Journal of Bodywork and Movement Therapies (2009) **13**, 121–127

ELSEVIER

Journal of
Bodywork and Movement Therapies

www.intl.elsevierhealth.com/journals/jbmt

FASCIA CONGRESS RESEARCH: MYOFIBROBLAST MINI-REVIEW

Myofibroblasts work best under stress

Pierre-Jean Wipff, Boris Hinz, PhD*

Laboratory of Cell Biophysics, Ecole Polytechnique Fédérale de Lausanne (EPFL), Bâtiment SG-AA-B143, Station 15, CH-1015 Lausanne, Switzerland

Received 31 March 2008; accepted 4 April 2008

KEYWORDS
Cell contraction;
Fibrosis;
Matrix compliance;
Transforming growth factor beta

Summary Myofibroblasts are reparative connective tissue cells that contribute to the reconstruction of injured tissue by secreting new extracellular matrix and by exerting high contractile force. Deregulation of these activities results in tissue contracture and development of fibrosis which makes the myofibroblast an important target for anti-fibrotic therapies. Two principle factors drive the development of myofibroblasts from different precursor cells and guarantee maintenance of the contractile phenotype: mechanical stress and transforming growth factor beta (TGFβ1). In this mini-review, we recapitulate the current understanding (1) of how myofibroblasts feel stress using specialized matrix adhesions, (2) of the level of stress that is required to induce their development and (3) of how myofibroblast mechanical activity can have a direct influence on the level of TGFβ1 activation. From these findings it emerges that the specific matrix adhesion structures of myofibroblasts are promising targets to modulate myofibroblast differentiation and activity.

Introduction

When tissues are injured, locally residing connective tissue fibroblasts and cells recruited from other sources acquire smooth muscle features by forming contractile stress fibers and by neo-expressing α-smooth muscle actin (α-SMA) (Hinz, 2007) (Figure 1). The high contractile activity generated by α-SMA in stress fibers plays a fundamental role in remodeling injured tissue such as during skin wound healing (Hinz et al., 2001a). Of high clinical relevance are the retractile phenomena, caused by excessive myofibroblast contractile and extracellular matrix (ECM) secreting activity, which characterize the vast majority of fibrocontractive diseases. This includes fibrosis affecting vital organs, such as heart (Virag and Murry, 2003), liver (Desmouliere et al., 2003), kidney (Lan, 2003)

Abbreviations: α-SMA, α-smooth muscle actin; ECM, extracellular matrix; FA, focal adhesion; LAP, latency-associated protein; LLC, large latent complex; LTBP, latent transforming growth factor β-binding protein; TGF, transforming growth factor.

*Corresponding author. Tel.: +41 21 6939703; fax: +41 21 6938305.
E-mail address: boris.hinz@epfl.ch (B. Hinz).

