Mechanical Aspects of Lung Fibrosis

A Spotlight on the Myofibroblast

Boris Hinz¹

¹Laboratory of Tissue Repair and Regeneration, Matrix Dynamics Group, Faculty of Dentistry, University of Toronto, Toronto, Ontario, Canada

Contractile myofibroblasts are responsible for the irreversible alterations of the lung parenchyma that hallmark pulmonary fibrosis. In response to lung injury, a variety of different precursor cells can become activated to develop myofibroblast features, most notably formation of stress fibers and expression of α -smooth muscle actin. Starting as an acute and beneficial repair process, myofibroblast secretion of collagen and contraction frequently becomes excessive and persists. The result is accumulation of stiff scar tissue that obstructs and ultimately destroys lung function. In addition to being a consequence of myofibroblast activities, the stiffened tissue is also a major promoter of the myofibroblast. The mechanical properties of scarred lung and fibrotic foci promote myofibroblast contraction and differentiation. One essential element in this detrimental feed-forward loop is the mechanical activation of the profibrotic growth factor transforming growth factor- β 1 from stores in the extracellular matrix. Interfering with myofibroblast contraction and integrin-mediated force transmission to latent transforming growth factor- β 1 and matrix proteins are here presented as possible therapeutic strategies to halt fibrosis.

Keywords: TGF- β 1 activation; stress fiber; contraction; matrix stiffness; integrin

Pulmonary fibrosis is a dysregulated reparative response of the lung that is characterized by uncontrolled myofibroblast proliferation and profibrotic activity, resulting in the excessive deposition, contraction, and stiffening of collagenous extracellular matrix (ECM) (1–4). Instrumental for the development of high contractile forces and the main defining feature of myofibroblasts is the neoexpression of α -smooth muscle actin (α -SMA) (Figure 1), which leads to a qualitative change in the organization and function of stress fibers (5, 6). Although a small population of α -SMA-negative but microfilament bundle-forming contractile fibroblasts resides in normal alveolar septa (7), myofibroblast differentiation and overactivity has been directly correlated with progression of lung fibrosis and deposition of collagen type I (8–10). Accumulation of this fibrotic scar tissue within the otherwise delicate interstitium results in both decreased lung compliance (i.e., increasing stiffness) and loss of gas exchange, ultimately rendering patients unable to breathe (2). A number of review articles recently elaborated on various aspects of myofibroblast biology and its contribution to pulmonary fibrosis (2, 11, 12). Here, I will summarize new findings and

concepts on how myofibroblast activities change the mechanical properties of lung tissue, and how in turn the mechanical environment in the fibrotic lung can control myofibroblast differentiation and action. It appears that this self-perpetuating mechanical loop is one major contributor to the progression of fibrosis.

ORIGINS OF THE MYOFIBROBLAST

By sharing contractile features with smooth muscle and collagen secreting activity with fibroblasts, myofibroblasts appear to be situated in a continuous differentiation spectrum ranging from fibroblastic to smooth muscle cells (SMCs). The controlled and transient appearance of myofibroblasts is important for restoring the integrity of damaged tissues by forming a collagenous scar (6). The scar protects injured organs from further damage upon mechanical challenge, such as the heart after myocardial infarction (13–15), skin after trauma (16), and tendon, bone, and cartilage after fracture or rupture (17–19). However, in many conditions, myofibroblasts go out of control and resist their physiological clearance through apoptosis (11, 20–24). In fibrosis, the excessive and detrimental myofibroblast activities turn beneficial tissue repair into devastating tissue deformation (25). Fibrosis can affect virtually all organs, including the lung (12, 26, 27), skin after burns (28) and in systemic sclerosis (29, 30), palmar fascia in Dupuytren's disease (31), heart (14), liver (32–34), and kidney (35, 36). Myofibroblasts activated during the stroma reaction to epithelial tumors generate the chemical and mechanical environment that promotes tumor progression (37, 38).

Healthy normal tissues usually do not contain myofibroblasts, which are formed *de novo* in response to an insult. Consistent with our body's need to rapidly repair injured organs and the great diversity of organs that are prone to fibrosis, myofibroblasts can be recruited from a multitude of different progenitor cells (38–40). Many myofibroblast precursors are mesenchymal and locally available, including fibroblasts and mesenchymal progenitor cells that reside in the connective tissue architecture of all organs. Myofibroblasts are also activated from SMCs in the arterial wall, pericytes in vascularized tissues, chondrocytes in cartilage, and osteoblasts in bone (39). In the diseased liver, hepatic stellate cells from meso/endodermal origin contribute to the myofibroblast population together with portal fibroblasts (41). In the lung, a number of different myofibroblast precursors have been identified in addition to local fibroblasts (42). Like in the kidney (43, 44) and tumors (45), epithelial and/or endothelial cells have been shown to generate lung myofibroblasts by mesenchymal transition (EMT) (46–49) (Figure 2). It has recently been suggested that acquisition of the myoid phenotype is associated with EMT but controlled by distinct signaling pathways (50, 51). Other studies seem to exclude a contribution of EMT to myofibroblast formation in human and mouse lungs (52, 53). Since EMT has also been questioned in kidney fibrosis (54), the debate about whether EMT occurs in the adult organism has recently been revived (55, 56). In addition to locally residing cells, myofibroblasts are generated from circulating progenitors, such as fibrocytes of

(Received in original form February 13, 2012; accepted in final form February 21, 2012)

Supported by the Canadian Institutes of Health Research (CIHR; grant #210820), the Collaborative Health Research Programme/Natural Sciences and Engineering Research Council of Canada (CIHR/NSERC) grant #1004005, the Canada Foundation for Innovation and Ontario Research Fund (grant #26653), the Heart and Stroke Foundation Ontario (grant #NA7086) (all to B.H.), and the Gebert Rf Foundation Switzerland (grant #531556).

Correspondence and requests for reprints should be addressed to Boris Hinz, Ph.D., Laboratory of Tissue Repair and Regeneration, Matrix Dynamics Group, Faculty of Dentistry, Fitzgerald Building, Room 241, University of Toronto, 150 College Street, Toronto, ON, M5S 3E2 Canada. E-mail: boris.hinz@utoronto.ca

Proc Am Thorac Soc Vol 9, Iss. 3, pp 137–147, Jul 15, 2012

Copyright 2012 by the American Thoracic Society

DOI: 10.1513/pats.201202-017AW

Internet address: www.atsjournals.org

Figure 1. Extracellular matrix and cytoskeletal features of cultured lung myofibroblasts. Rat lung myofibroblasts were stained for α -smooth muscle actin stress fibers (red), the transforming growth factor (TGF)- β 1 storage protein latent TGF- β -binding protein-1 (green), and the myofibroblast-associated extracellular matrix (ED-A) splice variant of fibronectin (blue) after 7 days of standard culture. The three-dimensional shading projection from a top view was reconstructed from z-series of confocal images. Scale bar = 100 μ m.

hematopoietic origin and bone marrow-derived mesenchymal stem cells (38, 57–63). However, differentiation of bone marrow-derived cells into myofibroblasts was not confirmed by other studies (60, 64, 65). Hence, to date it is still unclear which cell populations give rise to lung myofibroblasts and what their relative contributions to the development of lung fibrosis are. It is unlikely that this question can ever be answered in a definitive way. Conceivably, the type of injury, the local environment, and the progression of the disease will produce different myofibroblast populations.

MYOFIBROBLAST CONTRACTION LEADS TO TISSUE CONTRACTURE

A common feature of myofibroblasts arising from different conditions and precursors is the production of a collagen-rich ECM and its organization by applying high contractile forces (39). Although myofibroblasts phenotypically resemble SMCs, the irreversible outcome of myofibroblast contraction suggests that it is somewhat differently regulated. We have recently published an extensive overview on this subject (66) and here provide a synopsis of this review. Development of contractile force in SMCs, myofibroblasts, and fibroblasts is regulated at the level of myosin light chain (MLC) phosphorylation. Phosphorylated MLC enables the myosin head to interact with actin filaments and to generate force. In SMCs, increased levels of cytosolic Ca^{2+} enhance the activity of the MLC kinase via the Ca^{2+} /calmodulin pathway (67, 68). Force development is then terminated by MLC phosphatase activity. Conversely, in fibroblastic cells, the MLC phosphatase appears to be the key regulatory enzyme (69). Fibroblast contraction is achieved by inactivation of the MLC phosphatase through the Rho-(associated) kinase (ROCK or ROK), a downstream target of the small

GTPase RhoA (70). Whereas changes in cytosolic Ca^{2+} regulate SMC contraction and RhoA tunes baseline force development (71, 72), RhoA activity regulates fibroblast contraction and sufficiently high cytosolic Ca^{2+} levels ensure continued MLC activity (6).

