Tissue Stiffness, Latent TGF-β1 Activation, and Mechanical Signal Transduction: Implications for the Pathogenesis and Treatment of Fibrosis

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Tissue stiffening is a predominant feature of fibrosis and it obstructs organs whose mechanical properties are important for their function, such as the heart, lung, skin, and vessels. Stiff scar tissue further modulates the character of the healthy residing cells by driving the differentiation of a variety of precursor cells into fibrogenic myofibroblasts. This mechanical cue for myofibroblast differentiation establishes a vicious cycle because the excessive extracellular matrix–secreting and remodeling activities of myofibroblasts are cause and effect of further connective tissue contracture and stiffening. The second pivotal factor inducing myofibroblast development is transforming growth factor-β1. Recent findings suggest that transforming growth factor-β1 activity is partly controlled by myofibroblast contractile forces and tissue stiffness. This discovery opens new paths to prevent progression of fibrosis by specifically interfering with the stress perception and transmission mechanisms of the myofibroblast.

Introduction

Fibrosis is initiated by factors that trigger the physiologic repair mechanisms of the body, which are inflammatory response and connective tissue repair. When the normal wound healing and repair mechanisms become deregulated, fibrosis develops and progresses [1]. Common features of fibrosis are excess secretion and improper remodeling of extracellular matrix (ECM), which is predominantly composed of fibrous collagen. The cell most responsible for these detrimental effects is the myofibroblast, a phenotype that unifies ECM-producing fibroblastic features and cytoskeletal characteristics of smooth muscle cells [2]. It is the high contractile activity of the myofibroblast that generates connective tissue contracture and irreversible ECM remodeling, producing a stiff fibrotic scar. Formation of fibrotic tissue after injury can affect almost all organs, including skin [3], heart [4], vasculature [5], liver [6], lung [7], and kidney [8]. Cancer cells stimulate the formation of scar-like connective tissue and hijack this complex environment to promote tumor progression [9]. The intention of myofibroblast activities is the formation of a scar to rapidly restore the mechanical integrity of damaged tissues. However, because scar tissue is adapted to repair but not to regenerate damaged organs, it cannot restore organ function. Even worse, scar tissue most often obstructs and destructs the functionality of the intact remaining cells and tissue. Part of this destructive effect is due to the generation of a particular chemical and mechanical microenvironment that can turn healthy resident cells into fibrotic and/or dysfunctional cells. This article provides an overview on how tissue mechanics influence the development and progression of fibrosis by modulating the activity of two central elements in fibrotic diseases: the cytokine transforming growth factor-β1 (TGF-β1) and the myofibroblast. It will become clear that both the chemical cytokine milieu and the mechanical environment in the fibrotic scar are strongly interdependent.

The Relevance of Tissue Compliance for Fibrosis

One important feature of fibrotic scars is their stiffness compared with the relatively compliant tissue texture of most nonfibrotic organs. This particular mechanical property of scar tissue provides the names for some pathologies, such as connective tissue disease scleroderma (systemic

sclerosis [SSc]) and atherosclerosis of vessels ("skleros" being the Greek term for "hard"). In clinical practice, the stiff scar tissue that surrounds epithelial tumors can be used to detect tumors in situ with tissue elastography imaging methods. The physical dimension of tissue compliance or elasticity is expressed as the Young's modulus E (in units of Pascal [Pa]), which defines the force per unit area (stress) that is required to strain a given material. High stress has to be applied to lengthen materials with high Young's modulus. The Young's modulus of normal heart muscle, vascular smooth muscle, and skeletal muscle is approximately 10 kPa as measured with the subcellular precision of atomic force microscopy (AFM) [10]. Tissues that are macroscopically soft, such as liver, fat, and brain, exhibit low Young's moduli (between 0.1 and 1 kPa as determined by AFM) [10]. Although not yet measured on the subcellular level, the dermis of healthy human skin is estimated to be comparably compliant (Young's moduli of 1–5 kPa) [11]. In contrast, fibrotic tissue was found to be stiff, with Young's moduli of 20 to 100 kPa, which is close to the stiffness of collagen-dense tendon or the osteoid structure that precedes formation of the even stiffer bone matrix [10–12].

