Responsiveness of Dupuytren’s Disease Fibroblasts to 5α-Dihydrotestosterone

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Purpose: We recently showed that androgen receptors are expressed in Dupuytren’s contracture. The aim of the present work was to test the responsiveness of Dupuytren’s fibroblasts to 5α-dihydrotestosterone (5α-DHT), the active form of testosterone.

Methods: Cultured palmar fascia cells from 10 patients with Dupuytren’s contracture and 4 normal subjects were exposed to 5α-DHT (10 or 100 ng/mL) for 1, 3, 7, and 15 days. Their phenotype was analyzed immunohistochemically for α-smooth muscle actin and androgen receptor expression and proliferation rates were studied.

Results: At 15 days the higher concentration of 5α-DHT induced an increase in Dupuytren’s fibroblast proliferation, whereas anti-α-smooth muscle actin exhibited the strongest expression. At the same time point androgen receptor expression decreased with the lower concentration and disappeared altogether with the higher dose of 5α-DHT.

Conclusions: The palmar fascia is a target tissue for androgen action via androgen receptors. Further studies are required to determine whether control of androgen receptor may control the evolution of Dupuytren’s disease. (J Hand Surg 2003;28A:1029–1034. Copyright © 2003 by the American Society for Surgery of the Hand.)

Key words: Dupuytren’s contracture, myofibroblast, 5α-DHT, androgen receptor.

Dupuytren’s contracture is a fibroproliferative disease characterized by shortening of the palmar fascia leading to progressive digital flexion deformity. The pathogenesis of the disease is still debated. Clinical investigations have evidenced a strong, unexplained male predominance with a male:female ratio of about 6:1.1 Gabbiani and Maino2 were the first to hypothesize that myofibroblasts, specialized cells with phenotypical characteristics between fibroblasts and smooth muscle cells, could be responsible for the contracture. The myofibroblasts of Dupuytren’s contracture express the α-smooth muscle actin isoform,3 the ED-A and ED-B fibronectin isoforms,4 the filament protein desmin,3 and several growth factors.5,6 Recently we showed the expression of androgen receptors in tissue and cultured cells of Dupuytren’s nodules.7

The presence of androgen receptors suggests that Dupuytren’s fibroblast metabolism may be regulated partly by androgens. In the present work we tested the responsiveness of Dupuytren’s disease fibroblasts to stimulation with 5α-dihydrotestosterone (5α-DHT).

Testosterone is converted at the cellular level to 5α-DHT by the enzyme 5α-reductase, which subsequently binds to androgen receptors within cytosol.
and subsequently to nuclear DNA in a 2-step mechanism. Binding of the nuclear hormone receptor complex to nuclear chromatin promotes gene expression as a direct response to hormone stimuli. The myotropic activity of androgens is well known. They bind to nuclear receptors, stimulating skeletal and smooth muscle cell proliferation. The gene encoding the androgen receptor, also known as the dihydrotestosterone receptor, is located on the X chromosome in the region between the centromere and Xq13. The expression of androgen receptor in target cells is a prerequisite for testosterone action.

The aim of the present study was to investigate the response in terms of proliferation, differentiation, and androgen receptor expression of Dupuytren’s fibroblasts and normal palmar fascia fibroblasts to 5α-DHT.

Materials and Methods

Samples

Surgical samples were collected from 10 patients (8 men, 2 women; mean age, 65.8 y) undergoing surgery for Dupuytren’s contracture. They consisted of single or multiple nodules of variable size localized in palmodigital and digital areas. Disease stage was between 1 and 4 according to the scoring method of Tubiana. Samples of palmar fascia from 4 patients with carpal tunnel syndrome (2 men, 2 women; mean age, 59.8 y) were used for comparison.

Cell Cultures

Dupuytren’s nodular tissue was minced with microdissection scissors under aseptic conditions and washed extensively with serumless nutrient medium (Dulbecco’s modified Eagle medium [DMEM]; Gibco, Milan, Italy) to remove the blood component.

The chips were placed in 35-mm Petri dishes in DMEM supplemented with 10% heat-inactivated fetal calf serum and 1% penicillin-streptomycin solution (both from Gibco). Cultures were incubated at 37°C in a humidified atmosphere, 95% air, 5% CO2. The medium was changed at 2-day intervals. After a week the cells began to migrate from the chips and formed a monolayer. They were harvested using trypsin–ethylenediaminetetraacetic acid (Gibco) and subcultured until the third passage.

Normal palmar fascia cultures were grown in parallel under the same conditions.

Dupuytren’s and normal palmar fascia cells were allocated to 2 exposure groups that received DMEM supplemented with 10 ng/mL or 100 ng/mL 5α-DHT (Sigma, Milan, Italy) 3 times a week, and a control group that received DMEM only. Proliferation rates were assessed at 1, 3, 7, and 15 days using the 3-(4,5-dimethylthiazole-2-yl)-2,5-biphenyl tetrazolium bromide (MTT) method. Data were subjected to one-way analysis of variance (ANOVA).