Only a few studies have specifically addressed the regulation of myofibroblast contraction. Experiments with myofibroblast-populated wound granulation tissue strips suggest RhoA as the chief regulator of myofibroblast contractile activity. Inhibition of MLC phosphatase using the phosphatase inhibitor calyculin was shown to be sufficient to induce strip contraction (73), whereas increasing cytosolic Ca^{2+} by membrane depolarization and addition of Ca^{2+} ionophore had little effect on myofibroblast contraction of tissue strips and collagen gels (73). This view of RhoA taking center stage was further supported by studies using three-dimensional myofibroblast-populated collagen gels (74–77). Using two-dimensional deformable culture substrates that allow visualization of cell contractile forces, inhibition of Rho was shown to block myofibroblast contraction, whereas stimulation with lysophosphatic acid, an upstream effector of Rho, enhanced force development (78, 79). On the contrary, other studies claimed that the level of cytosolic Ca^{2+} is mainly regulating myofibroblast contraction. Application of calmodulin inhibitors to full-thickness rat skin wounds has been shown to impair wound closure (80), a process that is driven by myofibroblast contraction (81, 82). Stimulation with a variety of different agonists that all induce a Ca^{2+} response in SMCs induces contraction of myofibroblast-populated tissue strips (5, 73, 83–85). In three-dimensional collagen gel cultures of rat ventricular and colon myofibroblasts, contraction was substantially regulated by the levels of cytosolic Ca^{2+} (86–88), and interference with Ca^{2+} signaling had a more dramatic effect on collagen contraction by myofibroblastic hepatic stellate cells than modulation of RhoA-dependent processes (89). Similar induction of myofibroblast contraction was obtained when treating two-dimensional cell cultures with agonists that induce cytosolic Ca^{2+} transients (90, 91). In addition to different experimental setups, the heterogeneous provenance of myofibroblasts is one possible reason for the apparent discrepancy concerning the importance of Ca^{2+} and RhoA for myofibroblast contraction. It is conceivable that different tissue and cell origins as well as the level of differentiation will have an impact on myofibroblast physiology.

In a recent cell culture study, we have tested the hypothesis that myofibroblasts use both contraction regulation mechanisms by simultaneously assessing local and global contraction events at the single-cell level in culture (78). A key finding was that contractions of dorsal stress fibers engaged with ECM-coated microbeads were mediated by variations of cytosolic Ca^{2+} , whereas overall isometric cell tension was maintained through RhoA/ROCK-mediated contraction of ventral stress fibers engaged with an elastic rubber substrate (Figure 3). Contractile events following periodic cytosolic Ca^{2+} increases (~ 1 contraction/100 s) were acting on a short range (~ 400 nm/contraction) and comparably weak (~ 100 pN/contraction) as measured by atomic force microscopy (78). This was in contrast to isometric contraction of the rubber substrate over hours with a force development of several microneutons per cell (5, 78) or 5–10 nN/ μm^2 per adhesion site (92). It is tempting to speculate that the mechanical resistance (low for nonrestrained beads and high for rubber substrates) will determine what contraction mode is employed.

To date, it is unknown how myofibroblast contraction is regulated at the subcellular level in animal and human tissues. However, the *in vitro* findings have led to the development of an improved “lock-step” model of ECM remodeling (6). This

Figure 2. Myofibroblast origins. The main myofibroblast progenitors after lung injury appear to be locally residing mesenchymal cells. In the liver, myofibroblasts are additionally recruited from activated hepatic stellate cells. In tumors and in fibrotic lung, liver, and kidney, differentiated myofibroblasts have been described to derive from epithelial- and endothelial-to-mesenchymal transition (EMT), respectively. During atheromatous plaque formation, de-differentiating smooth muscle cells (i.e., that lose late smooth muscle cell markers) from the media are considered to be the major source of myofibroblastic cells. The relative contribution of bone marrow-derived circulating fibrocytes to the formation of differentiated myofibroblasts in different fibrotic lesions is unclear at present; it is conceivable that fibrocyte transdifferentiation also terminates at the proto-myofibroblast stage. Finally, mesenchymal stem cells have

been shown to acquire the differentiated myofibroblast phenotype *in vitro* and *in vivo*. TGF = transforming growth factor. Modified and reprinted from Reference 39 by permission from the American Society for Investigative Pathology.

model explains how global tissue remodeling can result from the contraction of single myofibroblasts and subsequent stabilization of tissues by secreted collagens and other ECM molecules (66, 78). RhoA/ROCK signaling is responsible for promoting long-lasting and strong isometric cell contractions, which will generate slack in individual collagen fibrils. Such tension-released fibrils are then accessible to the weak and short-ranged microcontractions generated by periodic cytosolic Ca^{2+} transients. The gradually raising tension in locally pulled fibrils will increasingly counteract further local translocation. At this point, the new fibril configuration has to be stabilized, possibly by local collagen digestion, deposition of new collagen fibrils, and cross-linking with the existent ECM. Although the details of this putative remodeling step are not explored, it has been shown that collagen remodeling by matrix metalloproteinases can be regulated by mechanical tension (93, 94). The stabilized ECM can then sustain tissue stress and myofibroblasts are able to respread. These processes would result in irreversible contractions characteristic for fibrosis rather than reversible contraction (6, 66, 78).

THE STIFFNESS OF SCAR TISSUE: CAUSE AND CONSEQUENCE OF FIBROSIS

As discussed above, the most evident outcome of myofibroblast activity is tissue stiffening (95). Myofibroblasts, in contrast to any of their precursors, produce excessive, poorly organized but dense collagen ECM. Scar tissue stiffness is expressed as Young's elastic modulus (in Pascals), which is defined as the force per unit area (stress) that is required to strain a material. High stress is needed to deform materials with high Young's modulus. The Young's modulus of normal lung tissue ranges between 1 and 5 kPa as measured by atomic force microscopy (96).

Fibrotic tissue in the lung (96–100) and in other organs is up to 30 times stiffer (20–100 kPa) (95, 101–106). The fact that myofibroblast-generated fibrotic scars are stiffer than healthy parenchyma has important consequences for lung function. Stiff scar tissue impedes elastic expansion and contraction of the lung during breathing cycles. Accumulation of dense collagenous scar obstructs the regulation of airway diameter by airway SMCs and prevents efficient gas exchange (107).

Moreover, mechanical cues inherent to the stiff fibrotic ECM have a profound influence on myofibroblast differentiation and progression of fibrosis. Mechanical stress is one of the most potent factors controlling myofibroblast fate and development (95, 108). In healing rat skin wounds, expression of α -SMA is accelerated by splinting the edges of full-thickness rat skin wounds with plastic frames, which increases tissue tension; the release of tension has the opposite effect (109). A similar result is found after stretching human burn scar tissue *in situ* (28). Comparable controlled experiments with animal and human lung tissues have not yet been performed. Myofibroblasts express α -SMA when grown in attached (stressed) three-dimensional collagen gel culture but not in free-floating (relaxed) gels (110). Application of mechanical force to ECM protein-coated magnetite beads that are attached to fibroblasts cultured on two-dimensional surfaces induces α -SMA expression (111). Similarly, application of cyclic stretching forces stimulates EMT in cultured alveolar type II epithelial cells and induces lung fibrosis in an animal model of mechanical ventilation (112).

The influence of substrate stiffness on the functional behavior of fibroblasts and myofibroblasts has been systematically studied using polymer culture substrates produced with stiffness within the elasticity range of normal and fibrotic tissue. Growing fibroblasts on pathologically stiff surfaces promotes myofibroblast activation, whereas exposure to healthy-soft substrates results in

Figure 3. Regulation of myofibroblast contraction. In a culture model of myofibroblasts grown on deformable (wrinkling) substrates, two modes of contraction function simultaneously but are controlled by different signaling pathways. Oscillating cytosolic Ca^{2+} controls stress fiber microcontractions that displace extracellular matrix-coated microbeads under low mechanical resistance on the cell surface. The RhoA/ROCK pathway controls overall isometric cell contraction, indicated by persisting wrinkles in the elastic substrate. Modified and reprinted by permission from Reference 198.

acute loss of the myofibroblast phenotype (92, 96, 113). Exposure of lung fibroblasts to pathological levels of ECM stiffness as seen during the end stages of fibrosis results in fibroproliferative behavior that is not unlike that seen in patients with idiopathic pulmonary fibrosis (96). Such changes are usually observed after hours or 1 to 2 days following the change in mechanical culture conditions. In a recent study, however, we could demonstrate a long-ranged imprinting effect of substrate mechanics on the fibrotic cell character. Continued exposure to fibrosis-stiff culture surfaces primed lung fibroblasts towards the profibrotic myofibroblast phenotype, and the preservation of this stiffness-dependent fibrotic response was independent of a sustained stiffness stimulus (114). Conversely, mechanically priming cells on healthy-soft culture substrates partly protected lung fibroblasts from myofibroblast activation by subsequent exposure to fibrosis-stiff substrates (Figure 4). By transplanting human lung fibroblasts from patients with lung fibrosis with different progression rates to humanized immunodeficient mouse, a recent study demonstrated that myofibroblasts can retain information to progress fibrosis (115). It remains elusive how fibroblasts store mechanosensed information and whether such a proposed “mechanical memory” will actively promote or prevent disease progression *in vivo*.

Tissue stiffness increases as a consequence of the ECM remodeling activities of fibroblasts and myofibroblasts (6, 110). Hence, actively contracting cells generate the conditions that make them more contractile in a detrimental mechanical feed-forward loop. How is this cycle started immediately after an injury when contractile fibroblasts are absent? Data obtained from an animal model of liver fibrosis demonstrated that local tissue stiffening occurs during inflammation and precedes the activation of fibroblastic cells; it appears that such early mechanical changes would be sufficient to trigger the contracture cascade (116). A number of mechanisms possibly account for early structural changes and tissue stiffening in these conditions. In the liver, collagen cross-linking by lysyl oxidases was implicated in initial tissue stiffening (116). Moreover, fibrosis-specific and stable collagen cross-links are formed by the activities of LOXL2 (117) and specific lysyl hydroxylases (118) in different fibrotic conditions. It remains elusive whether inflammatory cells or

activated epithelium (2, 119) in the injured lung can cause similar mechanical changes that will be sufficient to trigger the vicious cycle of myofibroblast activation and contraction.