What are the consequences of the fibrotic scar being stiffer than the host tissue? Apart from its diagnostic value, the stiff stroma surrounding cancer increases interstitial pressure in the tumor, thereby contributing to tumor progression and counteracting the systemic delivery of drugs [13,14]. The sheer mechanical obstacle represented by the fibrotic scar after myocardial infarct as well as its different electrical conduction fosters arrhythmia and heart failure. This is supported by recent findings that embryonic cardiomyocytes beat periodically when cultured on heart-soft substrate but not on fibrotic-stiff material [12]. In SSc, stiff fibrotic scars are the pathologic consequences of a series of events, including vascular dysfunction, autoimmune reactions, and inflammation [3]. SSc not only affects the skin but spreads over internal organs such as lung, kidney, and the heart. The leading causes of death of SSc patients are complications in the lung, such as pulmonary hypertension and lung fibrosis [3]. In both cases, changes in the mechanical properties of the tissue contribute to worsening of the disease and enhance lethality. In pulmonary hypertension, remodeling of the pulmonary arteries significantly decreases arterial compliance and thereby increases the working load for the heart. In pulmonary fibrosis, decreased alveolar and airway compliance is one of the major symptoms of irreversible connective tissue remodeling, leading to dramatically reduced lung function [7].

The Fibrotic Environment Drives Myofibroblast Development: A Fatal Affair

In addition to impeding organ functions that depend on specific mechanical properties of the intact tissue, stiff fibrotic scars can have even more profound influence on the progression of fibrosis by modulating the phenotype of the residing normal cells. In fibrotic skin, as in most other fibrotic organs, connective tissue fibroblasts acquire smooth muscle features by forming contractile stress fibers and by expressing α-smooth muscle actin (α-SMA). These molecular and structural features, together with upregulation of the cell contractile activity, are the hallmark of the myofibroblast phenotype [2]. In diseased liver, local hepatic stellate cells and portal fibroblasts are turned into myofibroblasts [6]. In fibrotic kidney, resident fibroblastic cells and epithelial cells that undergo epithelial to mesenchymal transition contribute to the myofibroblast population [15•]. Generation of fibroblasts and myofibroblasts by epithelial to mesenchymal transition was further demonstrated during tumor development and fibrosis of lung, heart, and liver [16,17]. During vessel repair and dermal scarring in SSc, pericytes have been shown to attain contractile myofibroblast features [18], and dedifferentiation of smooth muscle cells contributes to the generation of myofibroblasts in atheromatous plaques [5].

The influence of the mature scar microenvironment on myofibroblast fate as opposed to that of earlier inflammatory signals is difficult to decipher because local myofibroblast precursors are progressively recruited and then advance fibrosis development themselves [2]. The timing is different for the recruitment of blood circulating cells to sites of existing fibrotic tissue. Bone marrow–derived circulating cells with fibroblastic and monocyte characteristics, so-called fibrocytes, have been found to contribute to almost all fibrotic diseases, to the stroma reaction to epithelial tumors, and to normal skin wound healing [19]. Experiments with sex-mismatched and fluorescently labeled bone marrow transplants demonstrate that fibrocytes are selectively recruited to sites of fibrosis and mature into fibroblasts/myofibroblasts under the influence of the scar environment [19]. Therapeutically used mesenchymal stem cells (MSCs) are another cell type that is de novo exposed to scar tissue at even later stages of fibrosis. MSCs are envisaged to regenerate damaged organs by local injection or systemic delivery. Many of the potential therapeutic applications that have been proposed for MSCs imply their engraftment into fibrotic tissue, such as heart repair after myocardial infarct [20] and regeneration of fibrotic lung [21]. Nevertheless, the success of MSC therapy in clinical trials in regenerating the diseased organ is a matter of debate [22]; engrafting MSCs are at risk of the hostile fibrotic environment turning them into fibrogenic cells of myofibroblastic character rather than becoming regenerative cells. Indeed, MSCs were shown to turn into myofibroblasts when injected into the myocardial scar after infarct [23], when delivered to interstitial fibrosis following chronic renal disease [24], after being systemically transplanted into mice with acute liver injury and fibrosis [25], and when engrafting in a murine model

of chronic lung fibrosis [26]. In addition, these studies show that the timing of MSC transplantation with respect to the progression of fibrosis is critical for the success of regenerative therapy. When engrafting immediately after organ damage or in early stages of fibrosis, MSCs were capable of improving the healing; when delivered to a more mature scar, MSCs were rather prone to fibrogenesis and myofibroblast differentiation. The question remains: how important is the role of the mechanical property of the scar for the process of myofibroblast differentiation?

