

# Transforming Growth Factor- $\beta$ 1 Promotes the Morphological and Functional Differentiation of the Myofibroblast

Melville B. Vaughan,<sup>\*1</sup> Eric W. Howard,<sup>†</sup> and James J. Tomasek<sup>\*2</sup>

<sup>\*</sup>Department of Cell Biology and <sup>†</sup>Department of Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73104

The myofibroblast is responsible for the generation of contractile force associated with wound contraction and pathological contractures and is characterized by the presence of  $\alpha$ -smooth muscle ( $\alpha$ -sm) actin-containing stress fibers, vinculin-containing fibronexus adhesion complexes, and fibronectin fibrils containing the ED-A splice variant. Transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) can promote the expression of  $\alpha$ -sm actin in myofibroblasts, but the functional significance of this increased expression is unclear. In this study, we demonstrate, using the stress-relaxed collagen lattice contraction assay, that TGF- $\beta$ 1 promoted a dose-dependent increase in the generation of contractile force in myofibroblasts and a concomitant increase in the expression of  $\alpha$ -sm actin. We also demonstrate that TGF- $\beta$ 1 enhanced the formation of the structural elements important in myofibroblast contractile force generation and transmission, including stress fibers, vinculin-containing fibronexus adhesion complexes, and fibronectin fibrils, and that this enhancement occurred prior to, and independent of,  $\alpha$ -sm actin expression. This differentiated myofibroblast phenotype was not stable. Removal of TGF- $\beta$ 1 resulted in reduced expression of  $\alpha$ -sm actin as well as a decreased assembly of stress fibers and vinculin-containing adhesion complexes; however, there was no reduction in fibronectin fibrils. We conclude that TGF- $\beta$ 1 promotes the morphological and functional differentiation of the myofibroblast by first enhancing the formation of the structural elements characteristic of the myofibroblast followed by increased expression of  $\alpha$ -sm actin and contractile force generation.

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**Key Words:** myofibroblast; transforming growth factor- $\beta$ 1; collagen lattice; wound healing;  $\alpha$ -smooth muscle actin; fibronectin.

<sup>1</sup> Current address: Department of Cell Biology and Neuroscience, UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235.

<sup>2</sup> To whom correspondence and reprint requests should be addressed. Fax: (405) 271-3548. E-mail: james-tomasek@ouhsc.edu.

## INTRODUCTION

Tissue contraction is an integral part of normal wound closure; however, excessive or abnormal contraction of granulation tissue or other tissues leads to pathological contractures. An example is Dupuytren's disease, where contraction of the palmar fascia results in irreversible contracture with loss of function of the digits (for review, see [1]). Present in these tissues is the myofibroblast, a specialized fibroblast that has developed a contractile phenotype and contributes to tissue contraction [2–5]. With the development of a contractile phenotype, the myofibroblast acquires specific structural features associated with the generation and transmission of contractile force: bundles of actin microfilaments (termed stress fibers), vinculin-containing fibronexus adhesion complexes, and fibronectin (FN) fibrils [2, 4, 6–8]. These structural elements are what characterize myofibroblasts and distinguish them from normal connective tissue fibroblasts *in vivo*. In addition to these structural elements, the myofibroblast can also be identified based on the expression and assembly of  $\alpha$ -smooth muscle ( $\alpha$ -sm) actin into stress fibers [5, 9].

The cytokine transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) can be considered a direct inducer of the myofibroblast phenotype because it is capable of upregulating  $\alpha$ -sm actin in fibroblasts both *in vitro* and *in vivo* [10, 11]. Although the functional significance of  $\alpha$ -sm actin expression is unclear, studies have suggested that  $\alpha$ -sm actin may promote contractile force generation [12–15]. For this reason, we hypothesized that TGF- $\beta$ 1 could promote increased generation of contractile force in the myofibroblast and that this increase in force would correlate with increased expression of  $\alpha$ -sm actin. Previous studies have demonstrated that TGF- $\beta$ 1 can promote fibroblast-mediated collagen lattice contraction [16–24]; however, in all of these studies, collagen lattice contraction assays monitored collagen fibril reorganization that resulted from cell-generated tractional forces (migrational forces) rather than contractile force generation. In contrast, stress-relaxed collagen lattices provide a mechanism by which gener-

ation of contractile force can be directly measured [15, 25–28]. In this study, we have utilized stress-relaxed collagen lattices to test whether TGF- $\beta$ 1 can promote increased generation of contractile force by myofibroblasts and whether increased force generation is correlated with the expression of  $\alpha$ -sm actin.

TGF- $\beta$ 1, in addition to promoting the expression of  $\alpha$ -sm actin, can upregulate the expression of the extracellular glycoprotein FN [29]. Outside-in signals provided by FN have been shown to promote the formation of focal adhesions and stress fibers [30, 31]. We hypothesize that TGF- $\beta$ 1 can promote the formation of the structural elements important in myofibroblast contractile force generation and transmission, including stress fibers, vinculin-containing fibronexus adhesion complexes, and FN fibrils. Moreover, recent studies have demonstrated a temporal relationship between deposition of the ED-A isoform of FN (ED-A FN) and the expression of  $\alpha$ -sm actin, suggesting that deposition of ED-A FN may even be an intermediary step for the induction of  $\alpha$ -sm actin in response to TGF- $\beta$ 1 [32]. TGF- $\beta$ 1 may enhance the formation of the structural elements that characterize the myofibroblast independent of the expression of  $\alpha$ -sm actin, and the formation of these structural elements might even be a prerequisite for increased expression of  $\alpha$ -sm actin and contractile force generation in response to TGF- $\beta$ 1.

In this study, we demonstrate that TGF- $\beta$ 1 produced a dose-dependent increase in the generation of contractile force in myofibroblasts and that this correlated with increased expression of  $\alpha$ -sm actin. These results suggest that the functional consequence of increased expression of  $\alpha$ -sm actin is increased generation of contractile force. In addition, we found that TGF- $\beta$ 1 promoted the assembly of stress fibers, vinculin-containing fibronexus adhesion complexes, and FN-containing fibrils, all of which are structural elements postulated to be involved in force generation and transduction in the myofibroblast. Importantly, the formation of these structural elements appeared to be independent of  $\alpha$ -sm actin expression. We propose that TGF- $\beta$ 1 promotes the formation of the structural elements characteristic of the myofibroblast first and subsequently promotes increased expression of  $\alpha$ -sm actin and increased contractile force generation.

## MATERIALS AND METHODS

**Cell culture.** Human myofibroblasts were obtained as explant cultures [15, 33] or by collagenase digestion [34] of palmar aponeurosis from patients with Dupuytren's disease. Cells were cultured in complete culture medium composed of M-199 supplemented with 10% fetal bovine serum (FBS) (Irvine Scientific, Santa Ana, CA), 2 mM glutamine, and 1% antibiotic-antimycotic and used in all experiments between passages 5 and 12.