MECHANICAL ACTIVATION OF TRANSFORMING GROWTH FACTOR- β 1 PROVIDES A POSSIBLE CHECKPOINT IN FIBROSIS PROGRESSION

Myofibroblast mechanoregulation can occur at several levels. First, acute changes in the mechanical load determine the intracellular stress fiber localization of α -SMA (79, 92, 114). Second, stress directly modulates α -SMA promoter activity and protein expression (111, 120). Third, stress modulates the bioactivity of transforming growth factor (TGF)- β 1, the major cytokine inducing myofibroblast differentiation. Different recent studies demonstrated force-dependent activation of latent TGF- β 1 (79, 121–126). Together they support the concept that myofibroblasts can exert tensile forces on ECM-stored latent TGF- β 1 via specific integrins and thereby activate this profibrotic cytokine.

TGF- β 1 is the most potent profibrotic cytokine known (3, 127, 128). Tissue expression levels of TGF- β 1 are strongly elevated in different fibrotic lung diseases, including chronic airway remodeling in asthma (129), chronic obstructive pulmonary disease (130, 131), and interstitial pulmonary disease (132, 133). Delivery of adenovirus producing active TGF- β 1 to mouse lungs leads to accumulation of fibroblasts and collagen (134, 135). Inhibition of TGF- β 1 with function-blocking antibodies suppresses experimentally induced lung fibrosis (136). One predominant detrimental role of TGF- β 1 in causing lung fibrosis is the activation of myofibroblasts (4, 128). Active TGF- β 1 assembles a complex of TGF- β 1-receptor type I and II in the cell membrane. The kinase activity of the complex phosphorylates SMAD 2/3, which translocates to the nucleus and activates transcription factors that regulate fibrogenic genes (137, 138). TGF- β 1 induces the expression of α -SMA and other components of the myofibroblast contractile cytoskeleton (139, 140) as well as collagen production (141).

Despite the fact that TGF- β 1 is an important therapeutic target to halt fibrosis, blocking the already active TGF- β 1 in animal experiments and clinical tests had surprisingly little

Figure 4. Mechanical priming on fibrosis-stiff substrates results in persistent activation of lung myofibroblasts. Lung fibroblasts were explanted and subcultured for two passages on either 5-kPa healthy lung-soft or 100-kPa fibrotic lung-stiff collagen-coated silicone substrates. Cells were then transferred to soft or stiff surfaces and assessed after 12 hours and after 2 further passages (14 d). Cells were immunostained for α -smooth muscle actin (α -SMA) (red) and nuclei (blue). Although stiff substrate-primed myofibroblasts demonstrated acute decrease of α -SMA stress fiber organization 12 hours after a shift to soft substrates, myofibroblast presence was reestablished and persisted even after 14 days of soft culture (114). Scale bar = 200 μ m.

positive effect (142). Moreover, TGF- β 1 plays beneficial roles in controlling epithelial cell growth, inflammation, tissue homeostasis, and immune suppression in a variety of normal and pathologic adult tissues (138, 143–147). This additional risk of side effects has stimulated the search for alternatives to specifically inhibit TGF- β 1 profibrotic signaling. One possibility to achieve this aim is to interfere with the activation of latent TGF- β 1 (148–151).

TGF- β 1 is synthesized as part of a pro-protein that is intracellularly cleaved to produce the small latent complex (SLC) (Figure 5). Mature SLC consists of the TGF- β 1 dimer, noncovalently linked to the dimeric latency-associated peptide (LAP) (152). The majority of cell types secrete SLC together with latent TGF- β -binding protein-1 (LTBP-1) (152, 153). LTBP-1 targets latent TGF- β 1 as a large latent complex to the ECM by interacting with different proteins, including fibronectin and fibrillin (154–156). These ECM deposits of latent TGF- β 1 are accessible for cell-mediated activation (113, 157, 158). Activation of latent TGF- β 1 by its dissociation from LAP is promoted by various mechanisms, which differ according to cell type and physiological context (152, 158–160). Transmembrane integrins have been shown to bind to and activate latent TGF- β 1. Both LAP and LTBP-1 contain arginine-glycine-aspartic acid motifs for integrin docking (161–163). Binding comprises all α v integrins (α v β 1, α v β 3, α v β 5, α v β 6, and α v β 8) and integrins α 5 β 1, α 8 β 1, and α IIb β 3 (164, 165). Integrins α v β 5, α v β 6, α v β 8, and α v β 3 were shown to activate latent TGF- β 1 using two different modes of action (113, 164). The first depends on proteases that seem to be guided to the large latent complex by association mainly with integrin α v β 8 (150, 158, 166).

The second integrin-mediated activation mechanism is independent of proteolysis and involves transmission of cell-derived forces to the large latent complex (113, 123, 126, 158, 164). Integrin binding to LAP is essential for contraction-mediated TGF- β 1 activation (Figure 5) and inhibited by arginine-glycine-aspartic acid- and LAP-competitive peptides (79). TGF- β 1 activation efficiency increases with increasing ECM stiffness. Whereas compliant substrates appear to absorb the large deformations generated by myofibroblast contraction and thus protect the

latent complex against conformational changes, the complex is stretchable on stiff substrates (79). Hence, activation of TGF- β 1 via integrin-mediated myofibroblast contraction represents a potential checkpoint in the progression of fibrosis. In other words, in the absence of exogenous active TGF- β 1, autocrine generation of myofibroblasts only occurs after the ECM has been sufficiently pre-modeled. Consistently, the ECM stiffness threshold for TGF- β 1 activation by cultured myofibroblasts is lower (>5 kPa) (79) than for the induction and preservation of the myofibroblast phenotype (<16 kPa) (92).

Currently available data supports the notion that myofibroblast traction on the large latent complex induces a conformational change in LAP that liberates active TGF- β 1. We have recently measured the mechanical stress involved in TGF- β 1 activation at the molecular level and demonstrated TGF- β 1 release upon force transmission to LAP (122). Our findings are consistent with previous studies suggesting that TGF- β 1 activation involves conformational changes in the N-terminal portion of LAP, which confers latency by binding to TGF- β 1 (167–171). The recently published three-dimensional structure of the SLC (121) and force spectroscopy of SLC unfolding (122) revealed two structures that interact with TGF- β 1 in this LAP region: the α 1-helix and the latency lasso. Together, both domains form a “straitjacket” that traps TGF- β 1 (121) (Figure 5). In contrast to the remaining LAP molecule, which is packed in a force-resistant structure, α 1-helix and latency lasso are particularly susceptible to unfolding upon mechanical stretch due to their flexibility and their position opposite to the integrin binding site in the SLC (121). Using the atomic force microscope to pull on SLC and the large latent complex led to three main discoveries: (1) Mechanical force applied to LAP can unfold the α 1-helix and the latency lasso in the straitjacket. (2) Simultaneous unfolding of both domains and full opening of the straitjacket is only possible when LAP is bound to LTBP-1. (3) In the context of the large latent complex, fully unraveling the straitjacket requires lower forces ($\sim$ 40 pN) than unfolding of its individual stretches ($\sim$ 50 pN). Together these data favor an all-or-nothing mechanism of full TGF- β 1 release by mechanical force (Figure 5) and establish a direct link between the

Figure 5. Mechanical activation of transforming growth factor (TGF)- β 1. (A) Structural and force spectroscopy data suggest that the TGF- β 1/LTBP-1-binding domains of latency-associated peptide (LAP) act as a sensor in a mechanical model of integrin-mediated TGF- β 1 activation. Single-molecule mechanical stretch applied with the cantilever tip of an atomic force microscope opens the latent TGF- β 1 complex to release TGF- β 1. (B) Domains that are prone to unfolding within the strait-jacket are the latency lasso and the α -helix. (C) Typical force-extension profiles of large latent complex pulling are shown in which every saw tooth indicates unfolding of one protein domain. (D) All force-extension curves were analyzed for unfolding lengths (ΔLc) that correspond to the length of the whole 45-amino acid (aa) straitjacket, the 17-aa latency lasso, and the 25-aa α -helix domain. Values were plotted in a bivariate ΔLc -force contour map with red indicating high occurrence of unfolding events. (E) When cells are grown on a compliant ECM and/or develop low contractile activity, the lack of sufficient mechanical tension will prevent integrin-mediated conformational changes required to activate TGF- β 1 from the latent complex. (F) Conversely, on a stiff ECM, the transmission

of contractile forces via integrins to the LAP will favor unfolding of the straitjacket region, resulting in TGF- β 1 release. The minimal force required to unfold the entire SLC straitjacket and to liberate TGF- β 1 is ~ 40 pN. Reproduced by permission from Reference 122. AFM = atomic force microscope; ECM = extracellular matrix; EGF = epidermal growth factor; LLC = large latent complex; LTBP-1 = latent TGF- β -binding protein-1; RGD = arginine-glycine-aspartic acid; SLC = small latent complex.

mechanical and chemical factors regulating myofibroblast differentiation and ultimately causing fibrosis (113, 154, 155).

Inhibition of TGF- β 1-activating integrins has promising therapeutic potential (172). Knocking out integrin subunits that activate TGF- β 1, including β 6 (173), α v (174), and β 8 (175), and mutation of the integrin-binding site in LAP (176), recapitulates the TGF- β 1 knockout mouse (177). Knockout mice have defective vasculogenesis, are devoid of Langerhans cells, and spontaneously develop lung and skin inflammation. Importantly, the lungs of β 6 integrin knockout mice are protected from bleomycin-induced fibrosis (178). Controlled delivery of β 6 integrin-blocking antibodies reduces lung fibrosis without negative side effects (179). α v β 6 is the best studied integrin in the context of TGF- β 1 activation and lung fibrosis, but only epithelial cells express this integrin (157, 176, 178, 180). Epithelial cells are important in the onset of fibrosis in epithelialized tissues like the lung. Consequently, specific deletion of the TGF- β 1 receptor RII in epithelial cells alone was sufficient to reduce bleomycin-induced experimental lung fibrosis (181).