Myofibroblasts and Stiff Tissue: When the Going Gets Tough, the Tough Get Going

In vivo, it is difficult to discriminate mechanical from chemical cues exerted by the complex fibrotic tissue environment. However, the development over the past decade of novel culture materials with tuneable stiffness has led to a quantum leap in our understanding of how cells feel and respond to the mechanical properties of a given substratum. It emerges as a general theme that different cell types prefer the culture substrate stiffness that most closely resembles the stiffness of their origin tissue. Neuronal cells grow best on brain-soft polyacrylamide hydrogels that eliminate other contaminating cells of the tissue preparation [27]. Muscle precursor cells in culture differentiate most efficiently into functional muscle fibers on muscle-stiff hydrogels [10]. Cancer cells appreciate soft hydrogels and dislike stiff hydrogels [14]. Interestingly, the stiffness (or softness) of the culture substrate alone is sufficient to initiate the differentiation of MSCs along different lineages by mimicking the appropriate tissue stiffness in culture [28••]. In "brain-soft" gels with elastic modulus of approximately 1 kPa, MSCs express early neurogenic markers. Growth on "muscle-stiff" substrates of approximately 11 kPa induces early myogenic factors, and "bone-stiff" substrates of greater than 34 kPa induce osteogenic differentiation [28••].

Myofibroblasts and their precursor cells are mechano-responsive and, to a certain degree, plastic in their phenotype. Differentiation of myofibroblasts in the presence of profibrotic stimuli, such as active TGF-β1, requires a mechanically resistant substrate. In their seminal study, Arora et al. [29] used collagen culture substrates of different mechanical strength to show that TGF-β1 only upregulates the myofibroblast marker α-SMA in fibroblasts grown on stiff, but not on compliant, collagen. Later, the threshold ECM stiffness for occurrence of α-SMA in stress fibers was measured to be approximately 16 kPa, close to the Young's modulus of scar tissue demonstrated for myofibroblasts cultured on compliant silicone surfaces [11]. A comparable ECM stiffness of approximately 15 kPa is required to activate hepatic stellate cells into α-SMA–positive myofibroblasts under appropriate growth conditions [30]. In both experimental setups, even the strong fibrogenic stimulus of active TGF-β1 did not induce myofibroblast differentiation on softer substrate culture.

Experimental studies on normal wound healing have made it clear that mechanical stress, predominantly originating from the stiff ECM, also controls myofibroblast fate and development in vivo. The stiffness of the provisional ECM of early wounds is comparable with the elastic modulus of newly polymerized collagen gels (approximately 0.01–0.1 kPa), which are frequently used in vitro models of tissue repair [31]. In mechanically restrained gels, ECM stiffness is gradually increasing due to collagen remodeling, which forces migration and spreading of fibroblasts. This initially induces the formation of α-SMA–negative stress fibers, similar to fibroblasts residing in 5- to 6-day-old rat wound granulation tissue [32,33]. De novo stress fiber formation by fibroblasts on hydrogel requires a substrate's Young's modulus to be 3 to 6 kPa [27]. Expression of α-SMA and incorporation into stress fibers demands even higher ECM stiffness that begins to develop in anchored collagen gels after 2 to 3 days of remodeling and after 8 to 9 days in experimental rat wounds [32,33]. The in vivo ECM threshold stiffness corresponding to the occurrence of α-SMA in stress fibers is approximately 20 kPa as measured for contractile rat wound granulation tissue [11]. Mechanically preventing wound closure by splinting the edges of experimental rat wounds [32] and stretching human burn wound scar tissue [34] accelerates expression of α-SMA compared with normally healing wounds; stress release by removing the splint leads to reduced α-SMA expression [32]. ECM stress not only controls the level of myofibroblast differentiation but promotes their survival in wound granulation tissue and thereby contributes to hypertrophic scar formation [35].

