Correlation of α-Smooth Muscle Actin Expression and Contraction in Dupuytren's Disease Fibroblasts

James Tomasek, PhD, Ghazi M. Rayan, MD, Oklahoma City, OK

We studied 11 nodules from patients with Dupuytren's contracture to determine whether α-smooth muscle actin expression in Dupuytren's fibroblasts is related to the generation of contractile force. Tissue was placed into explant culture and fibroblast strains were obtained. The mean percent of cultured Dupuytren's fibroblasts expressing α-smooth muscle actin, as determined by immunofluorescence, was 14 ± 8 and ranged from 1% to 26%. The ability of Dupuytren's fibroblasts to generate contractile force was determined by using a previously described collagen lattice contraction assay. We observed a significant positive correlation between the expression of α-smooth muscle actin and the generation of contractile force in cell strains of Dupuytren's fibroblasts. In addition, six fibroblast strains from palmar fascia of individuals undergoing carpal tunnel release were examined. In six strains of palmar fibroblasts the mean percent of cells expressing α-smooth muscle actin was 5 ± 3 and ranged from 1% to 9%. Six Dupuytren's fibroblast strains, in which more than 15% of the cells expressed α-smooth muscle actin, were significantly more contractile than the palmar fibroblasts. These results suggest that Dupuytren's fibroblasts can acquire smooth muscle characteristics and that the acquisition of a smooth muscle-like phenotype correlates with increased contractility. (J Hand Surg 1995;20A:450-455.)

Dupuytren's disease is characterized by shortening of the palmar fascia leading to progressive, digital flexion deformity.[1] Specialized fibroblasts present within the diseased fascia have been proposed to be the active cellular agent responsible for the shortening of the palmar fascia.[2-4] These specialized fibroblasts can acquire morphologic and biochemical features similar to smooth muscle. The most prominent morphologic features demonstrated by these cells are the large bundles of actin microfilaments that traverse the cell along its long axis.[2,5,6] The extracellular matrix becomes rich in fibronectin, specifically in two isoforms of fibronectin that are associated with smooth muscle cells.[7] In addition, some of the cells begin to express the smooth muscle-type intermediate filament protein desmin.[4,8]

Some of the fibroblasts in Dupuytren's diseased fascia express the α-smooth muscle (sm) actin isoform.[4,9] Actin, a major component of microfilaments, plays an essential role in regulating cell contractility.[10] The β and γ cytoplasmic actins are expressed in all cell types; whereas those encoding muscle actin isoforms such as α-smooth, α-cardiac and α-skeletal muscle actin are expressed only in specialized cell types.[11,12] The expression of α-sm actin in fibroblasts of Dupuytren's disease is consistent with their acquisition of a smooth muscle-like phenotype.

The role of increased α-sm expression in fibroblasts is unknown but may be related to the ability of fibroblasts to generate contractile force. The pur-

pose of this study was to determine whether increased expression of α-sm actin by Dupuytren's fibroblasts correlated with increased generation of contractile force.

Materials and Methods

Fibroblast cell strains from Dupuytren's diseased and normal palmar fascia were examined for expression of α-smooth muscle actin by immunofluorescence. The relative amount of contractile force generated by fibroblasts was determined by an *in vitro* collagen lattice contraction model we have previously used to quantitate relative amounts of contractile force generated by fibroblasts.[13,14]

Cell Culture

Dupuytren's fibroblast explant cultures were obtained from 11 patients undergoing surgery for Dupuytren's contracture. Palmar fascia fibroblast explant cultures from six patients undergoing carpal tunnel release were used as controls. The Dupuytren's nodular tissue was dissected free from the surrounding cord and normal-appearing palmar fascia. Pieces of nodular tissue were placed onto 60-mm culture dishes (Falcon, Oxnard, CA). They were cultured in complete media containing M-199 (GIBCO Laboratories, Grand Island, NY) supplemented with 10% fetal bovine serum (Irvine Scientific, Santa Ana, CA), 2 mM glutamine, and 1% antibiotic-antimycotic solution. Cultured fibroblasts were harvested using trypsin-EDTA (GIBCO) and subcultured in 75 cm² tissue culture flasks (Falcon). Eleven cell strains of Dupuytren's fibroblasts and six cell strains of palmar fascia fibroblasts were obtained and used. All cell strains used in these experiments were subcultured less than ten passages.