However, progression to end-stage pulmonary fibrosis depends on α v β 6 integrin-negative myofibroblasts (182, 183).

The question remains which mesenchymal integrin is able to promote mechanical activation of latent TGF- β 1. In lung myofibroblast cultures, contraction-mediated TGF- β 1 activation is significantly reduced by inhibition of α v β 5 integrin (79, 123). Integrin α v β 5 is up-regulated in culture lung myofibroblasts and strongly associates with fibrotic foci of diseased lung (184). In airway SMCs, integrin α v β 5 is involved in TGF- β 1 activation and chronic airway remodeling in animal models of asthma (185). From these findings, one would expect that loss of α v β 5 integrin function protects from lung fibrosis similar to what has been shown for integrin α v β 6 (179). Despite the fact that the phenotype of the α v β 5 knockout mouse is mild (186, 187), these animals have yet to be challenged in fibrosis models in which TGF- β 1 activation relies on myofibroblasts. Another possible candidate for TGF- β 1 activation in the fibrotic lung is integrin α 8 β 1, which is up-regulated in conjunction with myofibroblast differentiation in pulmonary, hepatic, and cardiac fibrosis (188, 189).

In cultured cardiac fibroblasts, $\alpha 8\beta 1$ integrin is induced by TGF- $\beta 1$ and up-regulated on myofibroblasts (189, 190). However, the function of this integrin in lung fibroblasts is still unclear. When overexpressed in nonfibroblastic cells, $\alpha 8\beta 1$ integrin strongly interacts with LAP but does not activate TGF- $\beta 1$ (162). Currently it is unknown whether this function is gained when expressed in highly contractile myofibroblasts.

CONCLUSIONS AND PERSPECTIVES

Myofibroblasts are attractive therapeutic targets to stop the progression of pulmonary fibrosis and to reduce the devastating structural changes in the lung architecture caused by this cell (191). The fact that excessive contraction results in detrimental tissue contracture has nourished the idea to target contractile features of the myofibroblast. Specific inhibition of α -SMA with competitive peptides not only reduces myofibroblast contraction in culture and in rat wounds but decreases transcription of collagen type I (82). Other strategies currently evaluate the potential of less specific drugs acting on cell contraction. The peptide hormone relaxin reduces the contractile activity of cultured lung fibroblast and bleomycin-induced accumulation of myofibroblasts and collagen in the lung (192). Application of the ROCK-mediated contraction inhibitor Fasudil is effective in reducing myofibroblast-promoted experimental wound contraction (193) and development of kidney fibrosis (194). Inhibition of thrombin similarly reduces lung myofibroblast contraction and leads to suppression of the fibrogenic phenotype (195).

Interference with force transmission to and mechanosensing from the ECM at sites of integrins provides alternative potentials to modulate myofibroblast action and differentiation. The most recent development in this area is to target specific integrins that myofibroblasts use to liberate active TGF- $\beta 1$ by pulling on the large latent complex. Pharmacological interference with TGF- $\beta 1$ -activating integrins could specifically counteract the harmful activity of active TGF- $\beta 1$ but without inhibiting its beneficial effects on other cell types. Inhibition of specific integrins has already entered preclinical and clinical trials as therapy for other diseases, such as tumor treatment (196, 197). Hence most of the antagonistic peptides and antibodies that have been developed for cancer therapies would be readily available for the treatment of lung fibrosis.

Author disclosures are available with the text of this article at www.atsjournals.org.

References

- Thannickal VJ, Lee DY, White ES, Cui Z, Larios JM, Chacon R, Horowitz JC, Day RM, Thomas PE. Myofibroblast differentiation by transforming growth factor- $\beta 1$ is dependent on cell adhesion and integrin signaling via focal adhesion kinase. *J Biol Chem* 2003;278:12384–12389.
- Wynn TA. Integrating mechanisms of pulmonary fibrosis. *J Exp Med* 2011;208:1339–1350.
- Araya J, Nishimura SL. Fibrogenic reactions in lung disease. *Annu Rev Pathol* 2010;5:77–98.
- Phan SH. Biology of fibroblasts and myofibroblasts. *Proc Am Thorac Soc* 2008;5:334–337.
- Hinz B, Celetta G, Tomasek JJ, Gabbiani G, Chaponnier C. Alpha-smooth muscle actin expression upregulates fibroblast contractile activity. *Mol Biol Cell* 2001;12:2730–2741.
- Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown RA. Myofibroblasts and mechano-regulation of connective tissue remodelling. *Nat Rev Mol Cell Biol* 2002;3:349–363.
- Kapanci Y, Ribaux C, Chaponnier C, Gabbiani G. Cytoskeletal features of alveolar myofibroblasts and pericytes in normal human and rat lung. *J Histochem Cytochem* 1992;40:1955–1963.
- Zhang HY, Gharaee-Kermani M, Zhang K, Karmiol S, Phan SH. Lung fibroblast alpha-smooth muscle actin expression and contractile phenotype in bleomycin-induced pulmonary fibrosis. *Am J Pathol* 1996;148:527–537.
- Powell DW, Mifflin RC, Valentich JD, Crowe SE, Saada JI, West AB. Myofibroblasts. I. Paracrine cells important in health and disease. *Am J Physiol* 1999;277:C1–C9.
- King TE Jr, Schwarz MI, Brown K, Toozé JA, Colby TV, Waldron JA Jr, Flint A, Thurlbeck W, Cherniack RM. Idiopathic pulmonary fibrosis: relationship between histopathologic features and mortality. *Am J Respir Crit Care Med* 2001;164:1025–1032.
- Kis K, Liu X, Hagood JS. Myofibroblast differentiation and survival in fibrotic disease. *Expert Rev Mol Med* 2011;13:e27.
- Hardie WD, Glasser SW, Hagood JS. Emerging concepts in the pathogenesis of lung fibrosis. *American Journal of Pathology* 2009;175:3–16.
- Souders CA, Bowers SL, Baudino TA. Cardiac fibroblast: the Renaissance cell. *Circ Res* 2009;105:1164–1176.
- Porter KE, Turner NA. Cardiac fibroblasts: at the heart of myocardial remodeling. *Pharmacol Ther* 2009;123:255–278.
- Leask A. Potential therapeutic targets for cardiac fibrosis: TGF β , angiotensin, endothelin, CCN2, and PDGF, partners in fibroblast activation. *Circ Res* 2010;106:1675–1680.
- Hinz B. Formation and function of the myofibroblast during tissue repair. *J Invest Dermatol* 2007;127:526–537.
- Wong JK, Lui YH, Kapacee Z, Kadler KE, Ferguson MW, McGrouther DA. The cellular biology of flexor tendon adhesion formation: an old problem in a new paradigm. *Am J Pathol* 2009;175:1938–1951.
- Kinner B, Gerstenfeld LC, Einhorn TA, Spector M. Expression of smooth muscle actin in connective tissue cells participating in fracture healing in a murine model. *Bone* 2002;30:738–745.
- Spector M. Musculoskeletal connective tissue cells with muscle: expression of muscle actin in and contraction of fibroblasts, chondrocytes, and osteoblasts. *Wound Repair Regen* 2001;9:11–18.
- Kulasekaran P, Scavone CA, Rogers DS, Arenberg DA, Thannickal VJ, Horowitz JC. Endothelin-1 and transforming growth factor- $\beta 1$ independently induce fibroblast resistance to apoptosis via AKT activation. *Am J Respir Cell Mol Biol* 2009;41:484–493.
- de Andrade JA, Thannickal VJ. Innovative approaches to the therapy of fibrosis. *Curr Opin Rheumatol* 2009.
- Douglass A, Wallace K, Parr R, Park J, Durward E, Broadbent I, Bareille C, Porter AJ, Wright MC. Antibody-targeted myofibroblast apoptosis reduces fibrosis during sustained liver injury. *J Hepatol* 2008;49:88–98.
- Desmoulière A, Redard M, Darby I, Gabbiani G. Apoptosis mediates the decrease in cellularity during the transition between granulation tissue and scar. *Am J Pathol* 1995;146:56–66.
- Wynes MW, Edelman BL, Kostyk AG, Edwards MG, Coldren C, Groshong SD, Cosgrove GP, Redente EF, Bamberg A, Brown KK, et al. Increased cell surface fas expression is necessary and sufficient to sensitize lung fibroblasts to fas ligation-induced apoptosis: implications for fibroblast accumulation in idiopathic pulmonary fibrosis. *J Immunol* 2011;187:527–537.
- Wynn TA. Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. *J Clin Invest* 2007;117:524–529.
- Goodwin A, Jenkins G. Role of integrin-mediated TGF β activation in the pathogenesis of pulmonary fibrosis. *Biochem Soc Trans* 2009;37:849–854.
- Gharaee-Kermani M, Hu B, Phan SH, Gyetko MR. Recent advances in molecular targets and treatment of idiopathic pulmonary fibrosis: focus on TGF β signaling and the myofibroblast. *Curr Med Chem* 2009;16:1400–1417.
- Junker JP, Kratz C, Tollback A, Kratz G. Mechanical tension stimulates the transdifferentiation of fibroblasts into myofibroblasts in human burn scars. *Burns* 2008.
- Varga J, Abraham D. Systemic sclerosis: a prototypic multisystem fibrotic disorder. *J Clin Invest* 2007;117:557–567.
- Asano Y. Future treatments in systemic sclerosis. *Journal of Dermatology* 2010;37:54–70.
- Verjee LS, Midwood K, Davidson D, Essex D, Sandison A, Nanchahal J. Myofibroblast distribution in Dupuytren's cords: correlation with digital contracture. *J Hand Surg Am* 2009;34:1785–1794.
- Desmoulière A. Hepatic stellate cells: the only cells involved in liver fibrogenesis? A dogma challenged. *Gastroenterology* 2007;132:2059–2062.