1360-8592/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jbmt.2008.04.031

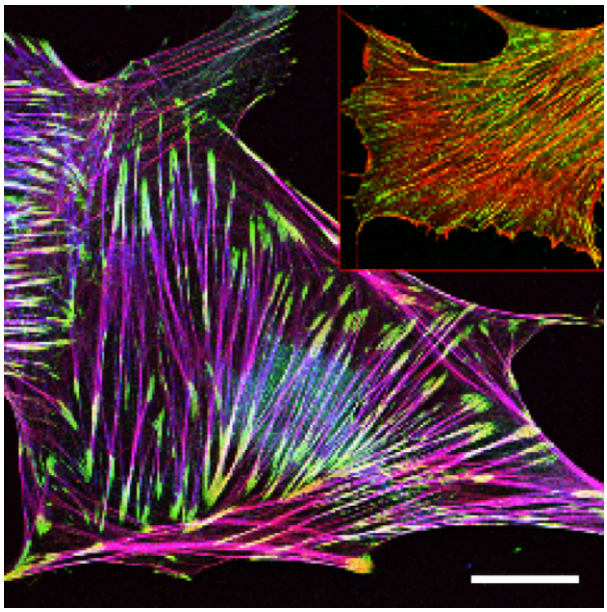

Figure 1 (On-line colour) Myofibroblast morphology differs on stiff and rigid culture substrates. Myofibroblasts cultured on rigid plastic surfaces and soft polymer substrates (inset) were immunostained for polymerized actin (red), α-SMA (blue) and FAs (vinculin, green). On rigid plastic, myofibroblasts develop thick stress fiber bundles that incorporate α-SMA (purple color) and that insert at sites of large supermature FAs. On soft polymer substrates large FA cannot develop and α-SMA is not recruited to stress fibers (red). Note that both cells are displayed at the same magnification. Bar: 50 μm.

and lung (Phan, 2002) and fibrosis reducing life quality in scleroderma (Rajkumar et al., 2005), hypertrophic scars (Desmouliere et al., 2003; Gabbiani, 2003), airway remodeling in asthma (Chiappara et al., 2001) and Dupuytren's disease (Tomasek et al., 1999). Moreover, myofibroblasts at the tumor invasion front are activated by the transformed epithelium to stimulate tumor growth and invasion by promoting angiogenesis and tumor cell migration (Desmouliere et al., 2004). But don't get fooled—the myofibroblast is not necessarily the 'bad guy' as it contributes to physiological wound healing and its absence has been associated with development of chronic wounds (Gabbiani, 2003). In addition, contractile fibroblasts have been suggested to generate a beneficial mechanical stress or 'tonus' in some normal tissues, such as in the alveolar septa in the lung and possibly the human dorsal fascia, as it has emerged from the first Fascia Research Congress in Boston 2007.

To develop novel strategies counteracting myofibroblast malfunction in fibrosis and controlling beneficial myofibroblast action in physiological tissue repair, it is important to understand the molecular mechanisms that regulate myofibroblast formation and activity. Over the past 15 years, the pro-fibrotic cytokine transforming growth factor beta1 (TGFβ1) has been established as the most potent inducer of myofibroblast differentiation (Desmouliere et al., 1993; Ronnov-Jessen and Petersen, 1993). However, during tissue repair, reparative fibroblasts are not only in contact with an altered chemical milieu but face a dramatically changed mechanical microenvironment. In fact, expression of α-SMA demands both, a mechanically restrained environment and the action of TGFβ1. In this mini-review, we will elaborate that mechanical stress and TGFβ1 are actually two sides of the myofibroblast differentiation coin, as recently reviewed in greater detail (Wipff and Hinz, 2008).

How stiff is the myofibroblast environment?

Having spent most of their lives shielded by a protective ECM, fibroblasts are exposed to considerable stress when the protective ECM structure is lost after tissue injury (Tomasek et al., 2002). In response to this mechanical challenge, fibroblasts develop tension on their own by building up a contractile stress fiber apparatus that is used to stiffen newly secreted ECM. What is a 'stiff' ECM in a physiological sense and how does ECM stiffness develop during tissue repair and remodeling? The stiffness of the provisional ECM of early wounds is comparable with the elastic modulus of $\sim$10–100 Pa of newly polymerized collagen gels, frequently used in vitro models of tissue repair (Carlson and Longaker, 2004; Grinnell, 2003). Fibroblasts initiated in such gels organize actin filaments in a sub-membrane cortex (Tamariz and Grinnell, 2002). In mechanically restrained gels, tension is gradually increasing and first induces the formation of α-SMA-negative stress fibers (Tamariz and Grinnell, 2002), similar to fibroblasts of 5–6 day-old rat wound granulation tissue (Hinz et al., 2001b). Gel and tissue stiffness at this stage have not been measured but neo-formation of stress fibers in fibroblasts grown on soft two-dimensional culture substrates occurs at an elastic modulus of $\sim$3000–6000 Pa (Discher et al., 2005; Yeung et al., 2005).

