Regulation of Proliferation and Platelet-Derived Growth Factor Expression in Palmar Fibromatosis (Dupuytren Contracture) by Mechanical Strain

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Summary: Palmar fibromatosis (Dupuytren contracture) causes fibrosis of specific palmar fascial bands. These bands are subjected to repetitive mechanical strain *in situ*. Primary cell cultures were derived from (a) palmar fibromatosis from eight patients, (b) uninvolved palmar fascia (Skoog's fibers) from four of these patients, and (c) normal palmar fascia from four additional patients. The cells were plated onto collagen-coated membranes either subjected to cyclic strain (25% maximal strain at 1 Hz) or without strain. Bromodeoxyuridine incorporation showed an increase in proliferation in all cultures subjected to strain. This increase was highest for palmar fibromatosis (10 to 40% nuclear incorporation, $p = 0.02$). Skoog's fibers and fascia from the normal individuals showed a trend (not significant) toward increase with strain (8 to 25%, $p = 0.15$ for Skoog's fibers, and 8 to 15%, $p = 0.45$ for normal fascia). Cyclic strain increased the expression of platelet-derived growth factor-A relative to glyceraldehyde-3-phosphate dehydrogenase in palmar fibromatosis (2.2 to 3.5, $p = 0.05$) and Skoog's fibers (0.8 to 2.0, $p = 0.04$). The expression of platelet-derived growth factor-B relative to glyceraldehyde-3-phosphate dehydrogenase was enhanced by cyclic strain only in the fibromatosis tissue (0.7 to 2.1, $p = 0.04$). The normal fascia did not express platelet-derived growth factor. Platelet-derived growth factor neutralizing antibody decreased bromodeoxyuridine incorporation in fibromatosis cultures subjected to cyclic strain to near levels for those grown in the absence of strain (38 to 16%, $p = 0.05$). Conditioned medium from fibromatosis cells grown under stain showed a trend toward increased proliferation in additional fibromatosis cultures compared with conditioned medium from fibromatosis cells grown without strain (9 to 15% nuclear incorporation, $p = 0.20$). The observed palmar fibromatosis contracture can be partially explained on the basis of the cell's response to cyclic strain, which may be mediated by platelet-derived growth factor.

Palmar fibromatosis (Dupuytren contracture) results in fibrosis and contracture of specific fascial bands in the palm of the hand (14). The fibrotic, contracted fascia limits motion of the joints of the fingers, often with significant functional loss. Clinically, the disorder progresses through stages, beginning with an "active nodule" and ending with a contracted band (21).

There is a higher prevalence of this disorder in individuals of Northern European descent. Alcohol use, antiseizure medications, and trauma may predispose to its development (23,24). Involved tissue demonstrates an abnormal ratio of type-I to type-III collagen (9); increased levels of prostaglandins (prostaglandin E_2 and prostaglandin alpha) (5); and expression of multiple growth factors, such as platelet-derived growth factor (PDGF) (26), basic fibroblast growth factor (19,32), and transforming growth factor beta (18,32). Cell culture study shows an abnormal response of cells derived from palmar fibromatosis to stimulation with several growth factors (1). Despite these studies, the underlying etiology remains unclear.

Only specific anatomic portions of the palmar fascia are involved in the process (14). The involved bands are those that are oriented longitudinally in the hand. One explanation for the observed anatomic distribution is that the involved bands are those that are subjected to repeated *in situ* stretch during daily activity (17). Other connective tissue cells, such as osteocytes and myocytes, demonstrate regulation in response to signal transducers and growth factors. Proliferation and differentiation have also been demonstrated as

responses to cellular mechanical strain (6,10-12,22). In palmar fibromatosis, cellular mechanical strain may be responsible for such changes in cellular behavior and may explain the anatomic location of the involved tissue.