**Preparation of stressed collagen lattices.** Cells were cultured within stabilized type I collagen lattices as previously described [15,

25, 33, 35], such that the final collagen concentration was 0.65 mg/ml and the cell concentration was  $1.25 \times 10^5$  cells/ml. A 250- $\mu$ l drop of the collagen/cell suspension was plated onto 35-mm tissue culture dishes. After 1 h, complete culture medium containing either recombinant human TGF- $\beta$ 1 (Sigma, St. Louis, MO) (1–1000 pg/ml) or the appropriate dilution of vehicle (1 mg/ml bovine serum albumin in 4 mM HCl) was added to the cultures. After 2.5 days, fresh TGF- $\beta$ 1 or vehicle was added to the culture. To determine the specificity of the effect of TGF- $\beta$ 1, an anti-TGF- $\beta$ 1 neutralizing antibody or a control IgY antibody (R&D Systems, Minneapolis, MN) was added along with the TGF- $\beta$ 1. After 5 days in culture, collagen lattices were prepared for the collagen lattice contraction assay, fluorescence microscopy, or Western immunoblotting. For some experiments, collagen lattices were cultured for 5 days in the absence of TGF- $\beta$ 1 followed by an additional 4 days with or without TGF- $\beta$ 1. For reversal experiments, collagen lattices were cultured for an additional 10 days with or without the addition of fresh TGF- $\beta$ 1 every 2.5 days.

**Collagen lattice contraction assay.** Collagen lattice contraction was measured as previously described [15, 25, 33, 35]. After 5 days in culture, collagen lattices were mechanically released from the bottom of the tissue culture dishes by gently pipetting medium at the lattice–dish interface. Rapid contraction was analyzed by measuring the lattice diameter before release and at specific times after release using a Nikon SMZ-1 stereoscope. The initial collagen lattice diameters ranged from 14 to 16 mm. The fraction of the original collagen lattice diameter was obtained by dividing the diameter at each time point by the initial diameter of the lattice. All contraction assays were carried out in triplicate, and every experiment was repeated three or more times. Data points and error bars in these figures represent averages  $\pm$  SD. Where error bars cannot be seen, the data points are overlapped.

**Whole-mount staining.** Collagen lattices were fixed and stained as whole mounts as previously described [36]. Primary antibodies used included a mouse anti- $\alpha$ -sm actin monoclonal antibody (1:1000 dilution, clone 1A4, Sigma), a mouse anti-vinculin monoclonal antibody (1:400 dilution; Sigma), a mouse anti-fibronectin monoclonal antibody recognizing all fibronectin isoforms (IST-4), and a mouse anti-fibronectin monoclonal antibody recognizing specifically the ED-A isoform (IST-9; both fibronectin antibodies were generous gifts of Luciano Zardi, Laboratory of Cell Biology, Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy) [37, 38]. Secondary staining was performed using a rhodamine-conjugated goat anti-mouse antibody (1:100 dilution; Organon Teknica Corp., Durham, NC) or an Alexa 488-conjugated goat anti-mouse antibody (1:200 dilution; Molecular Probes, Eugene, OR). For multiple labeling with primary mouse antibodies, an isotype-specific goat anti-mouse IgG2a antibody conjugated to rhodamine (Sigma) was used to label the  $\alpha$ -sm actin antibody [32]. For  $\alpha$ -sm actin quantitation, lattice pieces were subsequently stained with 4',6-diamidino-2-phenylindole HCl (DAPI) (1  $\mu$ g/ml; Molecular Probes) to visualize nuclei. To visualize f-actin, lattices were stained with a rhodamine-conjugated phalloidin as per the manufacturer's instructions (Molecular Probes).

**Light and confocal laser microscopy.** For light microscopy, whole mounts of collagen lattices were viewed with an Olympus BH-2 or Vanox-S microscope equipped with epifluorescence illumination. Images were collected using either a 66 series SIT camera (Dage-MTI, Michigan City, IN) or a digital camera (SPOT; Diagnostic Instruments, Sterling Heights, MI). For confocal microscopy, an imaging system was employed (Microradiance; Bio-Rad) consisting of a 25-mW argon ion laser emitting at 488 and 514 nm and a 1-mW green helium–neon laser emitting at 546 nm attached to a BX-50 microscope (Olympus). Output was digitally processed; images were generated using 24-bit three-dimensional rendering software (LaserSharp; Bio-Rad).

**Quantification of  $\alpha$ -sm actin and vinculin staining.** For  $\alpha$ -sm actin images, a computerized image analysis program (Bioquant System IV; R&M Biometrics, Nashville, TN) was used to maintain a constant threshold for determining positive immunostaining of cells

with the anti- $\alpha$ -sm actin antibody, while the number of cells in the field was determined based on DAPI-positive nuclei. The percentage of cells expressing  $\alpha$ -sm actin was determined by counting at least 200 cells per collagen lattice per treatment group. Each experiment was repeated at least three times. To quantitate vinculin staining, digital images were saved as 8-bit bitmaps and then analyzed as follows using NIH Image program 1.6 (<http://www.scioncorp.com>; Scion Image, Scion Corp., Fredricksburg, MD). The area occupied by the entire cell was selected and then translated from grayscale to black and white. The number of white pixels per total number of pixels was then determined. The white pixels per  $\mu\text{m}^2$  were determined by analyzing at least 3 cells per collagen lattice with 3 lattices per experimental group. Data points and error bars represent averages  $\pm$  SEM.

**Western immunoblotting.** The level of  $\alpha$ -sm actin expression in response to TGF- $\beta$ 1 was analyzed by immunoblotting. To isolate cells, collagen lattices were washed in PBS for 10 min at room temperature followed by incubation with 0.05% trypsin, 0.53 mM EDTA, in PBS (Life Technologies) for 10 min at 37°C and subsequently digested in 3 mg/ml collagenase (type I; Sigma) in buffer (130 mM NaCl, 10 mM Ca acetate, 20 mM Hepes, pH 7.2) for 8 min at 37°C [28]. The enzymatic reaction was stopped by adding 50  $\mu$ l of

**TABLE 1**  
Effects of TGF- $\beta$ 1 on Properties of Different Myofibroblast Cultures

Cell Culture	Contractility % change	Cell number % change	$\alpha$ -sm actin expression	
			% before	% after
DP124p11	32	-1	13.5	30.5
DP142Cp1	16	-14.5	14	33.5
DP142Ep2	34.5	33.5	4	33.5
DP142Ep6	31.5	-5	3	20
DP131p16	11	9.5	13	23

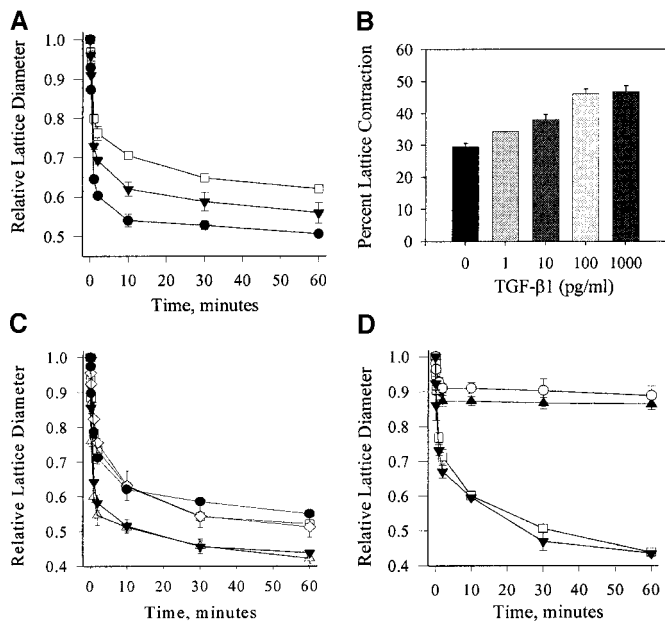
FBS and cell number was determined. Cells were pelleted and resuspended in sample buffer (5% SDS, 50% glycerol, 0.13 M Tris, pH 6.8, 0.1% bromophenol blue) at a concentration of  $2.5 \times 10^3$  cells/ $\mu$ l, followed by boiling for 3 min. A volume from each experimental group containing  $1.25 \times 10^4$  cells was electrophoresed on a 12.5% SDS gel. Separated proteins were transferred to PVDF membranes (Micron Separations Inc., Westborough, MA). Membranes were incubated for 4 h at 25°C with either a mouse anti- $\alpha$ -sm actin monoclonal antibody (1:400 dilution, clone 1A4; Sigma) or a rabbit polyclonal anti-actin antibody (1:250 dilution; Sigma) followed by a goat anti-mouse or a goat anti-rabbit alkaline phosphatase (Sigma) for 1 h at 25°C and subsequently developed with Immuno-Star chemiluminescence substrate (Bio-Rad, Hercules, CA) for 5 min at 25°C. X-ray film (X-OMAT AR5; Eastman Kodak, Rochester, NY) was exposed to chemiluminescence reaction for a period of 5–120 s at 25°C and film was developed.

