in this study.

**Myosin immunofluorescence staining.** The type of myosin present in Dupuytren’s myofibroblasts was determined by indirect immunofluorescence. Myofibroblasts were found to stain intensely with anti-nonmuscle myosin antibody (Fig. 3, a) but not with anti-smoothmuscle myosin antibody (Fig. 3, b). This myosin type specificity is no different from that found for fibroblasts in the normal palmar fascia, which also stain with only the anti-nonmuscle myosin antibody. Anti-smoothmuscle myosin antibody staining in Dupuytren’s nodules is seen only in vascular smooth muscle cells around arterioles (Fig. 3, b). Anti-nonmuscle myosin antibody stains myofibroblasts and also stains the other major nonmuscle tissue type in these cryosections, the vascular endothelium (Fig. 3, a).

**Laminin immunofluorescence staining.** The extracellular matrix that surrounds Dupuytren’s myofibroblasts was examined for laminin by indirect immunofluorescence. No staining with anti-laminin antibody was observed at the surface of Dupuytren’s myofibroblasts or in their surrounding matrix (Fig. 4, a). Similarly no staining was observed around fibroblasts in normal palmar fascia (Fig. 4, b).

**Fibronectin immunofluorescence staining.** The pattern of anti-fibronectin antibody staining was examined in cryosections of Dupuytren’s nodules and normal palmar fascia. Dupuytren’s nodules that contain myofibroblasts stain intensely with anti-fibronectin antibody (Fig. 5, a). The staining pattern with this antibody appears fibrillar and is restricted to the regions of the nodule that contain myofibroblasts. The normal palmar fascia showed little staining with anti-fibronectin antibody (Fig. 5, b), in contrast with Dupuytren’s nodules (compare Figs. 5, a and b).

**Discussion**

This study shows that Dupuytren’s nodular cells contain nonmuscle but not smoothmuscle-type myosin. In addition, the extracellular matrix contains abundant amounts of fibronectin but none of the smoothmuscle-associated extracellular glycoprotein, laminin. Although the morphologic similarity of these cells to smooth muscle has suggested that they might exert and transmit contractile forces by a smoothmuscle-like mechanism,\textsuperscript{5, 8, 22} the absence of a major smooth muscle cytoskeletal protein and a major smooth muscle cell-matrix attachment protein in the Dupuytren’s nodular cell strongly argues against this hypothesis. Instead, the presence of nonmuscle myosin in the myofibroblast’s cytoskeleton and the presence of fibronectin at its surface demonstrate the close resemblance of this specialized cell type to the fibroblast.

If the myofibroblast, as previously proposed, is the
agent of joint contraction,\textsuperscript{23} it must be capable of generating an intracellular contractile force and a means of transmitting this force to the surrounding tissue. Fibroblastic cells contain a cytoplasmic actomyosin system that is capable of generating contractile forces\textsuperscript{10} and a collagen- and fibronectin-rich extracellular matrix that is capable of transmitting such force across a tissue fabric.\textsuperscript{23} While normal fibroblasts do not display large bundles of actin microfilaments \textit{in situ}, such as those seen in myofibroblasts, they can be made to do so by culturing them on a planar substratum, such as a plastic tissue culture dish.\textsuperscript{26} These actin bundles, called stress fibers, can contract under appropriate conditions.\textsuperscript{27} Thus, a nonmuscle contractile system like that present in the myofibroblast has the potential to generate intracellular forces that could lead to joint contracture.

Fibronectin, which we detected in abundant amounts at the surfaces of Dupuytren’s myofibroblasts, could serve to transmit myofibroblast-generated forces across the contracting palmar fascia. Fibronectin is ideally suited for such a role, since it is divided into functional domains that can bind to both the surface of fibroblasts and the surrounding collagen.\textsuperscript{28} Such a role for fibronectin has been suggested in contracting granulation tissue, where fibronectin fibrils have been observed to run from the myofibroblast’s surface into the surrounding collagen-rich matrix.\textsuperscript{29} We have found similar fibrils in our preliminary ultrastructural observations of myofibroblasts in Dupuytren’s nodules.\textsuperscript{30}

We have shown that the Dupuytren’s myofibroblast is not a smooth-muscle-like cell with respect to its contractile cytoskeleton and extracellular matrix, and other studies have shown that it does not contain desmin, a cytoskeletal intermediate filament protein that is characteristic of many smooth muscle cells.\textsuperscript{23} In addition, results from this study have shown biochemical similarities between the cytoskeleton and extracellular matrix of Dupuytren’s myofibroblasts and fibroblasts in the normal palmar fascia. Therefore, we suggest that the Dupuytren’s myofibroblast represents a modification of either normal palmar fibroblasts or a subpopulation of tissue mesenchymal stem cells\textsuperscript{31} but is unrelated to smooth muscle cells.

Our inability to detect myofibroblasts by the criterion of intense actin staining in more than one half of the biopsy specimens of Dupuytren’s nodules examined is consistent with findings of other investigators\textsuperscript{4, 24} and raises several possibilities. The myofibroblasts may be a transient population in the nodule and decrease as the disease progresses. Alternatively, they may remain in the nodule and simply decline in activity with time, so that they are undetectable by standard criteria. In either case, the persistence of the contracture could be caused by the remodeling and shortening of the tissue fabric dependent on the initial effects of active myofibroblasts.\textsuperscript{32}

Finally, we suggest that the term \textit{myofibroblast} should be considered a misnomer when it is applied to the pathogenic cell type in the Dupuytren’s nodule. Its similarities to smooth muscle are apparently only morphologic and do not extend to the level of characteristic cytoskeletal or extracellular proteins (see also ref. 33). This cell type appears to resemble a fibroblastic cell that has reorganized its cytoskeleton and accumulated large amounts of fibronectin. Future studies on the causes and treatment of Dupuytren’s disease should take into account these new findings on the biosynthetic and cytologic properties of this interesting cell type.

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REFERENCES