PDGF is a dimeric protein (AA, AB, or BB dimers) that is a potent mitogen and chemotactic agent for fibrocytes and other mesenchymal cells (25). There are two PDGF receptors, alpha and beta. The A chain binds only the alpha receptor, whereas the B chain binds both receptors (30). Normal fibrocytes do not express PDGF except for a short period of time after local trauma (4). Fibrocyte-like cells from a variety of fibroproliferative lesions, including palmar fibromatosis, express PDGF (2,3,26). Involved fascia bands from palmar fibromatosis express mRNA for PDGF-B chain, whereas adjacent, uninvolved bands do not. Normal fibrocytes, as well as fibrocytes from palmar fibromatosis, express PDGF receptors (2,3,25,26,30).

The purpose of this study was to determine if mechanical strain at the cellular level is responsible for a proliferative effect in palmar fibromatosis and if this proliferative effect is mediated by PDGF.

METHODS

Primary cell cultures were established from (a) involved palmar fibromatosis tissue from eight patients, (b) adjacent, uninvolved fascial tissue from four of these patients (superficial transverse bands or Skoog's fibers), and (c) palmar fascia from four additional patients who did not have palmar fibromatosis. The tissues were mechanically dissociated and placed in RPMI-1640 medium with L-glutamine (BioWhittaker, Walkersville, MD, U.S.A.) and 50% fetal calf serum. The medium was changed after 4 days to RPMI-1640 with 10% fetal calf serum added. The cultures reached confluence in a 75 cm² flask in 2 weeks, after which they were divided, and an equal number of cells (0.5×10^4) were plated onto collagen-coated membrane-bottomed culture wells (Flexcell, McKeesport, PA, U.S.A.) that could be subjected to mechanical strain. All experiments were performed 4 days after plating the cells, while they were in their log phase of growth.

Mechanical strain was applied to the cultures with use of a computer-controlled vacuum device (Flexcell). Mechanical strain magnitude was chosen to approximate that to which the palmar fascia is subjected *in situ*. The palmar fascia is located deep to the palmar skin and undergoes a significant change in length as the finger is brought through a full range of motion. The change in length is less than the overlying skin, as the fascia is slightly closer to the center of rotation of the joints. The palmar skin at the proximal phalanx is subjected to a change in length of 38% as the finger goes through its full range of motion (29). Since the fascia should be subjected to a smaller change in length, we chose 25% maximal strain at 1 Hz. Our samples were harvested from the pretendinous band, which is located just proximal to the metacarpal phalangeal joint.

Proliferation was measured using bromodeoxyuridine incorporation (27). Ten micromolar bromodeoxyuridine (Sigma Chemical, St. Louis, MO, U.S.A.) per milliliter of culture medium was added to cell cultures. At the end of 12 hours, the cells were fixed with alcohol and paraformaldehyde. Horse serum was added as a blocking agent. The cells were incubated with a mouse anti-bromodeoxyuridine antibody (DAKO, Carpinteria, CA, U.S.A.) and DNase (Sigma Chemical). The primary antibody was detected

using a biotinylated secondary antibody with an avidin/biotinylated enzyme complex (Vector Laboratories, Burlingame, CA, U.S.A.). The percentage of bromodeoxyuridine incorporation was determined by counting the number of cells over 10 high-power fields (from the center to the periphery of the plate) that exhibited nuclear staining and comparing them with fields without staining. The percentage of bromodeoxyuridine incorporation for a given sample was determined by averaging the percentage of cells exhibiting nuclear incorporation over these 10 fields.

Proliferation experiments were performed with cells grown for 12 hours in medium containing bromodeoxyuridine either without mechanical strain or subjected to cyclic mechanical strain using the computer-controlled vacuum device. Bromodeoxyuridine incorporation was also measured in cells from palmar fibromatosis subjected to stretch for 12 hours with the addition of a PDGF neutralizing antibody (anti-human PDGF [AB] neutralizing antibody G5171; Promega, Madison, WI, U.S.A.) at a nearly complete neutralizing concentration (100 µl antibody/1 ml medium) or with the addition of control IgG (DAKO) at the same concentration. Additional palmar fibromatosis cultures were grown with and without stretch in serum-free medium (Ham's F-12 with insulin, selenium, and transferrin added) for 12 hours. The conditioned medium from the wells was used to grow additional palmar fibromatosis cultures with bromodeoxyuridine added. The proliferation rates of the cells in the conditioned media from stretched and not stretched cultures were compared.