## RESULTS

### TGF- $\beta$ 1 Increases Myofibroblast Contractility

We hypothesized that TGF- $\beta$ 1 would increase generation of contractile force in myofibroblasts. We found that TGF- $\beta$ 1 (0–1000 pg/ml) could increase contraction of myofibroblasts in stressed-relaxed collagen lattices in a dose-dependent manner (Figs. 1A and 1B). We examined five different myofibroblast cell strains from three different patients. All showed increased contraction in response to TGF- $\beta$ 1; however, the percentage increase in contraction varied between the different strains (Table 1). To determine whether increased contraction was a direct result of TGF- $\beta$ 1 treatment, TGF- $\beta$ 1-treated or control collagen lattices were incubated in the presence or absence of TGF- $\beta$ 1-neutralizing antibodies. Anti-TGF- $\beta$ 1 antibodies administered above 1  $\mu$ g/ml reduced contraction to control levels (Fig. 1C). To control against nonspecific antibody effect, similar lattices were incubated with 10  $\mu$ g/ml chicken IgY antibodies. These control antibodies had no effect on TGF- $\beta$ 1-promoted collagen lattice contraction (not shown). These results demonstrate that TGF- $\beta$ 1 can increase myofibroblast contraction of stress-relaxed collagen lattices.

We have previously demonstrated that the contrac-



**FIG. 1.** TGF- $\beta$ 1 increases myofibroblast contractility. Myofibroblasts were cultured within stressed collagen lattices for 5 days. (A) Lattices were incubated for the 5 days with 0 (□), 10 (▼), or 100 (●) pg/ml TGF- $\beta$ 1 and then released. (B) Lattices were incubated for the 5 days with varying concentrations of TGF- $\beta$ 1 and then released. Percent lattice contraction was determined 10 min after release. Increasing concentrations of TGF- $\beta$ 1 resulted in increasing generation of contractile force by myofibroblasts. (C) Lattices were incubated for the 5 days without TGF- $\beta$ 1 (□) or with 100 pg/ml TGF- $\beta$ 1 in the presence of 0 (△), 0.1 (▼), 1 (◇), or 10 (●)  $\mu$ g/ml anti-TGF- $\beta$ 1 antibodies and then released. Anti-TGF- $\beta$ 1 antibody administered at 1 or 10  $\mu$ g/ml inhibited TGF- $\beta$ 1-promoted contraction to the level of the no TGF- $\beta$ 1 control. (D) After 5 days in culture, lattices were washed for 5 min with serum-free media, treated with serum-free media (▲), 10% FBS (□), 1000 pg/ml TGF- $\beta$ 1 (○), or 1000 pg/ml TGF- $\beta$ 1 + 10% FBS (▼), and then released. TGF- $\beta$ 1 did not promote an increase in the reduction of collagen lattice diameter in the presence or absence of FBS.

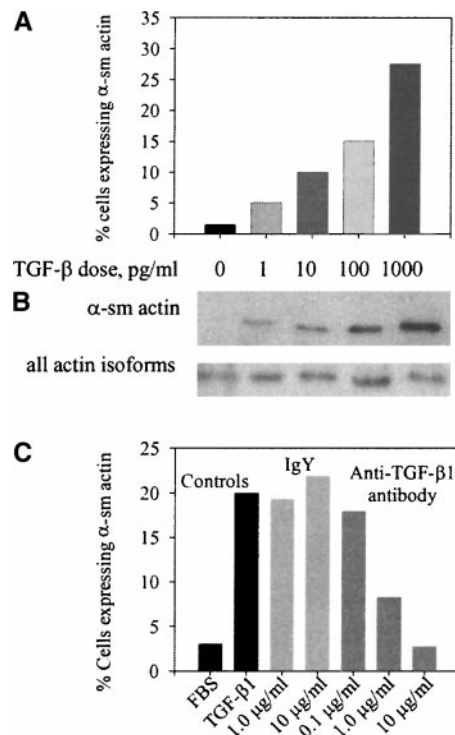


tion of stress-relaxed collagen lattices is dependent on cell number [25]. Since TGF- $\beta$ 1 can affect the proliferation rate of fibroblasts [39], we examined its effect on myofibroblast number in stressed collagen lattices. In initial studies, we found that TGF- $\beta$ 1 administered in the range of 5–50 ng/ml increased contraction of stress-relaxed collagen lattices; however, there was also a correlated increase in cell number compared with controls (M. B. Vaughan and J. J. Tomasek, not reported). TGF- $\beta$ 1 at lower concentrations (10–1000 pg/ml) promoted contraction and had no effect on cell number of the myofibroblast cell strain illustrated in Fig. 1 (Table 1, cell culture DP124). TGF- $\beta$ 1 had variable effects on the cell number of the other strains examined; however, collagen lattice contraction increased in all cases (Table 1). These results demonstrate that increased collagen lattice contraction in response to TGF- $\beta$ 1 is not the result of increased cell number.

Myofibroblast contraction of stress-relaxed collagen lattices can be stimulated by agonists such as FBS, LPA, and thrombin [25, 27, 33, 35]. To demonstrate that TGF- $\beta$ 1 does not act as an agonist to promote contraction, control stressed collagen lattices were washed with serum-free media immediately prior to addition of TGF- $\beta$ 1, FBS, or TGF- $\beta$ 1 + FBS and measurement of contraction. As previously described [25], FBS promoted collagen lattice contraction, while absence of agonist promoted little contraction (Fig. 1D). Addition of TGF- $\beta$ 1 did not increase contraction either in the absence or in the presence of FBS (Fig. 1D). These results demonstrate that TGF- $\beta$ 1 does not function as a contractile agonist, but appears to function by promoting the differentiation of a more contractile phenotype.