It appears that force controls myofibroblast differentiation and function on different levels. First, α-SMA only localizes to stress fibers under significant mechanical load, providing a mechanism to rapidly control myofibroblast contractile function [11,33]. Second, force application to myofibroblasts triggers nuclear translocation of the transcriptional co-activator myocardin-related transcription factor A and activation of the α-SMA promoter through mechanisms that entail Rho/Rho-associated kinase signaling and increased polymerization of actin [36•,37]. Third, the forces generated by the myofibroblasts themselves, in the context of a stiff ECM, can promote activation of TGF-β1, the central profibrotic cytokine.

TGF-β1 Activation: Stress Makes the Insoluble Soluble

If myofibroblasts are essential to generate stiff fibrotic tissue and if stiff ECM is pivotal for myofibroblast differentiation, what comes first? Part of this chicken-and-egg problem may be solved by the recent finding of mechanical activation of latent TGF-β1 by myofibroblast contraction [38••].

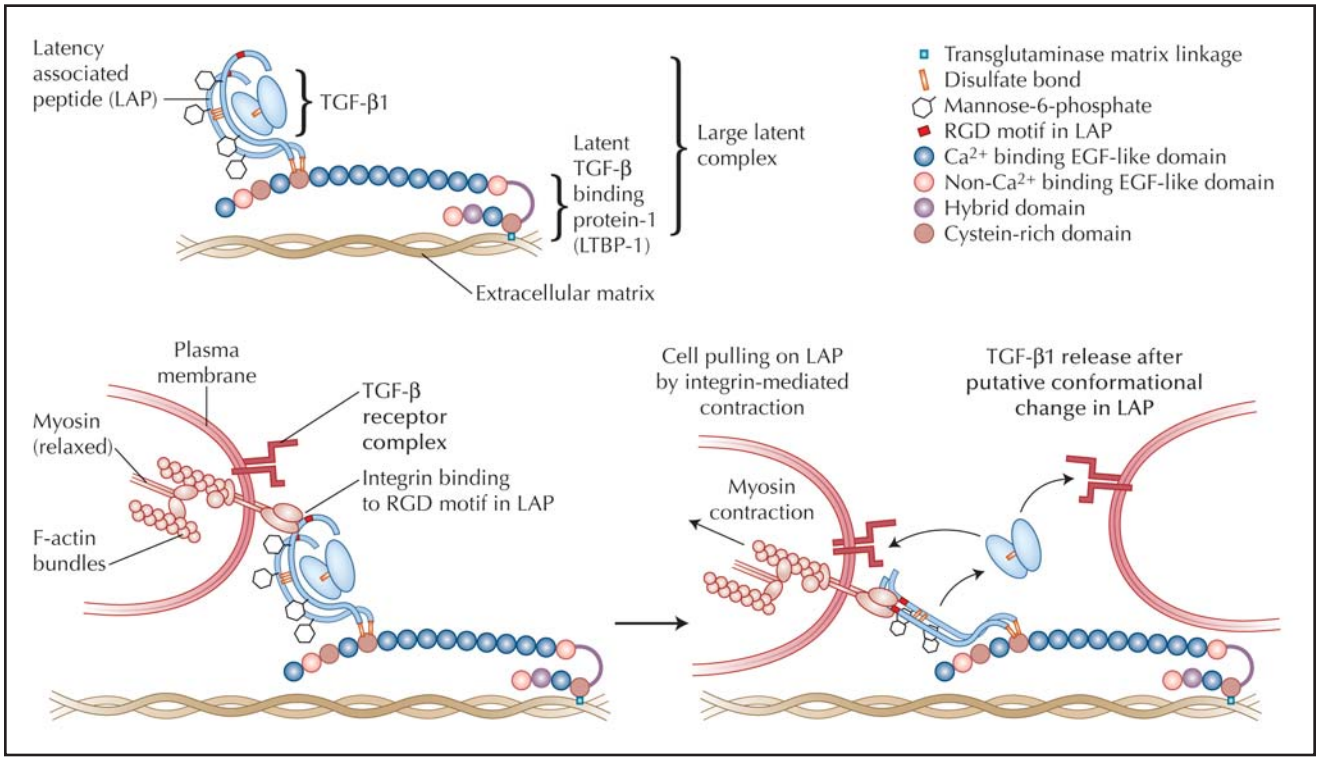

Figure 1. A model for activation of latent transforming growth factor-β1 (TGF-β1) by myofibroblast contraction. TGF-β1 is secreted in a large latent complex, consisting of TGF-β1 associated with the latency associated peptide (LAP) and the latent TGF-β1–binding protein (LTBP-1). The second cysteine-rich domain of LTBP-1 binds LAP via disulfide bonds, and the third cysteine-rich domain at the N-terminus of LTBP-1 promotes binding to the extracellular matrix (ECM) via transglutamination. When the large latent complex is covalently bound to a mechanically resistant ECM, cell traction forces exerted to LAP potentially result in a conformation change of the latent complex that liberates active TGF-β1. The integrin-mediated traction model predicts liberation of active TGF-β1 as demonstrated by contracting Triton X-100 cytoskeletons [38••]. However, no soluble active TGF-β1 is detected in cultures of contracting intact cells, which can be explained by presentation of the growth factor to the receptor [44] and/or by simultaneous binding of the receptor to the activating integrin rather than liberation. RGD—Arg-Gly-Asp. (*Adapted from* Wipff and Hinz [43].)