Cells for RNA analysis were grown in serum-free medium (Ham's F-12 with insulin, selenium, and transferrin added) on identical collagen-coated membranes either with cyclic mechanical strain or without strain for 2, 4, and 6 hours for the first two patients' samples and for 4 hours for the remainder of the samples. RNA was extracted from cells using guanidinium isothiocyanate homogenization followed by cesium trifluoride ultracentrifugation (13).

A semiquantitative reverse transcriptase-polymerase chain reaction technique was used to measure differences in specific mRNA expression (3). mRNA was converted to cDNA using reverse transcriptase with a poly-T primer. Specific oligonucleotide primers for PDGF-A, PDGF-B, pro-alpha 1(I) collagen, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used. Forward and reverse oligonucleotides for each specific mRNA species were chosen from the following base pair locations: GAPDH, 386-403 and 561-580 (16); PDGF-A, 624-643 and 829-848 (8); PDGF-B, 1227-47 and 1433-53 (15); and pro-alpha 1(I) collagen, 3439-58 and 3793-74 (28), respectively. Polymerase chain reaction was performed using Taq polymerase for a short number of cycles (25) to allow for less than maximal amplification using identical starting quantities of total RNA for each sample (0.5 µg). The resultant products were electrophoresed, stained with ethidium bromide, and photographed under ultraviolet light. The densities of each band were measured with a computer-aided densitometer, with total density of each band calculated by integrating the area under the density curve for that band as previously reported (3). Standard curves of starting amounts of specific mRNA diluted with yeast tRNA, compared with resulting density of electrophoresed amplicons, were developed for each of the primers to ensure that relative differences in expression could be detected. The relative expression of each growth factor was determined by dividing the density measurement for that factor by the density measurement obtained for GAPDH in that same sample.

The semiquantitative findings from polymerase chain reaction were confirmed using Northern blot technique for differences detected with polymerase chain reaction from one specimen of involved fascia from a patient with fibromatosis, one specimen of uninvolved fascia from a patient with fibromatosis, and one specimen of fascia from a patient without fibromatosis. Additional samples were tested to obtain sufficient RNA (12 wells, greater

TABLE 1. *Proliferation measured by nuclear incorporation of bromodeoxyuridine*

Tissue type and test conditions	Bromodeoxyuridine incorporation (%)	SD	P value[a]
Palmar fibromatosis			
Strained	40	25	
Not strained	10	8	0.02
Strained palmar fibromatosis			
With IgG added	38	26	
With PDGF antibody	16	12	0.05
Skoog's fibers			
Strained	25	18	
Not strained	8	9	0.15
Normal fascia			
Strained	15	12	
Not strained	8	10	0.45
Conditioned media			
From strained cells	15	10	
From not strained cells	9	6	0.20

PDGF = platelet-derived growth factor.

[a] P value for difference between values on this row and those on the row above. The p value for the change (between strained and not strained) in percentage of bromodeoxyuridine incorporation comparing palmar fibromatosis with normal fascia is 0.04.

than 15 μg). Northern blot was performed with previously reported techniques (2) but with use of a digoxigenin-labeled oligonucleotide probe (the sense probe from the polymerase chain reaction primer pair) for PDGF-A, PDGF-B, and GAPDH, which was detected using a chemiluminescent technique (Boehringer Mannheim, Indianapolis, IN, U.S.A.).