Cells Expressing α-Smooth Muscle Actin

The percentage of cells containing α-sm actin was determined for each cell strain. Fibroblasts were cultured on 16-mm glass coverslips for 2 days. Coverslips were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), rinsed in phosphate-buffered saline (PBS) and permeabilized for 5 minutes with ice cold-methanol. Fibroblasts were first stained by indirect immunofluorescence to visualize α-sm actin. The primary antibody was a monoclonal antibody specific for α-sm actin[15] (Sigma, St. Louis, MO) diluted at 1:200 in PBS followed by the secondary antibody rhodamine-labeled goat anti-mouse IgG (Cappel,

Organon Teknika, Durham, NC) diluted 1:100 in PBS. Next, 4',6-diamidino-2-phenylindole (DAPI; Calbiochem, San Diego, CA) was used to stain chromatin in all cells. The percentage of cells stained with anti-α-sm actin antibody was evaluated using a Bioquant II image analysis system (R-M Biometrics, Nashville, TN) connected to an intensified SIT series 66 camera (Dage-MTI, Michigan City, IN) attached to an Olympus epifluorescence microscope. The total number of cells in a field was determined by counting the number of DAPI stained nuclei, after which the number of α-sm actin-positive cells was determined. Ten random fields from each coverslip were counted. Each field contained approximately 50 cells. Two separate coverslips from each cell strain were examined and the mean percentage of cells stained with anti-α sm actin antibody was calculated.

Collagen Lattice Contraction Assay

Cells from each strain were cultured within stabilized type I collagen lattices as previously described.[13,14] The final collagen concentration was 0.65 mg/mL and the cell concentration was 1.25 × 10⁵ cells/mL. A 250 µL drop of the collagen/cell suspension was placed on a 35-mm plastic tissue culture dish (Falcon). After the collagen lattice was incubated for 1 hour at 37°C to allow for gelation of the collagen, 1.5 mL of complete medium was placed over the collagen lattice. Care was taken not to detach the lattices from the underlying plastic substratum. The stabilized lattices were then incubated for 5 days.

After 5 days, the stabilized lattices were mechanically released from the underlying substratum.[13,14] The edge of the collagen lattice was freed with forceps and the remaining lattice was released by gently pipetting media at the lattice-dish interface. Culture dishes were then returned to the incubator. Rapid contraction was analyzed by measuring the diameter of the lattice before and at various times after release, using a Nikon SMZ-1 stereoscope. Lattice diameters were normalized because of variation in the initial diameter of the lattices, which ranged from 14 to 16 mm. The relative lattice diameter was obtained by dividing the diameter of the collagen lattice at each time point by the initial diameter of the lattice. The percent contraction was obtained by subtracting the relative lattice diameter from one. All data were expressed as the mean ± standard deviation. For each individual cell strain the mean ± standard deviation was obtained by conducting each experiment in duplicate. Each experiment comprised at least six
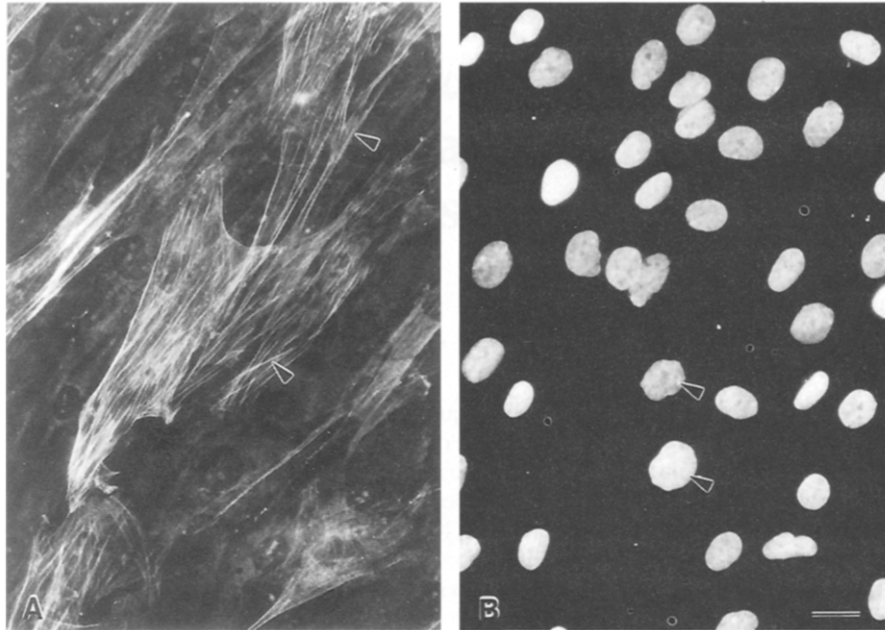
Figure 1. Fluorescence staining of a cultured cell strain of Dupuytren's fibroblasts. Fibroblasts were stained by indirect immunofluorescence with anti-α-smooth muscle actin antibody and with DAPI. (A) Bundles of actin microfilaments containing α-smooth muscle actin are brightly fluorescent after indirect immunofluorescence staining with anti-α-smooth muscle actin antibody (arrows). (B) Nuclei of fibroblasts in identical area as (A) are brightly fluorescent following DAPI staining (arrows). (bar = 2 μm).