Immunohistochemistry

Cells were replated in 4-well chamberslides (Nunc Inc., Naperville, IL) and maintained in DMEM-10% FBS.
fetal calf serum with or without 5α-DHT stimulation. After 15 days the cells were fixed with 2% phosphate-buffered formalin with 0.5% glutaraldehyde for 10 minutes at room temperature. Fixed cells were processed by the standard avidin-biotin peroxidase complex procedure (Vector, Burlingame, CA). Nonspecific binding was blocked with 3% normal goat serum in a phosphate-buffered saline solution, pH 7.4, for 30 minutes at room temperature, then cells were incubated with the primary antibodies overnight at 4°C. Antibodies were monoclonal anti–5α-smooth muscle actin (1:50) (Novocastra Laboratories, New Castle, UK) and anti-androgen receptor (1:30) (Biogenex, San Ramon, CA). The androgen receptor antibody binds to peptide SP-61 (301-320) of human androgen receptor and does not cross-react with human estrogen, progesterone, or glucocorticoid receptors.15 Rabbit and mouse immunoglobulins at the same dilutions as the primary antibodies were used as negative controls. Peroxidase activity was revealed by incubation with 0.05% 3,3′diaminobenzidine tetrahydrochloride (Sigma) in phosphate-buffered saline containing 0.03% peroxide for 5 minutes at room temperature. Slides then were washed, dehydrated, and mounted (Eukitt; O. Kindler GmbH & Co., Freiburg, Germany). For the double-labeling procedure the androgen receptor antibody was added first and peroxidase activity was visualized by incubation with 3,3′diaminobenzidine tetrahydrochloride; α-smooth muscle actin antibody was added second and revealed by 3-amino-9-ethylcarbazole (1 mL of a 4% solution of 3-amino-9-ethylcarbazole in dimethylformamide, 9 mL 50 mmol/L Na acetate, pH 5, and 0.01 mL 30% H₂O₂). Sections were mounted with Gel/mount (Biomedica Corp, Foster City, CA) and examined under a Zeiss Axiophot (Oberkochen, Germany) light microscope.

Histomorphometric Analysis
Histomorphometric measurements were performed to compare the number of immunoreactive cells/area in Dupuytren’s and normal palmar fascia cultures (Leitz, Quantimet 500 QWIN plus morphometric program; Leica, Cambridge Ltd, Cambridge, UK). Immunostaining was evaluated by counting, for each reaction, positive cells in 10 random fields from each patient (lens: 20×). Data were analyzed by using ANOVA.

Results
Proliferation Rate
The rates of proliferation of Dupuytren’s fibroblasts were similar at 1 and 3 days in all 3 groups of cells (p > .05). At 7 and 15 days they were significantly higher in the group stimulated with the higher dose of 5α-DHT (p < .05) (Fig. 1).

### Table 1. Histomorphometric Analysis of Dupuytren’s Fibroblast

<table>
<thead>
<tr>
<th></th>
<th>No DHT (cells/area)</th>
<th>10 ng/mL DHT (cells/area)</th>
<th>100 ng/mL DHT (cells/area)</th>
<th>Significance</th>
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</thead>
<tbody>
<tr>
<td>α-smooth muscle actin</td>
<td>5.9 ± 2.1</td>
<td>7.7 ± 3.5</td>
<td>10.3 ± 4.5</td>
<td>p &lt; .001</td>
</tr>
<tr>
<td>Androgen receptor</td>
<td>9.2 ± 3.3</td>
<td>2.3 ± 1.8</td>
<td>0</td>
<td>p &lt; .001</td>
</tr>
<tr>
<td>Androgen + α-actin</td>
<td>3.7 ± 1.5</td>
<td>1.4 ± 0.9</td>
<td>0</td>
<td>p &lt; .001</td>
</tr>
</tbody>
</table>

Evaluation of immunostaining of labeled cells/area (area: 0.78 mm²) in 10 random fields from 10 slides, one per patient. Differences between the 3 groups of cells for expression of α-actin (F₂,1,14 = 23.5; p < .001), androgen receptors (F₂,124 = 170; p < .001), and their colocalization (F₂,57 = 61.5; p < .001) were highly significant (ANOVA).
In normal palmar fascia fibroblasts the proliferation rates were not significantly different in the 3 groups at 1, 3, and 7 days (p > .05), whereas at 15 days the proliferation was significantly greater (p < .05) in the group treated with 100 ng 5α-DHT (Fig. 2).

Comparison of the proliferation rates of Dupuytren’s fibroblasts and normal palmar fibroblasts at 15 days evidenced significantly higher rates in the former in all cultures (Figs. 3, 4, 5).

No differences were observed between male and female samples (p > .05).

Immunohistochemistry
The number of Dupuytren’s fibroblasts immunostaining for α-smooth muscle actin increased in both groups of treated cells (Table 1). At 15 days the cytoplasmic labeling was observed in 30% of unexposed cells (5.9 ± 2.1 SD cells/area) (Fig. 6A), in 39% of cells stimulated with 10 ng/mL (7.7 ± 3.5 SD cells/area), and in 53% of cells treated with 100 ng/mL 5α-DHT (10.3 ± 4.5 SD cells/area) (Fig. 6B). Staining was scarce in untreated normal palmar fascia cells and more abundant in those treated with the higher dose of 5α-DHT (Table 2).