Statistical analysis was performed with the aid of a desktop computer. Data from replicate experiments were averaged so that a single data point was used for statistical analysis for each sample. Means and SDs of the percentage of bromodeoxyuridine incorporation and relative density of specific mRNA expression were calculated. Data were compared using the two-tailed *t* test. Statistical analysis was not performed for Northern blot data, since only one sample of each tissue type was tested.

RESULTS

Mechanical cyclic strain increased the proliferative response in all cultures but increased the percentage of bromodeoxyuridine staining to a significant degree only for the cells derived from palmar fibromatosis (Table 1). This increase was from 10% positive nuclear uptake to 40% (p = 0.02) in the palmar fibromatosis tissue (Fig. 1), from 8 to 25% (p = 0.15) in the Skoog's fibers, and from 8 to 15% (p = 0.45) in normal palmar fascia. Nuclear incorporation was highest at the region of maximal strain (near the periphery of the plates) for the cells subjected to mechanical strain.

PDGF neutralizing antibody decreased the nuclear incorporation of bromodeoxyuridine compared with IgG control from 38% positive bromodeoxyuridine staining to 16% positive staining (p = 0.05) (Fig. 1). The serum-free conditioned medium from cells subjected to cyclic mechanical strain resulted in a trend (not statistically significant) toward an increase in proliferation compared with the conditioned medium
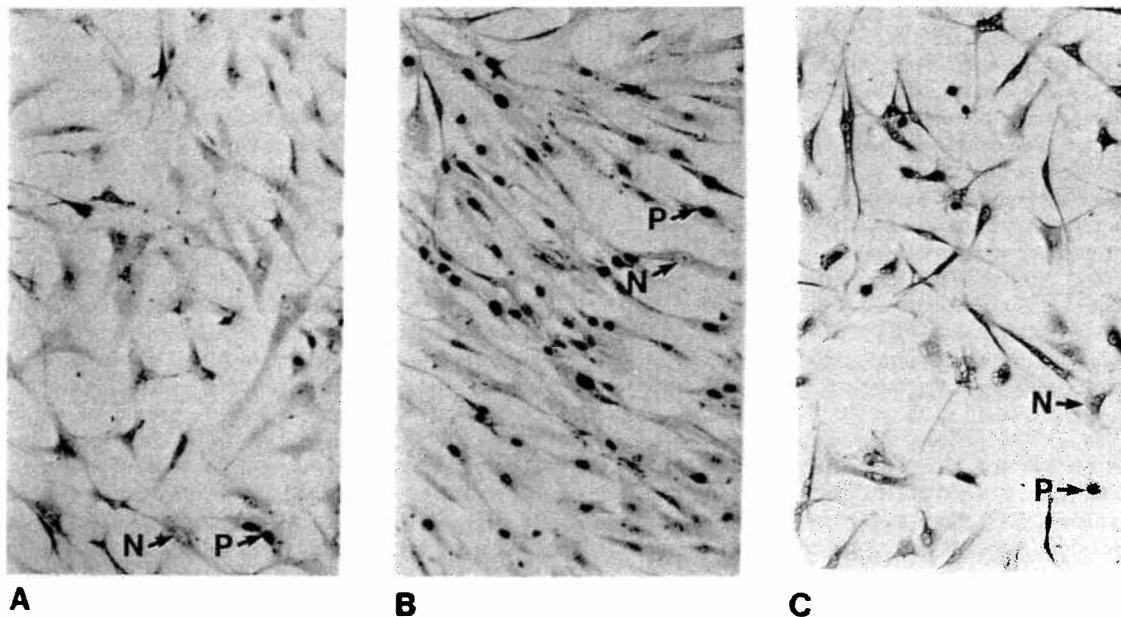

FIG. 1. Proliferation from primary cell culture derived from palmar fibromatosis. Bromodeoxyuridine was added to cultures for 12 hours and detected using immunohistochemistry. The black nuclei (P) took up bromodeoxyuridine during the test period, whereas the unstained nuclei (N) did not. **A:** Palmar fibromatosis cells grown on a collagen-coated membrane without mechanical strain, with an average of 10% of nuclei positive for bromodeoxyuridine uptake over 10 high-power fields. **B:** Cells grown in the presence of cyclic mechanical strain, with an average of 40% of nuclei positive for bromodeoxyuridine uptake over 10 high-power fields. **C:** Cells grown in the presence of mechanical strain with a platelet-derived growth factor (A and B) neutralizing antibody added. An average of 16% of nuclei were positive for bromodeoxyuridine uptake over 10 high-power fields.