#### *Increased Generation of Contractile Force Is Correlated with Increased Expression of $\alpha$ -sm Actin*

We wished to determine whether increased collagen lattice contraction that occurs in response to TGF- $\beta$ 1 is correlated with increased expression of  $\alpha$ -sm actin. The percentage of myofibroblasts immunostained for  $\alpha$ -sm actin was dramatically increased with TGF- $\beta$ 1 treatment in a dose-dependent manner (Fig. 2A). Immunostaining for  $\alpha$ -sm actin was restricted to bundles of actin microfilaments within the myofibroblasts (see Figs. 5A and 5C). An increase in the percentage of myofibroblasts expressing  $\alpha$ -sm actin was observed in all the cell strains examined (Table 1). The amount of  $\alpha$ -sm actin expressed in the entire cell population increased with increasing concentrations of TGF- $\beta$ 1 as measured by Western blot analysis (Fig. 2B). Similar to what was observed for collagen lattice contraction, neutralizing anti-TGF- $\beta$ 1 antibodies, when used at 1  $\mu$ g/ml or above, reduced the percentage of cells expressing  $\alpha$ -sm actin (Fig. 2C). These results demonstrate

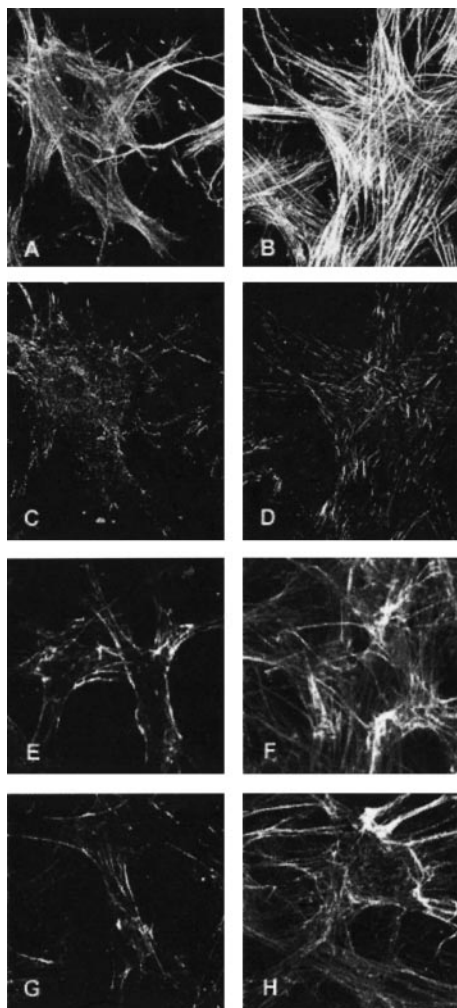


**FIG. 2.** TGF- $\beta$ 1 promotes increased expression of  $\alpha$ -sm actin expression in myofibroblasts in stressed collagen lattices. (A) Myofibroblasts within stressed collagen lattices were incubated for 5 days in the presence of increasing concentrations of TGF- $\beta$ 1 and then double stained with DAPI and anti- $\alpha$ -sm actin antibody. The percentage of myofibroblasts expressing  $\alpha$ -sm actin was increased with increasing concentration of TGF- $\beta$ 1. (B) Myofibroblasts within stressed collagen lattices were incubated for 5 days in the presence of increasing concentrations of TGF- $\beta$ 1;  $\alpha$ -sm actin and total actin (all actin isoforms) were then evaluated by Western blot analysis of equal numbers of cells. Treatment with TGF- $\beta$ 1 resulted in increased expression of  $\alpha$ -sm actin compared with total actin. (C) Myofibroblasts within stressed collagen lattices were incubated for 5 days in the presence of 100 pg/ml TGF- $\beta$ 1 containing either increasing concentrations of a TGF- $\beta$ 1-neutralizing antibody or a control chicken IgY antibody. Myofibroblasts were double stained with DAPI and anti- $\alpha$ -sm actin antibody. The increase in the percentage of cells expressing  $\alpha$ -sm actin in response to TGF- $\beta$ 1 was blocked by anti-TGF- $\beta$ 1 antibody.

that increased expression of  $\alpha$ -sm actin in response to TGF- $\beta$ 1 is correlated with increased force generation.

#### *TGF- $\beta$ 1 Promotes Formation of Myofibroblast Structural Elements*

We wanted to determine whether TGF- $\beta$ 1 could enhance the formation of the structural elements that characterize myofibroblasts. Stressed collagen lattices containing myofibroblasts were cultured in the presence or absence of 100 pg/ml TGF- $\beta$ 1 for 5 days followed by fixation. Staining with rhodamine phalloidin, a fluorescent probe that binds f-actin, revealed that the thickness and number of stress fibers increased with



**FIG. 3.** TGF- $\beta$ 1 enhances assembly of stress fibers, vinculin-containing adhesion complexes, and ED-A FN fibrils. Myofibroblasts within stressed collagen lattices were treated for 5 days in the absence (A, C, E, G) or presence (B, D, F, H) of 100 pg/ml TGF- $\beta$ 1. Myofibroblasts were double labeled with rhodamine phalloidin (A, B) and with anti-vinculin antibody (C, D) and visualized by confocal microscopy. When compared with controls, TGF- $\beta$ 1 increased the assembly of stress fibers and vinculin-containing adhesion complexes. Total fibronectin (E, F) and ED-A FN (G, H) were visualized using immunofluorescence. TGF- $\beta$ 1 promoted the assembly of both total and ED-A fibronectin. Magnification: A–D, 375 $\times$ ; E–H, 325 $\times$ .

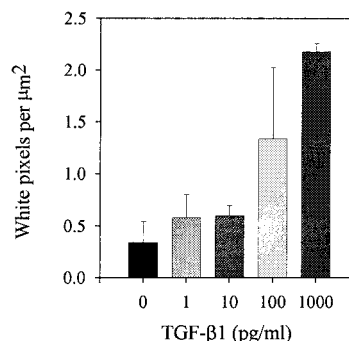
TGF- $\beta$ 1 treatment (Figs. 3A and 3B). To investigate the effect of TGF- $\beta$ 1 on the fibronexus adhesion complex, we examined the distribution of the actin-binding protein vinculin, which has previously been shown to be located in this complex [6]. Vinculin immunostaining in untreated cultures was dispersed over the cell body and organized into small linear vinculin-containing adhesion complexes at the periphery of the cell (Fig. 3C). In contrast, TGF- $\beta$ 1 treatment resulted in a dramatic increase in the number and size of the linear vinculin-containing adhesion complexes (Fig. 3D). To quantitate this size increase, we measured these com-

plexes in control and TGF- $\beta$ 1-treated stressed collagen lattices. The size of these fibronexus adhesion complexes increased with TGF- $\beta$ 1 treatment (Fig. 4). We also investigated whether FN fibril assembly was increased with TGF- $\beta$ 1 treatment. Immunofluorescence using a monoclonal antibody recognizing all FN isoforms demonstrated that FN fibril assembly was increased with TGF- $\beta$ 1 treatment (Figs. 3E and 3F). Previous work has demonstrated that the expression of the ED-A FN is increased with TGF- $\beta$ 1 treatment and is present in tissues containing myofibroblasts [32, 40]. Immunostaining with an antibody specific to ED-A FN showed increased assembly of this FN isoform in response to TGF- $\beta$ 1 (Figs. 3G and 3H).