collagen lattices. Statistical analysis was done using an independent group Student's t-test or Pearson r correlation test (Crunch Software, Oakland, CA).

Results

α-Smooth Muscle Actin Expression

Fibroblasts from Dupuytren's fibroblast strains and palmar fibroblast strains were double stained by indirect immunofluorescence for α-smooth muscle actin and histofluorescence using DAPI for nuclei (Fig. 1).

In 11 Dupuytren's fibroblast strains examined, the mean percent of cells stained with anti-α-sm actin antibody was 14 ± 8.1 (range, 1%–26%) (Table 1). In six strains greater than 15% of the cells expressed α-sm actin. In the remaining five strains less than 10% of the cells expressed α-sm actin. In six palmar fibroblast strains examined, the mean percent of cells expressing α-sm actin was 5 ± 2.8 (range 1%–9%) (Table 1). Dupuytren's fibroblasts have a significantly greater percentage of cells expressing α-sm actin compared with normal palmar fibroblasts (p < .05).

Table 1. Summary of Collagen Lattice Contraction and α-Smooth Muscle Actin Expression in Strains of Dupuytren's Fibroblasts and Palmar Fibroblasts

Dupuytren's Fibroblasts			Palmar Fibroblasts		
Strain	*% ASMA*	*% Contraction*	*Strain*	*% ASMA**	*% Contraction*
1	26	50	1	9	43
2	23	47	2	7	42
3	20	37	3	5	41
4	19	46	4	5	35
5	18	44	5	3	34
6	15	49	6	1	30
7	10	41			
8	8	42			
9	7	38			
10	5	38			
11	1	39			

ASMA, α-smooth muscle actin.

Collagen Lattice Contraction

Most of the lattice contraction occurs within the first 10 minutes after release (Fig. 2). The amount of collagen lattice contraction at 10 minutes after release was used to compare collagen lattice contraction among cell strains. In 11 Dupuytren's fibroblast strains examined, the mean percent of collagen lattice contraction 10 minutes after release was 43 ± 4.6 (range, 50%–37%) (Table 1). In six normal palmar fibroblast strains examined, the mean percent of collagen lattice contraction 10 minutes after release was 38 ± 5 (range, 42%–31%) (Table 1).

Dupuytren's fibroblast cell strains and normal palmar fibroblast cell strains were compared to determine differences in the abilities of these two cell populations to contract collagen lattices (Fig. 3). There was no significant difference between these two cell populations.

α-Smooth Muscle Actin Expression and Collagen Lattice Contraction

The percentage of cells expressing α-sm actin and that of collagen lattice contraction was compared for Dupuytren's fibroblasts (Fig. 4). These results demonstrate a moderate positive correlation between these two variables ($r = .68$; $p < .05$). As the number of cells expressing α-sm actin increased so did the percentage of collagen lattice contraction. A similar positive correlation was observed between percentage of normal palmar fibroblasts expressing α-sm actin and collagen lattice contraction ($r = .92$; $p < .05$).