TABLE 2. *Relative expression of PDGF compared with GAPDH as determined by semiquantitative polymerase chain reaction*

Tissue type and test conditions	Relative expression		SD		P value[a]	
	PDGF-A	PDGF-B	PDGF-A	PDGF-B	PDGF-A	PDGF-B
Palmar fibromatosis						
Strained	3.5	2.1	1.2	1.4		
Not strained	2.2	0.7	1.0	0.5	0.05	0.04
Skoog's fibers						
Strained	2.0	0	0.9	0		
Not strained	0.8	0	0.6	0	0.09	—
Normal fascia						
Strained	0	0	0	0		
Not strained	0	0	0	0	—	—

PDGF = platelet-derived growth factor and GAPDH = glyceraldehyde-3-phosphate dehydrogenase.

[a] P value for the difference between values on this row and those on the row above. The p values for the change (between strained and not strained) in relative levels of expression comparing palmar fibromatosis with normal fascia are less than 0.01 for PDGF-A and PDGF-B.

from the cells that were not subjected to mechanical strain from 9% nuclear bromodeoxyuridine incorporation to 15% (p = 0.20).

The initial two palmar fibromatosis samples showed increased expression of PDGF A and B chains with mechanical strain after 2 hours of cyclic strain; this peaked at 4 hours. The remainder of the samples were therefore studied for growth factor expression after a 4 hour period. The relative level of expression for the eight fibromatosis samples tested after 4 hours of cyclic strain showed a significant increase in the relative level of expression compared with GAPDH of 2.2 to 3.5 (p = 0.05) for the PDGF-A chain and 0.7 to 2.1 (p = 0.04) for the PDGF-B chain (Fig. 2, Table 2).

Uninvolved fascia from patients with palmar fibromatosis (Skoog's fibers) exhibited an increase in relative expression of the PDGF-A chain with cyclic strain (relative level of expression compared with GAPDH: 0.8 to 2.0, p = 0.09), but this difference did not achieve statistical significance. The Skoog's fibers did not express the PDGF-B chain (Fig. 2). Palmar fascia from the four individuals without palmar fibromatosis did not express A or B chains.

The relative level of expression of pro-alpha 1(I) collagen compared with GAPDH did not change with strain. Northern blot (Fig. 3) confirmed the results of semiquantitative reverse transcription-polymerase chain reaction for the single samples of each tissue type for which it was utilized.
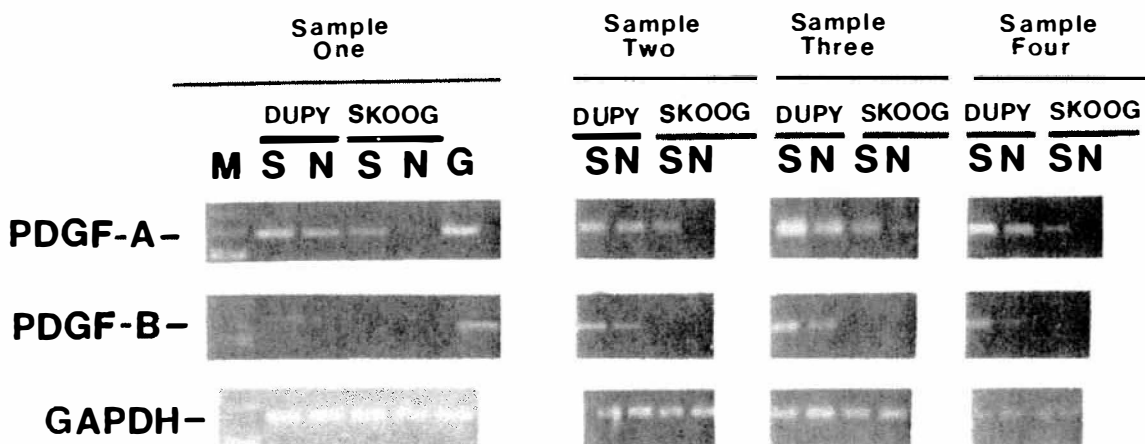