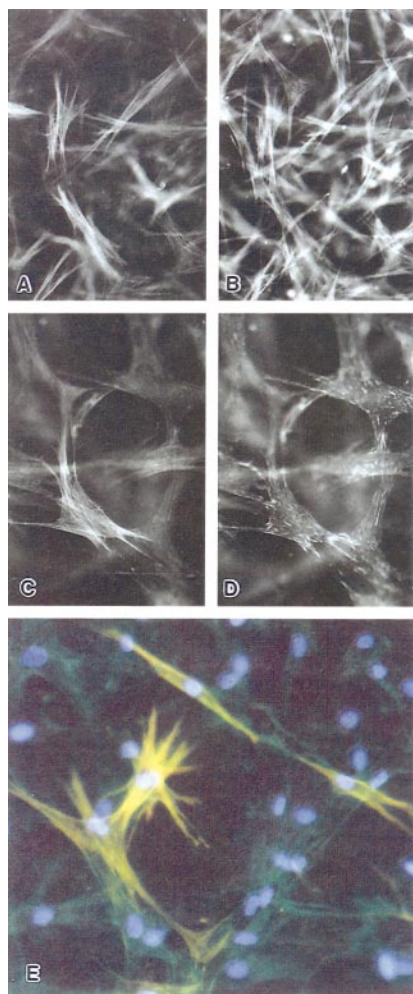
#### *TGF- $\beta$ 1-Enhanced Formation of Myofibroblast Structural Elements Is Independent of $\alpha$ -sm Actin Expression*

During our study, we noticed that all of the myofibroblasts treated with TGF- $\beta$ 1 appeared to show increased assembly of these structural elements, in contrast to immunostaining for  $\alpha$ -sm actin, which only stained a subset of the cells (20–30%). To further examine this finding, we performed double labeling of myofibroblasts that had been treated with TGF- $\beta$ 1. We found that increased assembly of stress fibers, vinculin-containing adhesion complexes, and ED-A FN fibrils could be found in both  $\alpha$ -sm actin positive and  $\alpha$ -sm actin negative myofibroblasts (Fig. 5). These results demonstrate that increased assembly of these structural elements occurs with TGF- $\beta$ 1 treatment; however, increased assembly of the structural elements is not correlated on an individual-cell basis with the expression of  $\alpha$ -sm actin.

We next examined whether there was a temporal correlation between the enhanced formation of these



**FIG. 4.** TGF- $\beta$ 1 increases the size of vinculin-containing adhesion complexes in myofibroblasts. Myofibroblasts within stressed collagen lattices were treated for 5 days with increasing concentrations of TGF- $\beta$ 1. Myofibroblasts were immunostained with anti-vinculin antibody and the size of focal adhesions determined. TGF- $\beta$ 1 increased the size of vinculin-containing adhesion complexes.



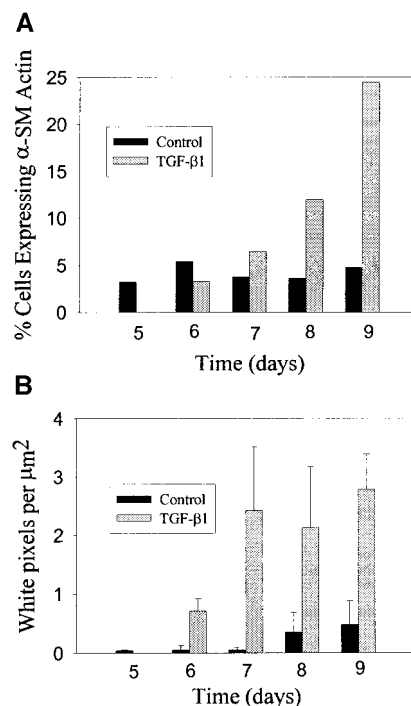
**FIG. 5.** Differential effect of TGF- $\beta$ 1 treatment on  $\alpha$ -sm actin expression and assembly of structural elements. Myofibroblasts within stressed collagen lattices were treated for 5 days with 100 pg/ml TGF- $\beta$ 1 followed by preparation for immunocytochemistry. Double staining for  $\alpha$ -sm actin (A, C) and stress fibers (B) or vinculin (D) shows that the majority of cells have increased stress fiber and vinculin staining, but only a subset of cells stain positively for  $\alpha$ -sm actin. Color photo (E) shows a small proportion of  $\alpha$ -sm actin positive cells (orange) and a large number of ED-A FN staining cells (green). Colocalization of staining appears yellow. Magnification: 125 $\times$  (A, B), 175 $\times$  (C-E).

structural elements and the expression of  $\alpha$ -sm actin in TGF- $\beta$ 1-treated myofibroblasts. Myofibroblasts were cultured in stressed collagen lattices for 5 days to allow for the initial formation of stress fibers, fibronexus adhesion complexes, and fibronectin fibrils followed by treatment with 100 pg/ml TGF- $\beta$ 1 for up to 4 days. Myofibroblasts were analyzed at days 1–4 post-TGF- $\beta$ 1 treatment for formation of these structural elements and percentage of cells expressing  $\alpha$ -sm actin. The percentage of cells expressing  $\alpha$ -sm actin did not increase until 2 days posttreatment and continued to increase at 4 days posttreatment (Fig. 6A). In contrast,

the size of vinculin-containing adhesion complexes was increased 1 day posttreatment and reached a maximum by 2 days posttreatment (Fig. 6B). The enhanced formation of stress fibers and FN fibrils in response to TGF- $\beta$ 1 followed a similar temporal pattern to that of the vinculin immunostaining (not illustrated). These results demonstrate that the enhanced formation of structural elements in myofibroblasts in response to TGF- $\beta$ 1 occurs prior to  $\alpha$ -sm actin expression.

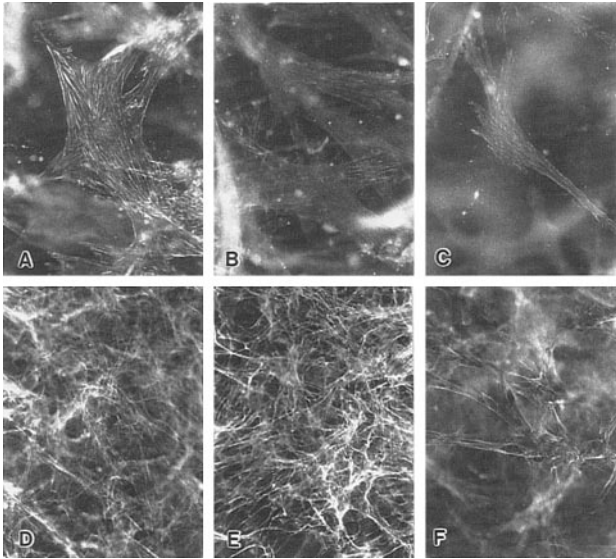
#### *The Myofibroblast Phenotype Is Partially Reversed after Removal of TGF- $\beta$ 1*

To determine whether the effects we observed in response to TGF- $\beta$ 1 could be reversed, TGF- $\beta$ 1-treated 5-day stressed collagen lattices were washed and incubated for an additional 10 days in the presence or absence of TGF- $\beta$ 1. Removal of TGF- $\beta$ 1 after 5 days of treatment lowered the percentage of myofibroblasts expressing  $\alpha$ -sm actin compared to 15-day TGF- $\beta$ 1



**FIG. 6.** TGF- $\beta$ 1-enhanced formation of vinculin-containing adhesion complexes occurs prior to  $\alpha$ -sm actin expression. Myofibroblasts were cultured for 5 days within stressed collagen lattices followed by treatment for 1–4 days with or without 100 pg/ml TGF- $\beta$ 1. (A) Myofibroblasts were double stained with DAPI and anti- $\alpha$ -sm actin antibody and the percentage of myofibroblasts expressing  $\alpha$ -sm actin determined. TGF- $\beta$ 1 did not increase  $\alpha$ -sm actin expression until 2 days posttreatment and expression continued to increase dramatically between 3 and 4 days posttreatment. (B) Myofibroblasts were immunostained with anti-vinculin antibody and the size of focal adhesions determined. TGF- $\beta$ 1 began to increase the size of vinculin-containing adhesion complexes 1 day posttreatment and reached a maximum by 2 days posttreatment.





**FIG. 7.** Removal of TGF- $\beta$ 1 decreases assembly of vinculin-containing adhesion complexes but not fibronectin fibrils. Myofibroblasts within stressed collagen lattices were cultured for 15 days with TGF- $\beta$ 1 (A, D), 5 days with TGF- $\beta$ 1 followed by 10 days without TGF- $\beta$ 1 (B, E), or 15 days without TGF- $\beta$ 1 (C, F). Myofibroblasts were fixed and stained with anti-vinculin antibody (A–C) or anti-total fibronectin antibody (D–F). Magnification: 275 $\times$  (A–C), 75 $\times$  (D–F).