Expression of α-SMA and incorporation into pre-existing stress fibers requires significantly higher ECM stiffness that begins to develop in anchored collagen gels after 2–3 days of remodeling (Hinz,

2006) and after 8–9 days in experimental rat wounds (Hinz et al., 2001b). The threshold ECM stiffness for occurrence of α-SMA in stress fibers ranges around 20,000 Pa as demonstrated for contractile wound granulation tissue and for myofibroblasts cultured on elastic substrates (Goffin et al., 2006) (Figure 1). A comparable ECM stiffness of ~15,000 Pa activates hepatic stellate cells into α-SMA-positive myofibroblasts in vitro and during development of liver fibrosis in vivo (Wells, 2005). In other fibrotic tissues and in granulation tissue toward the end of wound healing an ECM stiffness of greater than 50,000 Pa has been measured (Goffin et al., 2006). Using collagen and polyacrylamide gel substrates of varying stiffness, it has been shown that the level of α-SMA expression in cultured differentiated myofibroblasts increases with increasing ECM rigidity (Arora et al., 1999; Hinz et al., 2003) (Figure 1). In vivo, mechanically preventing wound closure by splinting the edges of experimental wounds accelerates expression of α-SMA compared with normally healing wounds; stress release by removing the splint leads to reduced α-SMA expression (Hinz et al., 2001b). The mechanisms and intracellular pathways through which tension potentially controls α-SMA transcription have recently been reviewed (Wang et al., 2006). In addition to being regulated on the expression level, α-SMA is beginning to be considered as mechano-sensitive protein. The fact that α-SMA only localizes to stress fibers under significant mechanical load may provide a mechanism to rapidly control myofibroblast contractile function (Goffin et al., 2006; Hinz, 2006). Reducing stress fiber tension by reducing substrate stiffness and by inhibiting myosin contraction leads to the removal of α-SMA from persisting stress fibers (Goffin et al., 2006) (Figure 1).

How do myofibroblasts feel the stress?

It is widely accepted that fibroblastic cells perceive mechanical signals from the ECM by utilizing integrins, i.e. the same transmembrane proteins that anchor stress fibers to the substrate through a complex of cytoplasmic proteins (Bershadsky et al., 2006; Chen et al., 2004; Giannone and Sheetz, 2006). Integrins are $\alpha\beta$ heterodimers that combine to 24 currently identified cell receptors in mammals binding to various ECM proteins (Hynes, 2002; Luo et al., 2007; Sheppard, 2000). The signaling role of integrin-based ECM adhesions becomes clear from their maturation in response to mechanical challenge. Reinforcement of adhesion sites under stress starts with integrin clustering into nascent adhesions that further develop into focal complexes when associating with actin filaments. Increasing intracellular stress that needs to be balanced by a mechanically resistant substrate then lead to the enlargement of ~1 μm^2 focal complexes into classical focal adhesions (FAs) (Bershadsky et al., 2006).

Ultrastructural analysis of myofibroblasts in fibrotic and wound granulation tissue has revealed massive cell–ECM contacts that are not formed by normal connective tissue fibroblasts (Eyden, 2005). Analogous to this so-called fibronexus, cultured myofibroblasts develop specialized 'supermature FAs', a designation that accounts for their significantly longer appearance (8–30 μm) (Dugina et al., 2001) compared with the FAs (2–6 μm) of α-SMA-negative fibroblasts (Geiger et al., 2001) (Figure 1). Supermature FAs exhibit a specific molecular composition which is subject of a more comprehensive recent review (Hinz, 2006). Establishment of supermature FAs depends both on the high intracellular contractile activity generated by α-SMA and on a stress-resistant ECM (Hinz, 2006). Myofibroblasts reduce supermature FAs to small classical FAs after inhibition of α-SMA-mediated contraction (Hinz et al., 2003) and when cultured on soft substrates and in newly polymerized collagen gels (Goffin et al., 2006; Hinz, 2006) (Figure 1). These studies suggest that fibroblasts gain information about the mechanical state of the ECM by assessing the level of tension in stress fibers, which is limited by the size of their ECM anchors. Myofibroblasts grown on arrays of FA adhesion islets ($\leqslant$6 μm long) rapidly lose α-SMA from stress fibers (Figure 2), which however is preserved on supermature FA islets ($>$8 μm long).