FIG. 2. Semiquantitative reverse transcription-polymerase chain reaction results for platelet-derived growth factor (PDGF)-A, PDGF-B, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from involved central band (DUPY) and uninvolved superficial transverse fibers (SKOOG) from four patients with palmar fibromatosis in whom both tissue types were available. Lanes labeled "S" were loaded with amplicons derived from samples subjected to 4 hours of cyclic mechanical strain before RNA extraction, whereas lanes labeled "N" were not subjected to mechanical strain. Lane "M" was loaded with a size marker and lane "G" was loaded with amplicons derived from RNA extracted from the A-172 glioblastoma cell line. There is increased relative expression of PDGF-A and PDGF-B in the palmar fibromatosis cultures (DUPY) subjected to strain compared with those not subjected to strain. Cultures from the superficial transverse fibers (SKOOG) also demonstrate increased relative expression of PDGF-A but not PDGF-B.
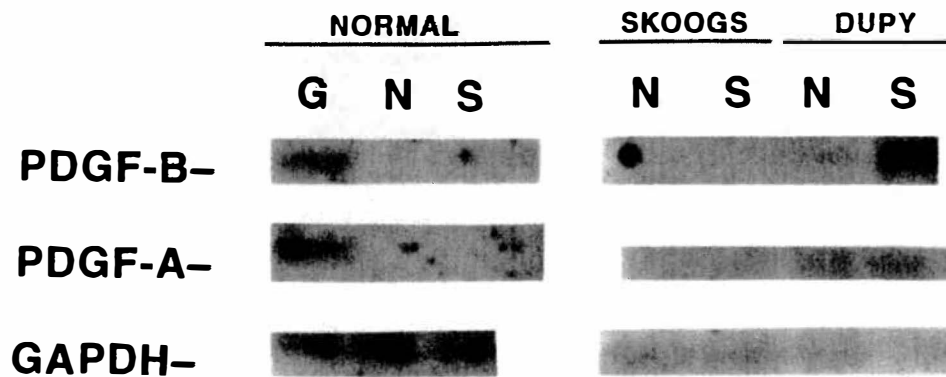
FIG. 3. Northern blot data from one sample of each tissue type. Lanes labeled "S" were loaded with RNA extracted from cultures subjected to cyclic mechanical strain for 4 hours, and lanes labeled "N" were loaded with RNA from cultures not subjected to mechanical strain. Lane "G" was loaded with RNA from the A-172 cell line (positive control for platelet-derived growth factor [PDGF]). "DUPY" is a primary cell culture from involved palmar fibromatosis, "SKOOGS" is a culture from the superficial transverse ligament (uninvolved tissues) from the same patient, and "NORMAL" is a palmar fascial culture from a patient without fibromatosis. These data confirm data obtained using semiquantitative polymerase chain reaction.

DISCUSSION

Cellular mechanical strain is responsible for a proliferative effect in palmar fibromatosis. The increase in proliferation can be partially reduced with a PDGF neutralizing antibody. Palmar fibromatosis and uninvolved fascial cells (Skoog's fibers) from patients with palmar fibromatosis will increase their levels of expression of the PDGF-A chain with mechanical strain, whereas fascial cells from patients without palmar fibromatosis do not express the PDGF-A chain. This points to an underlying abnormality, in affected individuals, in the cells forming the palmar fascia itself (that is, modulated by cellular strain).