33. Iredale JP. Models of liver fibrosis: exploring the dynamic nature of inflammation and repair in a solid organ. *J Clin Invest* 2007;117:539–548.
34. Friedman SL. Evolving challenges in hepatic fibrosis. *Nat Rev Gastroenterol Hepatol* 2010;7:425–436.
35. Hewitson TD. Renal tubulointerstitial fibrosis: common but never simple. *Am J Physiol Renal Physiol* 2009;296:F1239–1244.
36. Grande MT, Lopez-Novoa JM. Fibroblast activation and myofibroblast generation in obstructive nephropathy. *Nat Rev Nephrol* 2009;5:319–328.
37. De Wever O, Demetter P, Mareel M, Bracke M. Stromal myofibroblasts are drivers of invasive cancer growth. *Int J Cancer* 2008;123:2229–2238.
38. Hinz B. The myofibroblast in connective tissue repair and regeneration. In: Ralphs CAJ, editor. Regenerative medicine and biomaterials for the repair of connective tissues. Cambridge, UK: Woodhead Publishing Ltd.; 2010. pp. 39–82.
39. Hinz B, Phan SH, Thannickal VJ, Galli A, Bochaton-Piallat ML, Gabbiani G. The myofibroblast: one function, multiple origins. *Am J Pathol* 2007;170:1807–1816.
40. Rock JR, Hogan BL. Epithelial progenitor cells in lung development, maintenance, repair, and disease. *Annu Rev Cell Dev Biol* 2011;27:493–512.
41. Greenbaum LE, Wells RG. The role of stem cells in liver repair and fibrosis. *Int J Biochem Cell Biol* 2011;43:222–229.
42. Hoyles RK, Derrett-Smith EC, Khan K, Shiwen X, Howat SL, Wells AU, Abraham DJ, Denton CP. An essential role for resident fibroblasts in experimental lung fibrosis is defined by lineage-specific deletion of high-affinity type II transforming growth factor beta receptor. *Am J Respir Crit Care Med* 2010;183:249–261.
43. Inoue T, Okada H, Takenaka T, Watanabe Y, Suzuki H. A case report suggesting the occurrence of epithelial-mesenchymal transition in obstructive nephropathy. *Clin Exp Nephrol* 2009;13:385–388.
44. Quaggin SE, Kapus A. Scar wars: mapping the fate of epithelial-mesenchymal-myofibroblast transition. *Kidney Int* 2011;80:41–50.
45. Radisky DC, Kenny PA, Bissell MJ. Fibrosis and cancer: do myofibroblasts come also from epithelial cells via EMT? *J Cell Biochem* 2007;101:830–839.
46. Kasai H, Allen JT, Mason RM, Kamimura T, Zhang Z. TGF-beta1 induces human alveolar epithelial to mesenchymal cell transition (EMT). *Respir Res* 2005;6:56.
47. Kim KK, Kugler MC, Wolters PJ, Robillard L, Galvez MG, Brumwell AN, Sheppard D, Chapman HA. Alveolar epithelial cell mesenchymal transition develops in vivo during pulmonary fibrosis and is regulated by the extracellular matrix. *Proc Natl Acad Sci U S A* 2006;103:13180–13185.
48. Willis BC, Liebler JM, Luby-Phelps K, Nicholson AG, Crandall ED, du Bois RM, Borok Z. Induction of epithelial-mesenchymal transition in alveolar epithelial cells by transforming growth factor-beta1: potential role in idiopathic pulmonary fibrosis. *Am J Pathol* 2005;166:1321–1332.
49. Selman M, Pardo A, Kaminski N. Idiopathic pulmonary fibrosis: aberrant recapitulation of developmental programs? *PLoS Med* 2008;5:e62.
50. Masszi A, Speight P, Charbonney E, Lodyga M, Nakano H, Szaszi K, Kapus A. Fate-determining mechanisms in epithelial-myofibroblast transition: major inhibitory role for SMAD3. *J Cell Biol* 2010;188:383–399.
51. Xu J, Lamouille S, Derynck R. TGF-beta-induced epithelial to mesenchymal transition. *Cell Res* 2009;19:156–172.
52. Barth K, Reh J, Sturrock A, Kasper M. Epithelial vs myofibroblast differentiation in immortal rat lung cell lines—modulating effects of bleomycin. *Histochem Cell Biol* 2005;124:453–464.
53. Rock JR, Barkauskas CE, Cronic MJ, Xue Y, Harris JR, Liang J, Noble PW, Hogan BL. Multiple stromal populations contribute to pulmonary fibrosis without evidence for epithelial to mesenchymal transition. *Proc Natl Acad Sci U S A* 2011;108:E1475–1483.
54. Humphreys BD, Lin SL, Kobayashi A, Hudson TE, Nowlin BT, Bonventre JV, Valerius MT, McMahon AP, Duffield JS. Fate tracing reveals the pericyte and not epithelial origin of myofibroblasts in kidney fibrosis. *Am J Pathol* 2010;176:85–97.
55. Zeisberg M, Duffield JS. Resolved: EMT produces fibroblasts in the kidney. *J Am Soc Nephrol* 2010;21:1247–1253.
56. Popov Y, Schuppan D. Epithelial-to-mesenchymal transition in liver fibrosis: dead or alive? *Gastroenterology* 2010;139:722–725.
57. Herzog EL, Bucala R. Fibrocytes in health and disease. *Exp Hematol* 2010;38:548–556.
58. Abe R, Donnelly SC, Peng T, Bucala R, Metz CN. Peripheral blood fibrocytes: differentiation pathway and migration to wound sites. *J Immunol* 2001;166:7556–7562.
59. Phillips RJ, Burdick MD, Hong K, Lutz MA, Murray LA, Xue YY, Belperio JA, Keane MP, Strieter RM. Circulating fibrocytes traffic to the lungs in response to CXCL12 and mediate fibrosis. *J Clin Invest* 2004;114:438–446.
60. Hashimoto N, Jin H, Liu T, Chensue SW, Phan SH. Bone marrow-derived progenitor cells in pulmonary fibrosis. *J Clin Invest* 2004;113:243–252.
61. Brocker V, Langer F, Fellous TG, Mengel M, Brittan M, Bredt M, Milde S, Welte T, Eder M, Haverich A, et al. Fibroblasts of recipient origin contribute to bronchiolitis obliterans in human lung transplants. *Am J Respir Crit Care Med* 2006;173:1276–1282.
62. Andersson-Sjoland A, de Alba CG, Nihlberg K, Becerril C, Ramirez R, Pardo A, Westergren-Thorsson G, Selman M. Fibrocytes are a potential source of lung fibroblasts in idiopathic pulmonary fibrosis. *Int J Biochem Cell Biol* 2008;40:2129–2140.
63. Strieter RM. Pathogenesis and natural history of usual interstitial pneumonia: the whole story or the last chapter of a long novel. *Chest* 2005;128:526S–532S.
64. Kisseleva T, Uchinami H, Feirt N, Quintana-Bustamante O, Segovia JC, Schwabe RF, Brenner DA. Bone marrow-derived fibrocytes participate in pathogenesis of liver fibrosis. *J Hepatol* 2006;45:429–438.
65. Yokota T, Kawakami Y, Nagai Y, Ma JX, Tsai JY, Kincade PW, Sato S. Bone marrow lacks a transplantable progenitor for smooth muscle type alpha-actin-expressing cells. *Stem Cells* 2006;24:13–22.
66. Follonier Castella L, Gabbiani G, McCulloch CA, Hinz B. Regulation of myofibroblast activities: calcium pulls some strings behind the scene. *Exp Cell Res* 2010;316:2390–2401.
67. Hill-Eubanks DC, Werner ME, Heppner TJ, Nelson MT. Calcium signaling in smooth muscle. *Cold Spring Harb Perspect Biol* 2011;3:a004549.
68. Jude JA, Wylam ME, Walseth TF, Kannan MS. Calcium signaling in airway smooth muscle. *Proc Am Thorac Soc* 2008;5:15–22.
69. Katoh K, Kano Y, Amano M, Onishi H, Kaibuchi K, Fujiwara K. Rho-kinase-mediated contraction of isolated stress fibers. *J Cell Biol* 2001;153:569–584.
70. Kimura K, Ito M, Amano M, Chihara K, Fukata Y, Nakafuku M, Yamamori B, Feng JH, Nakano T, Okawa K, et al. Regulation of myosin phosphatase by Rho and Rho-associated kinase (rho-kinase). *Science* 1996;273:245–248.
71. Somlyo AP, Somlyo AV. Ca²⁺ sensitivity of smooth muscle and non-muscle myosin ii: modulated by G proteins, kinases, and myosin phosphatase. *Physiol Rev* 2003;83:1325–1358.
72. Loirand G, Guerin P, Pacaud P. Rho kinases in cardiovascular physiology and pathophysiology. *Circ Res* 2006;98:322–334.
73. Tomasek JJ, Vaughan MB, Kropp BP, Gabbiani G, Martin MD, Haaksma CJ, Hinz B. Contraction of myofibroblasts in granulation tissue is dependent on Rho/Rho kinase/myosin light chain phosphatase activity. *Wound Repair Regen* 2006;14:313–320.
74. Yee HF Jr, Melton AC, Tran BN. RhoA/Rho-associated kinase mediates fibroblast contractile force generation. *Biochem Biophys Res Commun* 2001;280:1340–1345.
75. Emmert DA, Fee JA, Goeckeler ZM, Grojean JM, Wakatsuki T, Elson EL, Herring BP, Gallagher PJ, Wysolmerski RB. Rho-kinase-mediated Ca²⁺-independent contraction in rat embryo fibroblasts. *Am J Physiol Cell Physiol* 2004;286:C8–21.
76. Nobe H, Nobe K, Fazal F, De Lanerolle P, Paul RJ. Rho kinase mediates serum-induced contraction in fibroblast fibers independent of myosin LC20 phosphorylation. *Am J Physiol Cell Physiol* 2003;284:C599–606.
77. Parizi M, Howard EW, Tomasek JJ. Regulation of LPA-promoted myofibroblast contraction: role of Rho, myosin light chain kinase, and myosin light chain phosphatase. *Exp Cell Res* 2000;254:210–220.
78. Follonier Castella L, Buscemi L, Godbout C, Meister JJ, Hinz B. A new lock-step mechanism of matrix remodelling based on subcellular contractile events. *J Cell Sci* 2010;123:1751–1760.
79. Wipff PJ, Rifkin DB, Meister JJ, Hinz B. Myofibroblast contraction activates latent TGF-beta1 from the extracellular matrix. *J Cell Biol* 2007;179:1311–1323.