The greatest percentage of cells expressing α-sm actin in normal palmar fibroblast cell strains was 9%. Therefore, we subdivided the Dupuytren's cell strains into two populations. One population contained six Dupuytren's fibroblast strains, in which more than 15% of the cells stained for α-sm actin. The average collagen lattice contraction for this population was 45 ± 4.6%. A second population contained five Dupuytren's fibroblast strains, in which more than 10% of the cells stained for α-sm actin. The average collagen lattice contraction for this population was 40 ± 1.8%. The six Dupuytren's fibroblast strains, in which more 15% of the cells expressed α-sm actin, were significantly more contractile than the five Dupuytren's fibroblast strains in which less than 10% of the cells stained for α-sm actin ($p < .05$) (Fig. 3).

These two populations of Dupuytren's fibroblasts were compared with normal palmar fibroblasts for their abilities to contract collagen lattices. The six strains of Dupuytren's fibroblasts with more than 15%
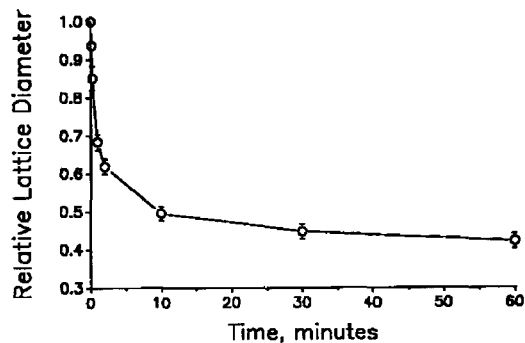
Figure 2. Fibroblasts rapidly contract a collagen lattice. Dupuytren's fibroblasts were cultured within stabilized collagen lattices for 5 days. Rapid contraction occurred immediately after release of the lattice from the underlying substratum. Most of the lattice contraction occurred within 10 minutes after release. All data are averages of six collagen lattices. Standard deviations are shown.

of cells expressing α-sm actin contracted collagen lattices to a significantly greater extent than normal palmar fibroblasts ($p < .05$) (Fig. 3). There was no significant difference in collagen lattice contraction between the five strains of Dupuytren's fibroblasts with less than 10% of cells expressing α-smooth muscle actin and normal palmar fibroblasts (Fig. 3).

Discussion

This study demonstrates that different cell strains of Dupuytren's fibroblasts express different levels of α-sm actin and that the expression of α-sm actin corre-
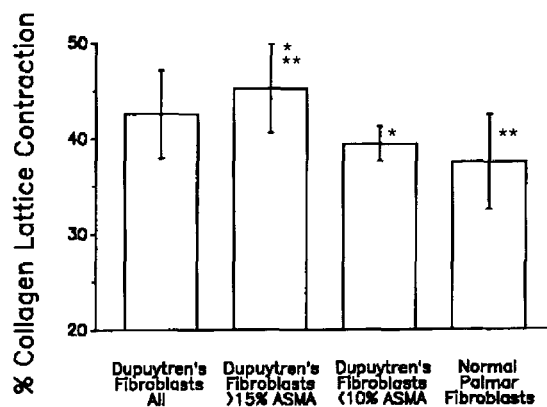
Figure 3. Bar graph representing the ability of different groups of fibroblast strains to promote collagen lattice contraction. The average percentage of collagen lattice contraction 10 minutes after release for each group of fibroblasts is illustrated. Standard deviations are shown. *$p < .05$; **$p < .05$.
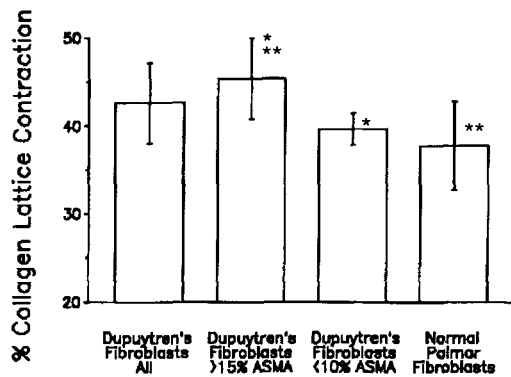
Figure 4. The percentage of collagen lattice contraction by Dupuytren's fibroblasts correlates positively with expression of α-smooth muscle actin. Each point represents one Dupuytren's fibroblast cell strain. *p<.05; **p<.05.