The mechanical liberation of this growth factor from stores in the ECM provides a novel mechanism to translate cell forces into biochemical signals. TGF-β1 is considered as the central regulatory cytokine for normal tissue repair and development of fibrosis. TGF-β1 mediates the inflammatory response, causes excessive ECM production, increases the synthesis of tissue inhibitors of metalloproteinases, decreases protease synthesis, and induces myofibroblast differentiation [2,39]. However, TGF-β1 signaling also ensures tissue homeostasis by controlling proliferation of epithelial cells, endothelial cells, immune cells, and fibroblasts [39–41]. Therefore, general inhibition of TGF-β1 is predicted to have many uncontrollable and unwanted effects and is thus problematic as an antifibrotic therapeutic strategy [42].

However, the diverse mechanisms leading to the activation of latent TGF-β1 provide several possibilities for a cell-specific inhibition of TGF-β1 action. Latent TGF-β1 is activated by its dissociation from the latency associated peptide (LAP) that is co-synthesized in conjunction with TGF-β1 [41]. Most cell types secrete TGF-β1 as part of the large latent complex, which consists of TGF-β1, LAP, and the latent TGF-β1 binding protein (LTBP-1) (Fig. 1) [41]. LTBP-1 is a member of the fibrillin family of ECM proteins and it binds to several other ECM components, including fibrillin-1, fibronectin, and vitronectin, thereby providing a reservoir of latent TGF-β1 in the ECM [41]. Activation of latent TGF-β1 by its dissociation from LAP is promoted by various mechanisms that differ according to the cell type and the physiologic context. Latent TGF-β1 activation can occur upon proteolytic cleavage, by interaction with thrombospondin 1, and with the mannose-6-phosphate receptor [41]. Moreover, integrins, representing the transmembrane components of cell-ECM adhesions have been reported to play a major role in activating latent TGF-β1 [43,44]. Two principal mechanisms of how integrins can activate a growth factor are experimentally supported. The first mechanism is sensitive to protease inhibitors, and current models propose integrins as a common docking point for latent TGF-β1 and its activating proteases. The second mechanism is independent from any proteolytic action and involves cell traction forces that are directly transmitted to the large latent complex via integrins [41,43,44].