80. Levinson H, Moyer KE, Siggers GC, Ehrlich HP. Calmodulin-myosin light chain kinase inhibition changes fibroblast-populated collagen lattice contraction, cell migration, focal adhesion formation, and wound contraction. *Wound Repair Regen* 2004;12:505–511.
81. Gabbiani G, Ryan GB, Majno G. Presence of modified fibroblasts in granulation tissue and their possible role in wound contraction. *Experientia* 1971;27:549–550.
82. Hinz B, Gabbiani G, Chaponnier C. The NH2-terminal peptide of alpha-smooth muscle actin inhibits force generation by the myofibroblast in vitro and in vivo. *J Cell Biol* 2002;157:657–663.
83. Goto T, Yanaga F, Ohtsuki I. Studies on the endothelin-1-induced contraction of rat granulation tissue pouch mediated by myofibroblasts. *Biochim Biophys Acta* 1998;1405:55–66.
84. Majno G, Gabbiani G, Hirschel BJ, Ryan GB, Statkov PR. Contraction of granulation tissue in vitro: similarity to smooth muscle. *Science* 1971;173:548–550.
85. Gabbiani G, Hirschel BJ, Ryan GB, Statkov PR, Majno G. Granulation tissue as a contractile organ: a study of structure and function. *J Exp Med* 1972;135:719–734.
86. Chilton L, Ohya S, Freed D, George E, Drohic V, Shibukawa Y, Maccannell KA, Imaizumi Y, Clark RB, Dixon IM, *et al.* K^+ currents regulate the resting membrane potential, proliferation, and contractile responses in ventricular fibroblasts and myofibroblasts. *Am J Physiol Heart Circ Physiol* 2005;288:H2931–2939.
87. Raizman JE, Komljenovic J, Chang R, Deng C, Bedosky KM, Rattan SG, Cunningham RH, Freed DH, Dixon IM. The participation of the Na^+-Ca^{2+} exchanger in primary cardiac myofibroblast migration, contraction, and proliferation. *J Cell Physiol* 2007;213:540–551.
88. Kernochan LE, Tran BN, Tangkijvanich P, Melton AC, Tam SP, Yee HF Jr. Endothelin-1 stimulates human colonic myofibroblast contraction and migration. *Gut* 2002;50:65–70.
89. Laleman W, Van Landeghem L, Severi T, Vander Elst I, Zeegers M, Bisschops R, Van Pelt J, Roskams T, Cassiman D, Fevery J, *et al.* Both Ca^{2+} -dependent and -independent pathways are involved in rat hepatic stellate cell contraction and intrahepatic hyperresponsiveness to methoxamine. *Am J Physiol Gastrointest Liver Physiol* 2007;292:G556–564.
90. Kawada N, Tran-Thi TA, Klein H, Decker K. The contraction of hepatic stellate (Ito) cells stimulated with vasoactive substances: possible involvement of endothelin 1 and nitric oxide in the regulation of the sinusoidal tonus. *Eur J Biochem* 1993;213:815–823.
91. Pinzani M, Failli P, Ruocco C, Casini A, Milani S, Baldi E, Giotti A, Gentilini P. Fat-storing cells as liver-specific pericytes: spatial dynamics of agonist-stimulated intracellular calcium transients. *J Clin Invest* 1992;90:642–646.
92. Goffin JM, Pittet P, Csucs G, Lussi JW, Meister JJ, Hinz B. Focal adhesion size controls tension-dependent recruitment of alpha-smooth muscle actin to stress fibers. *J Cell Biol* 2006;172:259–268.
93. Adhikari AS, Chai J, Dunn AR. Mechanical load induces a 100-fold increase in the rate of collagen proteolysis by MMP-1. *J Am Chem Soc* 2011.
94. Flynn BP, Bhole AP, Sacidi N, Liles M, Dimarzio CA, Ruberti JW. Mechanical strain stabilizes reconstituted collagen fibrils against enzymatic degradation by mammalian collagenase matrix metalloproteinase 8 (MMP-8). *PLoS One* 2010;5:e12337.
95. Hinz B. The myofibroblast: paradigm for a mechanically active cell. *J Biomech* 2010;43:146–155.
96. Liu F, Mih JD, Shea BS, Kho AT, Sharif AS, Tager AM, Tschumperlin DJ. Feedback amplification of fibrosis through matrix stiffening and COX-2 suppression. *J Cell Biol* 2010;190:693–706.
97. Liu F, Tschumperlin DJ. Micro-mechanical characterization of lung tissue using atomic force microscopy. *J Vis Exp* 2011.
98. Dolnikoff M, Mauad T, Ludwig MS. Extracellular matrix and oscillatory mechanics of rat lung parenchyma in bleomycin-induced fibrosis. *Am J Respir Crit Care Med* 1999;160:1750–1757.
99. Ebihara T, Venkatesan N, Tanaka R, Ludwig MS. Changes in extracellular matrix and tissue viscoelasticity in bleomycin-induced lung fibrosis: temporal aspects. *Am J Respir Crit Care Med* 2000;162:1569–1576.
100. Soucy PA, Werbin J, Heinz W, Hoh JH, Romer LH. Microelastic properties of lung cell-derived extracellular matrix. *Acta Biomater* 2011;7:96–105.
101. Janmey PA, Winer JP, Murray ME, Wen Q. The hard life of soft cells. *Cell Motil Cytoskeleton* 2009;66:597–605.
102. Buxboim A, Discher DE. Stem cells feel the difference. *Nat Methods* 2010;7:695–697.
103. Discher DE, Janmey P, Wang YL. Tissue cells feel and respond to the stiffness of their substrate. *Science* 2005;310:1139–1143.
104. Georges PC, Janmey PA. Cell type-specific response to growth on soft materials. *J Appl Physiol* 2005;98:1547–1553.
105. Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. *Cell* 2006;126:677–689.
106. Wells RG. The role of matrix stiffness in regulating cell behavior. *Hepatology* 2008;47:1394–1400.
107. Tschumperlin DJ, Boudreau F, Liu F. Recent advances and new opportunities in lung mechanobiology. *Journal of Biomechanics* 2010;43:99–107.
108. Arora PD, Narani N, McCulloch CA. The compliance of collagen gels regulates transforming growth factor-beta induction of alpha-smooth muscle actin in fibroblasts. *Am J Pathol* 1999;154:871–882.
109. Hinz B, Mastrangelo D, Iselin CE, Chaponnier C, Gabbiani G. Mechanical tension controls granulation tissue contractile activity and myofibroblast differentiation. *Am J Pathol* 2001;159:1009–1020.
110. Grinnell F, Petroll WM. Cell motility and mechanics in three-dimensional collagen matrices. *Annu Rev Cell Dev Biol* 2010;26:335–361.
111. Wang J, Chen H, Seth A, McCulloch CA. Mechanical force regulation of myofibroblast differentiation in cardiac fibroblasts. *Am J Physiol Heart Circ Physiol* 2003;285:H1871–1881.
112. Cabrera-Benitez NE, Parotto M, Post M, Han B, Spieth PM, Cheng WE, Valladares F, Villar J, Liu M, Sato M, *et al.* Mechanical stress induces lung fibrosis by epithelial-mesenchymal transition. *Crit Care Med* 2012;40:510–517.
113. Wipff PJ, Hinz B. Integrins and the activation of latent transforming growth factor beta1 - an intimate relationship. *Eur J Cell Biol* 2008;87:601–615.
114. Balestrini J, Chaudhry S, Sarrazo V, Koehler A, Hinz B. The mechanical memory of lung myofibroblasts. *Integrative Biology* 2012;4:410–421.
115. Trujillo G, Meneghin A, Flaherty KR, Sholl LM, Myers JL, Kazerooni EA, Gross BH, Oak SR, Coelho AL, Evanoff H, *et al.* TLR9 differentiates rapidly from slowly progressing forms of idiopathic pulmonary fibrosis. *Sci Transl Med* 2011;2:57ra82.
116. Georges PC, Hui JJ, Gombos Z, McCormick ME, Wang AY, Uemura M, Mick R, Janmey PA, Furth EE, Wells RG. Increased stiffness of the rat liver precedes matrix deposition: Implications for fibrosis. *Am J Physiol Gastrointest Liver Physiol* 2007;293:G1147–1154.
117. Barker HE, Chang J, Cox TR, Lang G, Bird D, Nicolau M, Evans HR, Gartland A, Erler JT. LOXL2-mediated matrix remodeling in metastasis and mammary gland involution. *Cancer Res* 2011;71:1561–1572.
118. van der Slot AJ, Zuurmond AM, van den Bogaardt AJ, Ulrich MM, Middelkoop E, Boers W, Karel Rouday H, DeGroot J, Huizinga TW, Bank RA. Increased formation of pyridinoline cross-links due to higher telopeptide lysyl hydroxylase levels is a general fibrotic phenomenon. *Matrix Biol* 2004;23:251–257.
119. Selman M, Pardo A. Role of epithelial cells in idiopathic pulmonary fibrosis: From innocent targets to serial killers. *Proc Am Thorac Soc* 2006;3:364–372.
120. Zhao XH, Laschinger C, Arora P, Szasz K, Kapus A, McCulloch CA. Force activates smooth muscle alpha-actin promoter activity through the rho signaling pathway. *J Cell Sci* 2007;120:1801–1809.
121. Shi M, Zhu J, Wang R, Chen X, Mi L, Walz T, Springer TA. Latent TGF-beta structure and activation. *Nature* 2011;474:343–349.
122. Buscemi L, Ramonet D, Klingberg F, Formey A, Smith-Clerc J, Meister JJ, Hinz B. The single-molecule mechanics of the latent TGF-beta1 complex. *Curr Biol* 2011;21:2046–2054.
123. Zhou Y, Hagood JS, Lu B, Merryman WD, Murphy-Ullrich JE. Thy-1-integrin alphavbeta5 interactions inhibit lung fibroblast contraction-induced latent TGF-beta1 activation and myofibroblast differentiation. *J Biol Chem* 2010.
124. Maeda T, Sakabe T, Sunaga A, Sakai K, Rivera AL, Keene DR, Sasaki T, Stavnezer E, Iannotti J, Schweitzer R, *et al.* Conversion of mechanical force into TGF-beta-mediated biochemical signals. *Curr Biol* 2011;21:933–941.