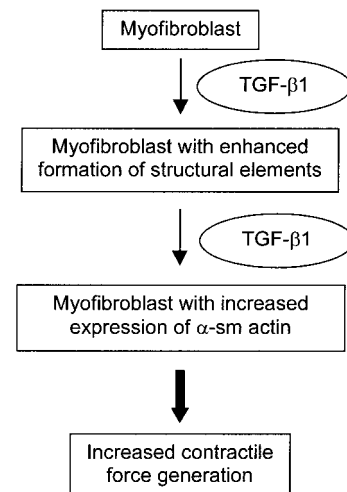
treatment (from 37 to 17%) to near levels of untreated 15-day stressed collagen lattices (15%) (data not shown). Similarly, the removal of TGF- $\beta$ 1 resulted in stress fibers and vinculin-staining adhesion complexes that resembled those in untreated, 15-day stressed lattices (Figs. 7A–7C). However, FN fibrils, stained with the anti-total FN antibody IST-4 were not reduced (Figs. 7D–7F). These results suggest that removal of TGF- $\beta$ 1 reduces the expression of  $\alpha$ -sm actin and the assembly of stress fibers and vinculin-containing adhesion complexes, but does not result in a loss of previously assembled FN fibrils.

## DISCUSSION

The expression of  $\alpha$ -sm actin and the formation of stress fibers, vinculin-containing adhesion complexes, and FN fibrils are the primary criteria for determining the differentiation of nonmuscle cells into contractile myofibroblasts. TGF- $\beta$ 1 will increase the expression of  $\alpha$ -sm actin in myofibroblasts cultured within three-dimensional stressed collagen lattices. The significance of this increased expression of  $\alpha$ -sm actin with regard to function has not been fully established. We demonstrate for the first time that TGF- $\beta$ 1 will increase generation of contractile force by myofibroblasts and that increased contractile force generation is correlated with increased expression of  $\alpha$ -sm actin. These results

strongly suggest that the function of increased expression of  $\alpha$ -sm actin is to increase the generation of contractile force. We also demonstrate that TGF- $\beta$ 1 will enhance the formation of stress fibers, vinculin-containing adhesion complexes, and FN fibrils by myofibroblasts and that this enhanced formation of structural elements is independent of  $\alpha$ -sm actin expression. The assembly of these structural elements may actually be a prerequisite for TGF- $\beta$ 1-increased  $\alpha$ -sm actin expression and force generation. In our culture conditions, this phenotype was not stable, in that withdrawal of TGF- $\beta$ 1 resulted in the reversal of all observed increases except for assembled FN fibrils, which remained in the ECM. We propose that TGF- $\beta$ 1 first promotes the enhanced formation of the structural elements that characterize the myofibroblast followed by increased expression of  $\alpha$ -sm actin and contractile force generation (Fig. 8).

The novel finding that TGF- $\beta$ 1 enhances the formation of stress fibers, vinculin-containing adhesion complexes, and FN fibrils independent of  $\alpha$ -sm actin expression is supported by two lines of evidence. First, all of the myofibroblasts in stressed collagen lattices treated with TGF- $\beta$ 1 have enhanced formation of the structural elements, while only a maximum of 35% have increased expression of  $\alpha$ -sm actin in response to TGF- $\beta$ 1. Second, enhanced formation of the structural elements occurs prior to increased expression of  $\alpha$ -sm



**FIG. 8.** Mechanism by which TGF- $\beta$ 1 promotes the morphological and functional differentiation of the myofibroblast. TGF- $\beta$ 1 is proposed to enhance the formation of the structural elements important in myofibroblast contractile force generation and transmission, including stress fibers, vinculin-containing fibronexus adhesion complexes, and fibronectin fibrils. The enhanced formation of these structural elements would occur prior to, and independent of,  $\alpha$ -sm actin expression. TGF- $\beta$ 1, in combination with the enhanced formation of structural elements, is proposed to increase the expression of  $\alpha$ -sm actin. This increased expression of  $\alpha$ -sm actin would result in increased generation of contractile force.

actin. The mechanism by which TGF- $\beta$ 1 enhances structural element formation is unclear. TGF- $\beta$ 1 has been previously demonstrated to increase the expression and assembly of FN [29, 41], which can provide outside-in signals promoting focal adhesion and stress fiber formation [30, 31]. TGF- $\beta$ 1 can also increase the expression of integrin receptors [42] and vinculin [43], both components of the fibronexus adhesion complex, and could potentially enhance the formation of these structural elements. Our results suggest that the formation of these structural elements may actually be a prerequisite for TGF- $\beta$ 1-induced expression of  $\alpha$ -sm actin, consistent with the observations of Serini and co-workers that increased expression of  $\alpha$ -sm actin in response to TGF- $\beta$ 1 requires the presence of fibrillar ED-A FN [32]. We propose a temporal relationship: TGF- $\beta$ 1 first promotes enhanced formation of the structural elements characterizing the myofibroblast followed by increased expression of  $\alpha$ -sm actin and increased force generation (Fig. 8).

We have also demonstrated that TGF- $\beta$ 1 increases the generation of contractile force by myofibroblasts, as measured using stress-relaxed collagen lattices. We specifically chose the stressed-relaxed collagen lattice model for this study because it measures the relative amount of contractile force generated by the myofibroblasts within the collagen lattice [25–27]. Myofibroblasts within the collagen lattice undergo isotonic contraction upon stress release, most likely the result of stress fiber contraction, resulting in the rapid contraction of the collagen lattice [25–27]. Numerous other investigators have demonstrated that TGF- $\beta$ 1 can promote collagen lattice contraction; however, all of these studies were examining either free-floating [16–22] or compliant collagen lattices [23, 24]. Lattice contraction in these models occurs over hours or days and is the result of tractional force-mediated matrix remodeling due to cell motility and appears to be regulated by a different signaling pathway than contraction of stress-relaxed collagen lattices [26, 27, 44]. In addition, fibroblasts in floating collagen lattices lack the structural elements defining the myofibroblastic phenotype [26]. Our study directly demonstrates for the first time that TGF- $\beta$ 1 can increase contractile force generation in myofibroblasts.

Our results demonstrating a correlation between  $\alpha$ -sm actin expression and contractile force generation strongly support the hypothesis that increased expression of  $\alpha$ -sm actin promotes increased force generation. Consistent with these results, we have previously demonstrated a correlation between  $\alpha$ -sm actin expression and contraction of stress-relaxed collagen lattices in different strains of cultured Dupuytren's myofibroblasts [15]. Other studies have presented evidence that increased  $\alpha$ -sm actin expression correlates with increased collagen lattice contraction; however, these are

difficult to interpret since the lattice contraction in these studies was the result of tractional force rather than contractile force [16, 22, 24, 45]. Different actin isoforms may have functional differences [13].  $\alpha$ -sm actin is functionally sorted to contractile stress fibers, while  $\beta$ -actin predominates in the highly motile lamellipodia of cultured pericytes [12]. Similarly, the expression of  $\alpha$ -sm actin has been inversely correlated with the motility of myofibroblasts; antisense oligonucleotides to  $\alpha$ -sm actin will enhance the migration of cultured myofibroblasts [14]. While we propose that increased expression of  $\alpha$ -sm actin is responsible for increased force generation, it is possible that the enhanced formation of myofibroblast structural elements accounts for at least some of the increased force generation. Currently, it is not possible to determine how much each of these contribute to increased force generation; however, it is clear that together they result in increased generation of contractile force by myofibroblasts in response to TGF- $\beta$ 1.