In contrast, androgen receptor expression was lowest with the higher concentration of 5α-DHT (Table 1). Perinuclear immunoreaction was detected in 67% (9.2 ± 3.3 SD cells/area) of unstimulated Dupuytren’s fibroblasts (Fig. 7A) and in few cells (5.4%) treated with 10 ng/mL, whereas the cells exposed to 100 mg/mL testosterone were unlabeled (Fig. 7B). Untreated normal palmar fascia cells displayed sparse labeling and treated cells displayed no labeling at all (Table 2).

Double-labeling for androgen receptors and α-smooth muscle actin was detected in Dupuytren’s untreated cells (3.7 ± 1.8 SD cells/area) (Fig. 8) and in those receiving the lower dose of testosterone.

No differences were observed for androgen receptor expression between men and women (p > .05).

**Discussion**

In this study the cultured cells from patients with Dupuytren’s contracture and from normal subjects were stimulated with 5α-DHT and their response was analyzed in terms of proliferation, differentiation, and androgen receptor expression.

We previously showed the presence of androgen receptors in the palmar fascia in tissue sections and cell cultures of Dupuytren’s disease.7 Dupuytren’s tissue showed diffuse labeling for androgen receptors.

![Figure 6. Immunostaining for α-smooth muscle actin in untreated myofibroblasts (arrows) (A, 20× counterstained with hematoxylin-eosin) and in those exposed to 100 ng/mL 5α-DHT (arrows) (B, 20× counterstained with hematoxylin-eosin).](image)

<table>
<thead>
<tr>
<th>Normal Palmar Fascia Fibroblasts</th>
<th>No DHT (cells/area)</th>
<th>10 ng/mL DHT (cells/area)</th>
<th>100 ng/mL DHT (cells/area)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-smooth muscle actin</td>
<td>2.6 (±1.5)</td>
<td>5.3 (±3.5)</td>
<td>9.6 (±4.8)</td>
<td>p &lt; .001</td>
</tr>
<tr>
<td>Androgen receptor</td>
<td>1.4 (±1.2)</td>
<td>0</td>
<td>0</td>
<td>p &lt; .001</td>
</tr>
<tr>
<td>Androgen + α-actin</td>
<td>0.2 (±0.4)</td>
<td>0</td>
<td>0</td>
<td>p &lt; .001</td>
</tr>
</tbody>
</table>

Differences between the 3 groups of cells for α-actin (F2,37 = 19.8; p < .001), androgen receptor (F2,144 = 58.4; p < .001), and actin receptor (F2,141 = 12.3; p < .001) expression were highly significant.
in the proliferative areas that was significantly higher than in normal palmar fascia. *In vitro*, Dupuytren’s fibroblasts strongly expressed androgen receptors, whereas few normal fascia cells were immunoreactive. The colocalization of α-smooth muscle actin and androgen receptors in tissues and cultured cells of Dupuytren’s nodules showed that myofibroblasts are a target cell type for androgens. In the present study, exposure to increasing concentrations of 5α-DHT resulted in increased myofibroblast proliferation, which was associated with the strongest expression of α-smooth muscle actin, a highly reliable marker of myofibroblast differentiation. The importance of the expression of the α-actin isoform is unknown but may be related to the ability of fibroblasts to generate contractile force.

With regard to the expression of androgen receptors, it decreased in the group treated with the lower testosterone concentration and disappeared altogether at 100 ng/mL. This down-regulation of androgen receptors might be explained with a feedback mechanism controlling and limiting the increased proliferation.

Androgen exposure of Dupuytren’s diseased fascia induced myofibroblast differentiation from fibroblasts, myofibroblast proliferation, and down-regulation of androgen receptors consistently with the observation that testosterone can increase α-actin synthesis and stimulate smooth muscle cell proliferation. Similarly, in normal fascia androgens can induce myofibroblast differentiation from fibroblasts and down-regulate receptor expression.

Similar to the skin and prostate gland the palmar fascia might thus be an androgen-dependent tissue and its fibroproliferative disease, Dupuytren’s contracture, might be receptor regulated. In effect, one therapeutic option for acne and hirsutism, which are androgen-dependent diseases of sebaceous glands and hair follicles, and for prostate cancer and benign prostatic hyperplasia, which are regulated by androgen receptors, is treatment with antiandrogens.

Because androgen receptors were expressed similarly in male and female patients, it is far from clear whether the androgen-responsive state of the palmar fascia can account for the male predominance of Dupuytren’s disease. Because only 2 female patients were studied, however, differences may have been underestimated.

The palmar fascia is a target tissue for androgen action via androgen receptors. This action leads to cell proliferation, it enhances α-smooth muscle actin synthesis, and down-regulates androgen receptor ex-
pression. Further studies should address the question of whether the control of androgen receptors is able to control the evolution of Dupuytren’s disease.

References