125. Ahamed J, Burg N, Yoshinaga K, Janczak CA, Rifkin DB, Collier BS. In vitro and in vivo evidence for shear-induced activation of latent transforming growth factor-beta1. *Blood* 2008;112:3650-3660.
126. Giacomini MM, Travis MA, Kudo M, Sheppard D. Epithelial cells utilize cortical actin/myosin to activate latent TGF-beta through integrin alpha(v)beta(6)-dependent physical force. *Exp Cell Res* 2012.
127. Gauldie J, Bonniaud P, Sime P, Ask K, Kolb M. TGF-beta, smad3 and the process of progressive fibrosis. *Biochem Soc Trans* 2007;35:661-664.
128. Sheppard D. Transforming growth factor beta: a central modulator of pulmonary and airway inflammation and fibrosis. *Proc Am Thorac Soc* 2006;3:413-417.
129. Ueda T, Niimi A, Matsumoto H, Takemura M, Yamaguchi M, Matsuoka H, Jinnai M, Chin K, Minakuchi M, Cheng L, et al. TGFbetab1 promoter polymorphism C-509t and pathophysiology of asthma. *J Allergy Clin Immunol* 2008;121:659-664.
130. Takizawa H, Tanaka M, Takami K, Ohtoshi T, Ito K, Satoh M, Okada Y, Yamasawa F, Nakahara K, Umeda A. Increased expression of transforming growth factor-beta1 in small airway epithelium from tobacco smokers and patients with chronic obstructive pulmonary disease (COPD). *Am J Respir Crit Care Med* 2001;163:1476-1483.
131. de Boer WI, van Schadewijk A, Sont JK, Sharma HS, Stolk J, Hiemstra PS, van Krieken JH. Transforming growth factor beta1 and recruitment of macrophages and mast cells in airways in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1998;158:1951-1957.
132. Khalil N, O'Connor RN, Unruh HW, Warren PW, Flanders KC, Kemp A, Berezney OH, Greenberg AH. Increased production and immunohistochemical localization of transforming growth factor-beta in idiopathic pulmonary fibrosis. *Am J Respir Cell Mol Biol* 1991;5:155-162.
133. Xaubet A, Marin-Arguedas A, Lario S, Ancochea J, Morell F, Ruiz-Manzano J, Rodriguez-Becerra E, Rodriguez-Arias JM, Inigo P, Sanz S, et al. Transforming growth factor-beta1 gene polymorphisms are associated with disease progression in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2003;168:431-435.
134. Sime PJ, Xing Z, Graham FL, Csaky KG, Gauldie J. Adenovector-mediated gene transfer of active transforming growth factor-beta1 induces prolonged severe fibrosis in rat lung. *J Clin Invest* 1997;100:768-776.
135. Kenyon NJ, Ward RW, McGrew G, Last JA. TGF-beta1 causes airway fibrosis and increased collagen i and iii mRNA in mice. *Thorax* 2003;58:772-777.
136. Giri SN, Hyde DM, Hollinger MA. Effect of antibody to transforming growth factor beta on bleomycin induced accumulation of lung collagen in mice. *Thorax* 1993;48:959-966.
137. Shi Y, Massague J. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. *Cell* 2003;113:685-700.
138. Moustakas A, Heldin CH. The regulation of TGFbeta signal transduction. *Development* 2009;136:3699-3714.
139. Malmstrom J, Lindberg H, Lindberg C, Bratt C, Wieslander E, Delander EL, Sarnstrand B, Burns JS, Mose-Larsen P, Fey S, et al. Transforming growth factor-beta 1 specifically induce proteins involved in the myofibroblast contractile apparatus. *Mol Cell Proteomics* 2004;3:466-477.
140. Roy SG, Nozaki Y, Phan SH. Regulation of alpha-smooth muscle actin gene expression in myofibroblast differentiation from rat lung fibroblasts. *Int J Biochem Cell Biol* 2001;33:723-734.
141. Breen EC. Mechanical strain increases type I collagen expression in pulmonary fibroblasts in vitro. *J Appl Physiol* 2000;88:203-209.
142. Varga J, Pasche B. Transforming growth factor beta as a therapeutic target in systemic sclerosis. *Nat Rev Rheumatol* 2009;5:200-206.
143. Ikushima H, Miyazono K. TGFbeta signalling: a complex web in cancer progression. *Nat Rev Cancer* 2010;10:415-424.
144. Yang L, Pang Y, Moses HL. TGF-beta and immune cells: an important regulatory axis in the tumor microenvironment and progression. *Trends Immunol* 2010;31:220-227.
145. Li MO, Wan YY, Sanjabi S, Robertson AK, Flavell RA. Transforming growth factor-beta regulation of immune responses. *Annu Rev Immunol* 2006;24:99-146.
146. Wakefield LM, Stuelten C. Keeping order in the neighborhood: new roles for TGFbeta in maintaining epithelial homeostasis. *Cancer Cell* 2007;12:293-295.
147. ten Dijke P, Arthur HM. Extracellular control of TGFbeta signalling in vascular development and disease. *Nat Rev Mol Cell Biol* 2007;8:857-869.
148. Howell JE, McNulty RJ. TGF-beta: its role in asthma and therapeutic potential. *Curr Drug Targets* 2006;7:547-565.
149. Meier K, Nanney LB. Emerging new drugs for scar reduction. *Expert Opin Emerg Drugs* 2006;11:39-47.
150. Nishimura SL. Integrin-mediated transforming growth factor-beta activation, a potential therapeutic target in fibrogenic disorders. *Am J Pathol* 2009;175:1362-1370.
151. Worthington JJ, Klementowicz JE, Travis MA. TGFbeta: a sleeping giant awoken by integrins. *Trends Biochem Sci* 2010;36:47-54.
152. Annes JP, Munger JS, Rifkin DB. Making sense of latent TGFbeta activation. *J Cell Sci* 2003;116:217-224.
153. Hyytiainen M, Penttinen C, Keski-Oja J. Latent TGF-beta binding proteins: extracellular matrix association and roles in TGF-beta activation. *Crit Rev Clin Lab Sci* 2004;41:233-264.
154. Ramirez F, Rifkin DB. Extracellular microfibrils: contextual platforms for TGFbeta and BMP signaling. *Curr Opin Cell Biol* 2009;21:616-622.
155. Hynes RO. The extracellular matrix: not just pretty fibrils. *Science* 2009;326:1216-1219.
156. Todorovic V, Rifkin DB. LTBP's, more than just an escort service. *J Cell Biochem* 2012;113:410-418.
157. Annes JP, Chen Y, Munger JS, Rifkin DB. Integrin [alpha]v[beta]6-mediated activation of latent TGF-[beta] requires the latent TGF-[beta] binding protein-1. *J Cell Biol* 2004;165:723-734.
158. Jenkins G. The role of proteases in transforming growth factor-beta activation. *Int J Biochem Cell Biol* 2008;40:1068-1078.
159. Sweetwyne MT, Murphy-Ullrich JE. Thrombospondin1 in tissue repair and fibrosis: TGF-beta-dependent and independent mechanisms. *Matrix Biol* 2012.
160. Crawford SE, Stellmach V, Murphy-Ullrich JE, Ribeiro SM, Lawler J, Hynes RO, Boivin GP, Bouck N. Thrombospondin-1 is a major activator of TGF-beta1 in vivo. *Cell* 1998;93:1159-1170.
161. Derynck R, Jarrett JA, Chen EY, Goeddel DV. The murine transforming growth factor-beta precursor. *J Biol Chem* 1986;261:4377-4379.
162. Lu M, Munger JS, Steadale M, Busald C, Tellier M, Schnapp LM. Integrin alpha8beta1 mediates adhesion to LAP-TGFbeta1. *J Cell Sci* 2002;115(Pt 23):4641-4648.
163. Munger JS, Harpel JG, Giancotti FG, Rifkin DB. Interactions between growth factors and integrins: latent forms of transforming growth factor-beta are ligands for the integrin alphavbeta1. *Mol Biol Cell* 1998;9:2627-2638.
164. Sheppard D. Integrin-mediated activation of latent transforming growth factor beta. *Cancer Metastasis Rev* 2005;24:395-402.
165. Ludbrook SB, Barry ST, Delves CJ, Horgan CM. The integrin alphavbeta3 is a receptor for the latency-associated peptides of transforming growth factors beta1 and beta3. *Biochem J* 2003;369:311-318.
166. Mu D, Cambier S, Fjellbirkeland L, Baron JL, Munger JS, Kawakatsu H, Sheppard D, Broaddus VC, Nishimura SL. The integrin alpha(v)beta8 mediates epithelial homeostasis through MT1-MMP-dependent activation of TGF-beta1. *J Cell Biol* 2002;157:493-507.
167. Schultz-Cherry S, Lawler J, Murphy-Ullrich JE. The type 1 repeats of thrombospondin 1 activate latent transforming growth factor-beta. *J Biol Chem* 1994;269:26783-26788.
168. McMahon GA, Dignam JD, Gentry LE. Structural characterization of the latent complex between transforming growth factor beta 1 and beta 1-latency-associated peptide. *Biochem J* 1996;313:343-351.
169. Young GD, Murphy-Ullrich JE. Molecular interactions that confer latency to transforming growth factor-beta. *J Biol Chem* 2004;279:38032-38039.
170. Sha X, Yang L, Gentry LE. Identification and analysis of discrete functional domains in the pro region of pre-pro-transforming growth factor beta 1. *J Cell Biol* 1991;114:827-839.
171. Walton KL, Mankanji Y, Chen J, Wilce MC, Chan KL, Robertson DM, Harrison CA. Two distinct regions of latency-associated peptide coordinate stability of the latent transforming growth factor-beta1 complex. *J Biol Chem* 2010;285:17029-17037.
172. Aluwihare P, Munger JS. What the lung has taught us about latent TGF-beta activation. *Am J Respir Cell Mol Biol* 2008;39:499-502.