The mechanism by which TGF- $\beta$ 1 promotes increased force generation in myofibroblasts is different from what has been reported for other contractile agonists, such as FBS, thrombin, and LPA. These agonists appear to promote contraction by the activation of specific intracellular signaling cascades resulting in increased myosin light chain phosphorylation and contractility [27, 46, 47]. TGF- $\beta$ 1 by itself does not promote contraction; rather, it enhances the contractile responsiveness of stress-relaxed collagen lattices to these contractile agonists. We propose that TGF- $\beta$ 1 promotes the differentiation of a more contractile phenotype, including increased formation of structural elements and expression of  $\alpha$ -sm actin, both resulting in enhanced myofibroblast contraction.

The myofibroblast phenotype that develops in response to TGF- $\beta$ 1 is not stable. It has been previously demonstrated that removal of TGF- $\beta$ 1 results in decreased expression of  $\alpha$ -sm actin [48]. We show here that removal of TGF- $\beta$ 1 also results in a return of stress fibers and vinculin-containing adhesion complexes to control levels. In contrast, assembly of FN fibrils, including ED-A FN, did not return to control levels after TGF- $\beta$ 1 removal. These results suggest that the presence of ED-A FN fibrils, in the absence of TGF- $\beta$ 1, was insufficient to maintain the enhanced myofibroblast phenotype.

The role of TGF- $\beta$ 1 during wound healing may be biphasic. Initially, TGF- $\beta$ 1 may function to increase fibroblast migration into the wound, based on its ability to stimulate fibroblast chemotaxis and cell migration [49]. Tractional forces exerted by these migratory fibroblasts could promote initial wound closure. With time, however, resistance would increase and, similar to stressed collagen lattices, tension would begin to develop [26]. Under these conditions, the fibroblasts



would begin to acquire the myofibroblast phenotype, with the formation of stress fibers, fibronexus adhesion complexes, and FN fibrils. TGF- $\beta$ 1 would enhance the formation of these structural elements similar to myofibroblasts in stressed collagen lattices. ED-A FN expressed in response to TGF- $\beta$ 1 would become assembled into FN fibrils. TGF- $\beta$ 1 and ED-A FN may act synergistically to promote  $\alpha$ -sm actin expression [32], which could serve as a brake for cell migration, favoring increased force generation and increased tension. In addition, increased tension could provide a positive-feedback mechanism to enhance both ED-A FN assembly and signaling from TGF- $\beta$ 1. If the signaling from ED-A FN and TGF- $\beta$ 1 were to continue, a pathological contracture, such as Dupuytren's disease, would occur. This model provides a basis by which these steps can now be tested both *in vitro* and *in vivo*.

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## REFERENCES

- Tomasek, J. J., Vaughan, M. B., and Haaksma, C. J. (1999). Cellular structure and biology of Dupuytren's disease. *Hand Clin.* **15**, 21–34.
- Gabbiani, G., and Majno, G. (1972). Dupuytren's contracture: Fibroblast contraction? *Am. J. Pathol.* **66**, 131–146.
- Darby, I., Skalli, O., and Gabbiani, G. (1990). Alpha-smooth muscle actin is transiently expressed by myofibroblasts during experimental wound healing. *Lab. Invest.* **63**, 21–29.
- Tomasek, J. J., and Haaksma, C. J. (1991). Fibronectin filaments and actin microfilaments are organized into a fibronexus in Dupuytren's diseased tissue. *Anat. Rec.* **230**, 175–182.
- Desmouliere, A., and Gabbiani, G. (1994). Modulation of fibroblastic cytoskeletal features during pathological situations: The role of extracellular matrix and cytokines. *Cell Motil. Cytoskeleton* **29**, 195–203.
- Singer, I. I. (1979). The fibronexus: a transmembrane association of fibronectin-containing fibers and bundles of 5 nm microfilaments in hamster and human fibroblasts. *Cell* **16**, 675–685.
- Singer, I. I., Kawka, D. W., Kazazis, D. M., and Clark, R. A. (1984). *In vivo* co-distribution of fibronectin and actin fibers in granulation tissue: Immunofluorescence and electron microscope studies of the fibronexus at the myofibroblast surface. *J. Cell Biol.* **98**, 2091–2106.
- Welch, M. P., Odland, G. F., and Clark, R. A. (1990). Temporal relationships of F-actin bundle formation, collagen and fibronectin matrix assembly, and fibronectin receptor expression to wound contraction. *J. Cell Biol.* **110**, 133–145.
- Schur, W., Skalli, O., and Gabbiani, G. (1990). Cellular biology. In "Dupuytren's Disease" (R. M. McFarlane, D. A. McGrouther, and M. H. Flint, Eds.), Churchill Livingstone, London, 31–47.
- Desmouliere, A., Geinoz, A., Gabbiani, F., and Gabbiani, G. (1993). Transforming growth factor-beta 1 induces alpha-smooth muscle actin expression in granulation tissue myofibroblasts and in quiescent and growing cultured fibroblasts. *J. Cell Biol.* **122**, 103–111.
- Ronnov-Jessen, L., and Petersen, O. W. (1993). Induction of alpha-smooth muscle actin by transforming growth factor-beta 1 in quiescent human breast gland fibroblasts. Implications for myofibroblast generation in breast neoplasia. *Lab. Invest.* **68**, 696–707.
- DeNofrio, D., Hoock, T. C., and Herman, I. M. (1989). Functional sorting of actin isoforms in microvascular pericytes. *J. Cell Biol.* **109**, 191–202.
- Herman, I. M. (1993). Actin isoforms. *Curr. Opin. Cell Biol.* **5**, 48–55.
- Ronnov-Jessen, L., and Petersen, O. W. (1996). A function for filamentous alpha-smooth muscle actin: Retardation of motility in fibroblasts. *J. Cell Biol.* **134**, 67–80.
- Tomasek, J. J., and Rayan, G. M. (1995). Correlation of alpha-smooth muscle actin expression and contraction in Dupuytren's disease fibroblasts. *J. Hand Surg. [Am.]* **20**, 450–455.
- Moulin, V., Castilloux, G., Auger, F. A., Garrel, D., O'Connor-McCourt, M. D., and Germain, L. (1998). Modulated response to cytokines of human wound healing myofibroblasts compared to dermal fibroblasts. *Exp. Cell Res.* **238**, 283–293.
- Montesano, R., and Orci, L. (1988). Transforming growth factor beta stimulates collagen-matrix contraction by fibroblasts: Implications for wound healing. *Proc. Natl. Acad. Sci. USA* **85**, 4894–4897.
- Finesmith, T. H., Broadley, K. N., and Davidson, J. M. (1990). Fibroblasts from wounds of different stages of repair vary in their ability to contract a collagen gel in response to growth factors. *J. Cell. Physiol.* **144**, 99–107.
- Tingstrom, A., Heldin, C. H., and Rubin, K. (1992). Regulation of fibroblast-mediated collagen gel contraction by platelet-derived growth factor, interleukin-1 alpha and transforming growth factor-beta 1. *J. Cell Sci.* **102**, 315–322.
- Piscatelli, S. J., Michaels, B. M., Gregory, P., Jennings, R. W., Longaker, M. T., Harrison, M. R., and Siebert, J. W. (1994). Fetal fibroblast contraction of collagen matrices *in vitro*: The effects of epidermal growth factor and transforming growth factor-beta. *Ann. Plast. Surg.* **33**, 38–45.
- Reed, M. J., Vernon, R. B., Abrass, I. B., and Sage, E. H. (1994). TGF-beta 1 induces the expression of type I collagen and SPARC, and enhances contraction of collagen gels, by fibroblasts from young and aged donors. *J. Cell. Physiol.* **158**, 169–179.
- Yokozeki, M., Moriyama, K., Shimokawa, H., and Kuroda, T. (1997). Transforming growth factor-beta 1 modulates myofibroblastic phenotype of rat palatal fibroblasts *in vitro*. *Exp. Cell Res.* **231**, 328–336.
- Fukamizu, H., and Grinnell, F. (1990). Spatial organization of extracellular matrix and fibroblast activity: Effects of serum, transforming growth factor beta, and fibronectin. *Exp. Cell Res.* **190**, 276–282.
- Kurosaka, H., Kurosaka, D., Kato, K., Mashima, Y., and Tanaka, Y. (1998). Transforming growth factor-beta 1 promotes contraction of collagen gel by bovine corneal fibroblasts through differentiation of myofibroblasts. *Invest. Ophthalmol. Vis. Sci.* **39**, 699–704.
- Tomasek, J. J., Haaksma, C. J., Eddy, R. J., and Vaughan, M. B. (1992). Fibroblast contraction occurs on release of tension in attached collagen lattices: Dependency on an organized actin cytoskeleton and serum. *Anat. Rec.* **232**, 359–368.
- Grinnell, F. (1994). Fibroblasts, myofibroblasts, and wound contraction. *J. Cell Biol.* **124**, 401–404.
- Parizi, M., Howard, E. W., and Tomasek, J. J. (2000). Regulation of LPA-promoted myofibroblast contraction: Role of Rho, myosin light chain kinase and myosin light chain phosphatase. *Exp. Cell Res.* **254**, 210–220.