lates positively with the generation of contractile force. Previous studies have demonstrated that fibroblasts can express α-sm actin.[15,16] The expression of α-sm actin is not due to a contamination by smooth muscle cells or pericytes. The expression of α-sm actin occurs in subpopulations of fibroblasts irrespective of the source of these cells and even after cloning or subcloning.[17] The percentage of Dupuytren's fibroblasts expressing α-sm actin varies depending on the stage of the disease.[4,9,18] The greatest percentage of these fibroblasts expressing α-sm actin occurs in the proliferative stage. There is a slight decrease of expression during the involutional stage, and an almost absence of expression in the residual stage.[18] The variation in expression of α-sm actin by different cell strains of Dupuytren's fibroblasts we observed in our study may reflect explant cultures at varying stages of the disease. Explant cultures from proliferative and involutional stage tissues would be expected to yield cell strains expressing a greater percentage of α-sm actin than explant cultures from residual stage tissues. Future studies should examine whether this relationship does occur.

We have previously used a collagen lattice contraction model to demonstrate that fibroblasts can generate contractile force and to quantitate relative amounts of contractile force generated by fibroblasts.[13,14] Dupuytren's fibroblasts with a greater percentage of cells expressing α-sm actin were more contractile than Dupuytren's fibroblasts with a smaller percentage of cells expressing α-sm actin. The increased contractility of Dupuytren's fibroblasts may be a result of increased expression of α-sm actin. This is consistent with findings that increased expression of α-sm actin is observed in fibroblasts in tissues undergoing con-

traction.[18] Alternatively, the increased expression of α-sm actin may reflect a change in a number of other proteins, which represent a modulation to a more contractile phenotype. The relationship between α-sm actin expression and fibroblast contraction observed in Dupuytren's disease may provide a diagnostic aid in determining the aggressiveness of the disease.

We previously have demonstrated that there is no difference in the ability of Dupuytren's fibroblasts and normal palmar fibroblasts to contract a collagen lattice.[14] This was based on a comparison of the mean contraction of all Dupuytren's cell strains and all normal palmar fibroblast cell strains examined. Similar results were obtained in this study with increased numbers of cell strains. However, when the Dupuytren's cell strains were divided into two populations based on percentage of cells expressing α-sm actin, we observed differences between these two populations and normal palmar fibroblasts. These results suggest that, at least with respect to collagen lattice contraction, cell strains of Dupuytren's fibroblast with less than 10% of the cells expressing α-sm actin are similar to normal palmar fibroblasts.

The expression of large amounts of α-sm actin does not appear to be required for cellular contraction. Normal palmar fibroblasts, which express little α-sm actin, can still generate considerable amounts of contractile force, as do strains of Dupuytren's fibroblasts expressing little α-sm actin. Previous studies have demonstrated that β and γ isoforms of actin, present in fibroblasts, can interact with myosin and generate contractile force.[10,11] The presence of α-sm actin may add to the amount of force that can already be generated by the interaction of β and γ actin with myosin.

Extracellular factors such as transforming growth factor-β, heparin, and γ-interferon can regulate the expression of α-sm actin in fibroblasts.[17,19,20] The presence of transforming growth factor-β in Dupuytren's diseased tissues suggests a role for this agent in promoting the expression of α-sm actin in fibroblasts during the active disease process.[21] Future studies correlating the levels of α-sm actin expression and contractile force generation will confirm that the expression of this contractile protein plays a role in the contraction of the palmar fascia observed in Dupuytren's disease.

References

1. McFarlane RM, McGrouther DA, Flint MH. Dupuytren's disease. Edinburgh: Churchill Livingstone, 1990:31–98.
2. Gabbiani G, Majno G. Dupuytren's contracture: fibroblast contraction? Am J Pathol 1972;66:131–8.