173. Huang XZ, Wu JF, Cass D, Erle DJ, Corry D, Young SG, Farese RV Jr., Sheppard D. Inactivation of the integrin beta 6 subunit gene reveals a role of epithelial integrins in regulating inflammation in the lung and skin. *J Cell Biol* 1996;133:921–928.
174. Bader BL, Rayburn H, Crowley D, Hynes RO. Extensive vasculogenesis, angiogenesis, and organogenesis precede lethality in mice lacking all alpha v integrins. *Cell* 1998;95:507–519.
175. Zhu J, Motejlek K, Wang D, Zang K, Schmidt A, Reichardt LF. Beta8 integrins are required for vascular morphogenesis in mouse embryos. *Development* 2002;129:2891–2903.
176. Yang Z, Mu Z, Dabovic B, Jurukovski V, Yu D, Sung J, Xiong X, Munger JS. Absence of integrin-mediated TGF[beta]1 activation in vivo recapitulates the phenotype of tgfbeta1-null mice. *J Cell Biol* 2007;176:787–793.
177. Shull MM, Ormsby I, Kier AB, Pawlowski S, Diebold RJ, Yin M, Allen R, Sidman C, Proetzel G, Calvin D, *et al.* Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. *Nature* 1992;359:693–699.
178. Munger JS, Huang X, Kawakatsu H, Griffiths MJ, Dalton SL, Wu J, Pittet JF, Kaminski N, Garat C, Matthay MA, *et al.* The integrin alpha v beta 6 binds and activates latent TGF beta 1: a mechanism for regulating pulmonary inflammation and fibrosis. *Cell* 1999;96:319–328.
179. Horan GS, Wood S, Ona V, Li DJ, Lukashev ME, Weinreb PH, Simon KJ, Hahm K, Allaire NE, Rinaldi NJ, *et al.* Partial inhibition of integrin alpha(v)beta6 prevents pulmonary fibrosis without exacerbating inflammation. *Am J Respir Crit Care Med* 2008;177:56–65.
180. Jenkins RG, Su X, Su G, Scotton CJ, Camerer E, Laurent GJ, Davis GE, Chambers RC, Matthay MA, Sheppard D. Ligation of protease-activated receptor 1 enhances alpha(v)beta6 integrin-dependent TGF-beta activation and promotes acute lung injury. *J Clin Invest* 2006;116:1606–1614.
181. Li M, Krishnaveni MS, Li C, Zhou B, Xing Y, Banfalvi A, Li A, Lombardi V, Akbari O, Borok Z, *et al.* Epithelium-specific deletion of TGF-beta receptor type ii protects mice from bleomycin-induced pulmonary fibrosis. *J Clin Invest* 2011;121:277–287.
182. Phan SH. The myofibroblast in pulmonary fibrosis. *Chest* 2002;122:286S–289S.
183. Thannickal VJ, Toews GB, White ES, Lynch JP 3rd, Martinez FJ. Mechanisms of pulmonary fibrosis. *Annu Rev Med* 2004;55:395–417.
184. Scotton CJ, Krupiczkoj MA, Konigshoff M, Mercer PF, Lee YC, Kaminski N, Morser J, Post JM, Maher TM, Nicholson AG, *et al.* Increased local expression of coagulation factor X contributes to the fibrotic response in human and murine lung injury. *J Clin Invest* 2009;119:2550–2563.
185. Tatler AL, John AE, Jolly L, Habgood A, Porte J, Brightling C, Knox AJ, Pang L, Sheppard D, Huang X, *et al.* Integrin alphavbeta5-mediated TGF-beta activation by airway smooth muscle cells in asthma. *J Immunol* 2011;187:6094–6107.
186. Sheppard D. Roles of alphav integrins in vascular biology and pulmonary pathology. *Curr Opin Cell Biol* 2004;16:552–557.
187. Huang X, Griffiths M, Wu J, Farese RV Jr, Sheppard D. Normal development, wound healing, and adenovirus susceptibility in beta5-deficient mice. *Mol Cell Biol* 2000;20:755–759.
188. Levine D, Rockey DC, Milner TA, Breuss JM, Fallon JT, Schnapp LM. Expression of the integrin alpha8beta1 during pulmonary and hepatic fibrosis. *Am J Pathol* 2000;156:1927–1935.
189. Bouzeghrane F, Mercure C, Reudelhuber TL, Thibault G. Alpha8beta1 integrin is upregulated in myofibroblasts of fibrotic and scarring myocardium. *J Mol Cell Cardiol* 2004;36:343–353.
190. Thibault G, Lacombe MJ, Schnapp LM, Lacasse A, Bouzeghrane F, Lapalme G. Upregulation of alpha(8)beta(1)-integrin in cardiac fibroblast by angiotensin ii and transforming growth factor-beta1. *Am J Physiol Cell Physiol* 2001;281:C1457–1467.
191. Scotton CJ, Chambers RC. Molecular targets in pulmonary fibrosis: the myofibroblast in focus. *Chest* 2007;132:1311–1321.
192. Huang X, Gai Y, Yang N, Lu B, Samuel CS, Thannickal VJ, Zhou Y. Relaxin regulates myofibroblast contractility and protects against lung fibrosis. *Am J Pathol* 2011;179:2751–2765.
193. Bond JE, Kokosis G, Ren L, Selim MA, Bergeron A, Levinson H. Wound contraction is attenuated by fasudil inhibition of Rho-associated kinase. *Plast Reconstr Surg* 2011;128:438e–450e.
194. Takeda Y, Nishikimi T, Akimoto K, Matsuoka H, Ishimitsu T. Beneficial effects of a combination of Rho-kinase inhibitor and ACE inhibitor on tubulointerstitial fibrosis induced by unilateral ureteral obstruction. *Hypertens Res* 2010;33:965–973.
195. Bogatkevich GS, Ludwicka-Bradley A, Silver RM. Dabigatran, a direct thrombin inhibitor, demonstrates antifibrotic effects on lung fibroblasts. *Arthritis Rheum* 2009;60:3455–3464.
196. Tucker GC. Inhibitors of integrins. *Curr Opin Pharmacol* 2002;2:394–402.
197. Weinreb PH, Simon KJ, Rayhorn P, Yang WJ, Leone DR, Dolinski BM, Pearse BR, Yokota Y, Kawakatsu H, Atakilit A, *et al.* Function-blocking integrin alphavbeta6 monoclonal antibodies: distinct ligand-mimetic and nonligand-mimetic classes. *J Biol Chem* 2004;279:17875–17887.
198. Hinz B, Gabbiani G. The role of myofibroblasts in Dupuytren's disease: fundamental aspects of contraction and therapeutic perspectives. In: Eaton C, Seegenschmiedt MH, Bayat A, Gabbiani G, Werker G, Wach W, editors. Dupuytren's disease and related hyperproliferative disorders: principles, research, and clinical perspectives. Springer Verlag; 2012.