The mechanism by which strain induces these cellular changes is probably complex. Studies of osteocytes and myocytes have shown that stretch alters calcium channels within a few milliseconds (22), signal transducers (such as inositol-3-phosphate) within seconds (11), and growth factor expression in hours (31). These changes lead to proliferation and differentiation (10,12). It is likely that mechanical strain activates many of these pathways in palmar fibromatosis cells.

Other cell types exhibit mechanical strain-induced proliferation mediated by PDGF. Wilson et al. (31) showed that cyclic mechanical strain caused a similar PDGF mediated response in vascular smooth-muscle cells, and Liu et al. (20) demonstrated a similar mechanism in fetal lung organ culture. Despite this mechanism's presence in a number of cell types, PDGF's expression is not enhanced by mechanical strain in cells from normal palmar fascia. The response of the fascia from involved individuals is, therefore, pathologic. Banes et al. (7) showed that mechanical strain and PDGF-BB act synergistically to increase DNA synthesis in tendon cells. Such an effect may play a role in palmar fibromatosis, in which the demonstrated expression of PDGF-B in vivo (26) may enhance the cells' proliferative response to mechanical strain.

There are several potential shortcomings to the use of this system to apply mechanical strain to palmar fibromatosis cells. One potential problem is that the observed effects could be caused by stirring of the media. However, if this were the case, one would expect proliferation to be greatest in the center (the site of greatest media motion) rather than near the periphery (the site of maximal cellular strain). The increased proliferation near the periphery that we observed points to mechanical strain as the more likely cause. The mechanical environment on the membranes is not in the same direction and may not be of the same magnitude as that in vivo. Despite this, we observed a difference in cellular response to mechanical strain between cells from pathologic and normal fascia, and the difference likely holds true for cells subjected to mechanical strain in vivo.

The semiquantitative reverse transcriptase-polymerase chain reaction technique is not as sensitive a technique for detecting differences as traditional Northern blot. It is plagued by variations in conditions that result in day-to-day and sample-to-sample differences in results. Its main advantage is that relatively small quantities of RNA can be used for analysis, such as a single well from a Flexcell plate, which does not yield sufficient RNA for Northern blot. Despite these disadvantages, semiquantitative reverse transcriptase-polymerase chain reaction can be used to detect relative differences between samples that are analyzed at the same time, under similar conditions (3). Thus, the results from the reaction are best thought of as qualitative rather than quantitative, despite the statistical significance of the results we obtained.

The lack of statistical significance in differences between the samples from normal fascia and Skoog's fibers is likely due to the number of samples tested.

This is supported by our observation that each of the samples individually showed an increase in nuclear incorporation with strain, and each of the Skoog's fiber samples showed an increase in PDGF-A expression compared with GAPDH with strain.

Cultures from the superficial transverse ligament (Skoog's fibers) demonstrated increased expression of PDGF-A chain but no expression of PDGF-B chain with cyclic mechanical strain. The explanation for the intermediate behavior of the uninvolved fascia from involved individuals (expression of PDGF-A but not PDGF-B) is unclear. Perhaps PDGF-B would be expressed with a strain of longer duration. Alternatively another event, or exogenous factor, may be necessary for PDGF-B expression. Further investigation is required to determine the underlying reason for this difference.

This study explains the observed longitudinal palmar anatomic location of the disease and demonstrates that there is an abnormality in the palmar fascia of individuals with this disorder. The proliferative response to mechanical strain in these tissues may be mediated by PDGF or enhanced by underlying PDGF expression. One potential treatment modality would be to block PDGF locally. However, palmar fibromatosis goes through different clinical stages, and proliferation may play a role only in the earlier stages, when an "active nodule" is present. In the later stages, it is possible that extracellular matrix formation may play a more important role. Therefore, the different factors expressed by the diseased tissues in different disease stages need to be delineated further before a trial of a PDGF-blocking agent in patients can be recommended.

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