3. Schultz RJ, Tomasek JJ. Cellular structure and interconnections. In: McFarlane RM, McGrouther DA, Flint MH, eds. Dupuytren's disease. Edinburgh: Churchill Livingstone, 1990:86–98.

4. Schürch W, Skalli O, Gabbiani G. Cellular biology. In: McFarlane RM, McGrouther DA, Flint MH, eds. Dupuytren's disease. Edinburgh: Churchill Livingstone, 1990:31–47.

5. Chiu HF, McFarlane RM. Pathogenesis of Dupuytren's disease. J Hand Surg 1978;3:1–10.

6. Tomasek JJ, Haaksma CJ. Fibronectin filaments and actin microfilaments are organized into a fibronexus in Dupuytren's diseased tissue. Anat Rec 1991; 230:175–82.

7. Halliday NL, Rayan GM, Zardi L, Tomasek JJ. Distribution of ED-A and ED-B fibronectin isoforms in Dupuytren's disease. J Hand Surg 1994;19A:428–34.

8. Shum DT, McFarlane RM. Histogenesis of Dupuytren's disease: an immunohistochemical study of 30 cases. J Hand Surg 1988;13A:61–66.

9. Skalli O, Schürch W, Seemayer T et al. Myofibroblasts from diverse pathologic settings are heterogeneous in their content of actin isoforms and intermediate filament proteins. Lab Invest 1989; 60:275–85.

10. Goldman R, Pollard T, Rosenbaum J. Cell motility. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1976:689–710.

11. Vandekerckhove J, Weber K. At least six different actins are expressed in higher mammals: an analysis based on the amino acid sequence of the amino-terminal tryptic peptide. J Mol Biol 1978;126:783–802.

12. Skalli O, Vandekerckhove J, Gabbiani G. Actin-isoform pattern as a marker of normal or pathological smooth-muscle and fibroblastic tissues. Differentiation 1987;33:232–8.

13. Tomasek JJ, Haaksma CJ, Eddy RJ, Vaughan MB. Fibroblast contraction occurs in release of tension in attached collagen lattices: dependency in an organized actin cytoskeleton and serum. Anat Rec 1992; 232:359–68.

14. Rayan GM, Tomasek JJ. Generation of contractile force by cultured Dupuytren's disease and normal palmar fibroblasts. Tiss Cell 1994;26:747–56.

15. Skalli O, Ropraz P, Trzeciak A, Benzonana G, Gillessen D, Gabbiani G. A monoclonal antibody against a-smooth muscle actin: a new probe for smooth muscle differentiation. J Cell Biol 1986; 103:2787–96.

16. Schürch W, Skalli O, Seemayer TA, Gabbiani G. Intermediate filament proteins and actin isoforms as markers for soft tissue tumor differentiation and origin. Am J Pathol 1987;128:91–103.

17. Desmouliere A, Rubbia-Brandt L, Abdiu A, Walz T, Macieira-Coelho A, Gabbiani G. a-Smooth muscle actin is expressed in a subpopulation of cultured and cloned fibroblasts and is modulated by g-interferon. Exp Cell Res 1992;201:64–73.

18. Schürch W, Seemayer TA, Gabbiani G. Myofibroblast. In: Sternberg SS, ed. Histology for pathologists. New York: Raven, 1992:109–44.

19. Desmouliere A, Rubbia-Brandt L, Grau G, Gabbiani G. Heparin induces a-smooth muscle actin expression in cultured fibroblasts and in granulation tissue myofibroblasts. Lab Invest 1992;67:716–26.

20. Desmouliere A, Geinoz A, Gabbiani F, Gabbiani G. Transforming growth factor-b1 induces a-smooth muscle actin expression in granulation tissue myofibroblasts and in quiescent and growing cultured fibroblasts. J Cell Biol 1993;122:103–11.

21. Baird KS, Crossan JF, Ralston SH. Abnormal growth factor and cytokine expression in Dupuytren's contracture. J Clin Pathol 1993;46:425–8.