The first evidence that integrins can directly activate latent TGF-β1 independently from proteolytic action was

provided for the LAP-binding epithelial integrin αvβ6, which is involved in the initiation of lung fibrosis and [45,46••]. Functional knockout of this integrin produces a mouse phenotype resembling that of the TGF-β1 knockout [47•], and the lungs of β6 knockout mice are protected from bleomycin-induced fibrosis [44]. Implication of cell-generated forces in αvβ6 integrin-mediated latent TGF-β1 activation was hypothesized because incubating purified αvβ6 integrin with latent TGF-β1 alone does not release the active growth factor. Moreover, disruption of actin filament bundles with cytochalasin D and truncation of the αvβ6 integrin cytoplasmic tail (associating with the cytoskeleton) both prevent latent TGF-β1 activation [44]. Subsequent studies carried out with β6 integrin-transfected fibroblasts, overexpressing either constitutively active or dominant-negative forms of the small guanosine triphosphatase RhoA, consolidated the role of the contractile cytoskeleton in the latent TGF-β1 activation process [46••]. Activation of RhoA is a central element in promoting actin-myosin contraction, and the level of RhoA activity was shown to correlate with the level of latent TGF-β1 activation [46••]. During fibrosis of epithelialized tissues, such as kidney and lung, αvβ6 integrin-mediated latent TGF-β1 activation appears to play a role in generating myofibroblasts through epithelial mesenchymal transition [17]. However, the evolution of pulmonary fibrosis toward end stage fibrotic lung disease depends on the persistence αvβ6 integrin-negative myofibroblasts that continue to express and to activate latent TGF-β1. Furthermore, myofibroblasts drive fibrosis in a variety of other organs that neither exhibit pronounced epithelium nor express αvβ6 integrin.

The contribution of mechanical stress to latent TGF-β1 activation was first directly demonstrated in experiments with myofibroblasts that activate latent TGF-β1 as a function of their contractile activity and ECM stiffness. Inducing myofibroblast contraction with thrombin, angiotensin-II, and endothelin-1 leads to the release of active TGF-β1 from latent stores in the ECM [38••]. From this study, the following model has been proposed for direct and integrin-mediated activation of latent TGF-β1 by contractile myofibroblasts: 1) myofibroblast integrins establish an axis between the extracellular LAP portion of the large latent complex and intracellular contractile stress fibers; 2) the forces generated by stress fibers pull on the latent complex via its integrin-binding moiety; and 3) anchorage of LTBP-1 to the ECM provides mechanical resistance to the pulling, which leads to opening of the complex and release/presentation of active TGF-β1 (Fig. 1).

The physical link between TGF-β1 and the ECM is provided by the LTBP-1 moiety of the large latent complex. An engineered construct of LTBP-1, comprising only its LAP-binding domain and its ECM-binding hinge region (Fig. 1) is sufficient to substitute for the full-length LTBP-1, with respect to integrin-mediated activation of latent TGF-β1 [45]. Consequently, deletion of the ECM-binding hinge region from LTBP-1 or complete absence of LTBP-1 abolishes this function [45]. Importantly, the contraction activation of latent TGF-β1 by myofibroblasts is not functional on latent TGF-β1–rich substrates that are soft, with a Young's modulus of below 10 kPa [38••]. This threshold ECM stiffness for latent TGF-β1 activation (5 kPa) is lower than the minimal stiffness required for incorporation of α-SMA into stress fibers (16 kPa) measured with the same experimental system [11]. This makes physiologic sense during development of fibrosis when ECM stiffness is gradually increasing with ongoing fibroblast remodeling activity [33]. It is possible that the stress-dependent release of TGF-β1 represents a crucial checkpoint in the further progression of fibrosis by restricting myofibroblast differentiation to a microenvironment that has been sufficiently pre-remodeled and stiffened by being efficiently contracted.

Another precondition for latent TGF-β1 activation by myofibroblast contraction is the transmission of intracellular force to the large latent complex via integrins. All integrins that were shown to activate latent TGF-β1 (either directly or by implying proteolysis) physically interact with the LAP portion of the large latent complex [44]. Inhibition or deletion of the integrin binding sequence Arg-Gly-Asp (RGD) in LAP abolishes latent TGF-β1 activation in cultured epithelial cells and phenocopies the TGF-β1 knockout [47]. In cultured myofibroblasts, inhibition of LAP-RGD similarly reduces latent TGF-β activation. Myofibroblasts do not express αvβ6 integrin but rather a series of other LAP binding candidates and function-blocking antibodies directed against integrin αvβ5, and to some extent β1 and αvβ3 integrin inhibit contraction-mediated latent TGF-β1 activation [38••]. Interestingly, integrins αvβ5 and αvβ3 are upregulated in SSc fibroblasts, and their deletion or inhibition prevents myofibroblast differentiation in a latent TGF-β1–dependent manner [48•].