28. Mochitate, K., Pawelek, P., and Grinnell, F. (1991). Stress relaxation of contracted collagen gels: Disruption of actin filament bundles, release of cell surface fibronectin, and down-regulation of DNA and protein synthesis. *Exp. Cell Res.* **193**, 198–207.
29. Igotz, R., and Massague, J. (1986). Transforming growth factor- $\beta$  stimulates the expression of fibronectin and collagen and their incorporation into the extracellular matrix. *J. Biol. Chem.* **261**, 4337–4345.
30. Woods, A., Couchman, J. R., Johansson, S., and Hook, M. (1986). Adhesion and cytoskeletal organisation of fibroblasts in response to fibronectin fragments. *EMBO J.* **5**, 665–670.
31. Woods, A., McCarthy, J. B., Furcht, L. T., and Couchman, J. R. (1993). A synthetic peptide from the COOH-terminal heparin-binding domain of fibronectin promotes focal adhesion formation. *Mol. Biol. Cell* **4**, 605–613.
32. Serini, G., Bochaton-Piallat, M. L., Ropraz, P., Geinoz, A., Borsi, L., Zardi, L., and Gabbiani, G. (1998). The fibronectin domain ED-A is crucial for myofibroblastic phenotype induction by transforming growth factor-beta 1. *J. Cell Biol.* **142**, 873–881.
33. Rayan, G. M., Parizi, M., and Tomasek, J. J. (1996). Pharmacologic regulation of Dupuytren's fibroblast contraction *in vitro*. *J. Hand Surg. [Am.]* **21**, 1065–1070.
34. Ronnov-Jessen, L., Petersen, O. W., Koteliansky, V. E., and Bissell, M. J. (1995). The origin of the myofibroblasts in breast cancer. Recapitulation of tumor environment in culture unravels diversity and implicates converted fibroblasts and recruited smooth muscle cells. *J. Clin. Invest.* **95**, 859–873.
35. Pilcher, B. K., Kim, D. W., Carney, D. H., and Tomasek, J. J. (1994). Thrombin stimulates fibroblast-mediated collagen lattice contraction by its proteolytically activated receptor. *Exp. Cell Res.* **211**, 368–373.
36. Halliday, N. L., and Tomasek, J. J. (1995). Mechanical properties of the extracellular matrix influence fibronectin fibril assembly *in vitro*. *Exp. Cell Res.* **217**, 109–117.
37. Borsi, L., Castellani, P., Risso, A. M., Leprini, A., and Zardi, L. (1990). Transforming growth factor-beta regulates the splicing pattern of fibronectin messenger RNA precursor. *FEBS Lett.* **261**, 175–178.
38. Carnemolla, B., Balza, E., Siri, A., Zardi, L., Nicotra, M. R., Bigotti, A., and Natali, P. G. (1989). A tumor-associated fibronectin isoform generated by alternative splicing of messenger RNA precursors. *J. Cell Biol.* **108**, 1139–1148.
39. Grande, J. P. (1997). Role of transforming growth factor-beta in tissue injury and repair. *Proc. Soc. Exp. Biol. Med.* **214**, 27–40.
40. Balza, E., Borsi, L., Allemanni, G., and Zardi, L. (1988). Transforming growth factor beta regulates the levels of different fibronectin isoforms in normal human cultured fibroblasts. *FEBS Lett.* **228**, 42–44.
41. Allen-Hoffmann, B. L., Crankshaw, C. L., and Mosher, D. F. (1988). Transforming growth factor beta increases cell surface binding and assembly of exogenous (plasma) fibronectin by normal human fibroblasts. *Mol. Cell. Biol.* **8**, 4234–4242.
42. Heino, J., Igotz, R. A., Hemler, M. E., Crouse, C., and Massague, J. (1989). Regulation of cell adhesion receptors by transforming growth factor-beta. Concomitant regulation of integrins that share a common beta 1 subunit. *J. Biol. Chem.* **5**, 380–388.
43. Tada, A., Kato, H., Takenaga, K., and Hasegawa, S. (1997). Transforming growth factor beta 1 increases the expressions of high molecular weight tropomyosin isoforms and vinculin and suppresses the transformed phenotypes in human lung carcinoma cells. *Cancer Lett.* **121**, 31–31.
44. Grinnell, F., Ho, C. H., Lin, Y. C., and Skuta, G. (1999). Differences in the regulation of fibroblast contraction of floating versus stressed collagen matrices. *J. Biol. Chem.* **274**, 918–923.
45. Arora, P. D., and McCulloch, C. A. (1994). Dependence of collagen remodelling on alpha-smooth muscle actin expression by fibroblasts. *J. Cell. Physiol.* **159**, 161–175.
46. Kolodney, M. S., and Elson, E. L. (1993). Correlation of myosin light chain phosphorylation with isometric contraction of fibroblasts. *J. Biol. Chem.* **268**, 23850–23855.
47. Chrzanowska-Wodnicka, M., and Burridge, K. (1996). Rho-stimulated contractility drives the formation of stress fibers and focal adhesions. *J. Cell Biol.* **133**, 1403–1415.
48. Arora, P. D., Narani, N., and McCulloch, C. A. (1999). The compliance of collagen gels regulates transforming growth factor-beta induction of alpha-smooth muscle actin in fibroblasts. *Am. J. Pathol.* **154**, 871–882.
49. Postlethwaite, A. E., Keski-Oja, J., Moses, H. L., and Kang, A. H. (1987). Stimulation of the chemotactic migration of human fibroblasts by transforming growth factor beta. *J. Exp. Med.* **165**, 251–256.

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