Conclusions

During development and progression of fibrosis, myofibroblasts adapt their harmful contractile activity to the level of stress in the surrounding tissue. They behave analogous to a bodybuilder in the gym: the more weight put on the bar the harder they have to work, and as a consequence the stronger they become by gradually building up muscle (ie, α-SMA–positive stress fibers). Just as some bodybuilders use (sometimes illegal) supplements to support muscle growth, myofibroblasts are addicted to TGF-β1 consumption. TGF-β1 is not easily available and does come in a stable package only if the cells pull strong enough and only when the package is mechanically anchored in the stiff fibrotic scar does the TGF-β1 become available.

Myofibroblasts use the same integrin-containing ECM adhesions that ultimately transmit stress fiber contraction to the ECM to feel out the mechanical properties of the constantly changing microenvironment. Rather than changing ECM mechanics to improve fibrosis (an approach that is difficult to envision), we propose to target the myofibroblast stress sensors, rendering them blind for mechanical inputs. Moreover, interfering with the integrins that are implicated in latent TGF-β1 activation is a possible therapeutic strategy to counteract the harmful activity of TGF-β1 in a myofibroblast-specific manner. Blocking the integrin αvβ6 was recently shown to prevent tumor progression by reducing latent TGF-β1 activation by carcinoma cells [49•]. In this study, the fibrotic stroma reaction against the tumor was not prevented and it is tempting to speculate that this could be done with additional anti–αvβ5 integrin therapy. Combined integrin αvβ6/αvβ5 inhibition was shown to be successful in reducing interleukin-1–induced acute lung injury through a TGF-β1–dependent pathway [50].

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No potential conflict of interest relevant to this article was reported.

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In the context of experimental lung fibrosis, latent TGF-β1 is activated by αvβ6 integrin through stimulation of the intracellular small GTPase Rho pathway. Although not directly assessed, the results of this study imply that cell contraction is involved in latent TGF-β1 activation because Rho is a major regulator of stress fiber contraction. Moreover, high-tidal ventilation of the lungs (ie, enhanced mechanical stress) is pivotal for efficient TGF-β1 release in this model.

47.• Yang Z, Mu Z, Dabovic B, et al.: **Absence of integrin-mediated TGF{beta}1 activation in vivo recapitulates the phenotype of TGF{beta}1-null mice.** *J Cell Biol* 2007, **176:**787–793.

Mutation of the integrin-binding site in the latent TGF-β1 complex recapitulates the complete knockout of TGF-β1. This follows and confirms earlier studies showing that the physiologic consequences of the αvβ6 integrin knockout are all related to TGF-β1 activation defects. The article makes the case that the major, if not only, function of this integrin is latent TGF-β1 activation.

48.• Asano Y, Ihn H, Yamane K, et al.: **Increased expression of integrin alphavbeta5 induces the myofibroblastic differentiation of dermal fibroblasts.** *Am J Pathol* 2006, **168:**499–510.

This article shows that integrins αvβ5 and αvβ3 play important roles in establishing autocrine TGF-β1 signaling in cultured dermal and SSc fibroblasts. This function depends on integrin binding of latent TGF-β1. Inhibition of this interaction prevents myofibroblast differentiation and expression of collagen and matrix metalloprotease, which are the hallmarks of fibrosis. The authors propose specific anti-integrin treatment to reduce skin fibrosis.

49.• Van Aarsen LA, Leone DR, Ho S, et al.: **Antibody-mediated blockade of integrin alpha v beta 6 inhibits tumor progression in vivo by a transforming growth factor-beta–regulated mechanism.** *Cancer Res* 2008, **68:**561–570.

By using self-developed function-blocking antibodies against the integrin αvβ6, the authors prevented progression of epithelial tumor cells in vitro and in vivo. This effect was related to the reduced activation of latent TGF-β1 by the epithelial integrin without effect on the surrounding stromal cells, demonstrating that integrin inhibition is a possible therapy for the treatment of TGF-β1–related diseases in a cell-specific manner.

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