It is believed that specific proteins in the cytoplasmic adhesion plaque serve as mechanosensors and act as molecular switches that change their conformation/activation state when force is applied (Giannone and Sheetz, 2006). However, adhesion-mediated mechanical switches and signal generators are not necessarily localized within the cell. The first ECM protein to be identified as a mechano-sensitive protein was fibronectin, which reveals cryptic sites for auto-fibrillogenesis when unfolded upon cell traction (Zhong et al., 1998). ECM protein unfolding may similarly reveal specific integrin-binding sites and thus change cell adhesion-dependent responses such as cell migration, proliferation, survival, and differentiation as well as ECM organization and remodeling (Vogel and Sheetz, 2006).

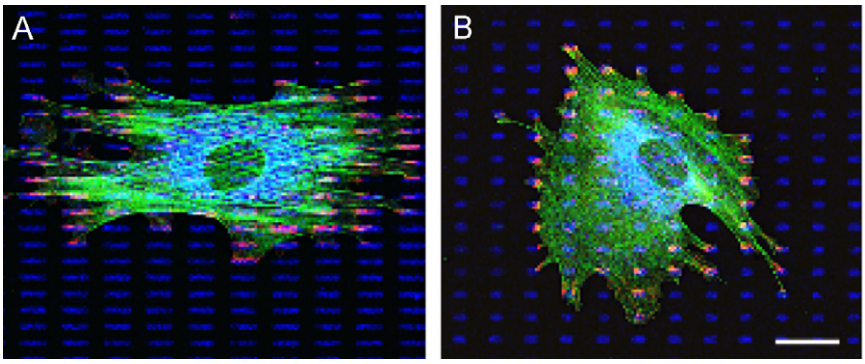

Figure 2 (On-line colour) Focal adhesion size controls recruitment of α-smooth muscle actin to stress fibers. Arrays of fibronectin islets with a size of (A) $4 \times 1.25\,\mu m$ and (B) $10 \times 1.25\,\mu m$ were created on glass by microcontact printing; non-printed regions were passivated against cell attachment (Goffin et al., 2006). Differentiated myofibroblasts were stained for vinculin (red), fibronectin (blue), and α-SMA (green). α-SMA is only recruited to contractile fibers on islets that exceed the length of FAs and that exhibit supermature FA features. Bar: $25\,\mu m$

TGFβ1 activation—when the insoluble becomes soluble

A novel mechanism to translate cell forces into biochemical signals is the liberation of growth factors from stores in the ECM. Such a mechanism was recently demonstrated for the activation of the pro-fibrotic cytokine TGFβ1 by epithelial cells (Jenkins et al., 2006) and by myofibroblasts (Wipff et al., 2007). TGFβ1 is synthesized as a homodimeric protein together with the latency-associated protein (LAP); activation of TGFβ1 requires its dissociation from LAP (Annes et al., 2003). The majority of cell types secrete TGFβ1 as part of the large latent complex (LLC), consisting of TGFβ1, LAP and the latent TGFβ1 binding protein (LTBP-1) (Annes et al., 2003; Todorovic et al., 2005) (Figure 3). LTBP-1 is an ECM protein that binds to several other ECM components, including fibrillin-1, fibronectin and vitronectin, thereby providing a reservoir of latent TGFβ in the ECM (Annes et al., 2003; Todorovic et al., 2005).

Activation of TGFβ1 by its dissociation from LAP and/or the ECM-bound LLC is promoted by various mechanisms, which differ according to the cell type and the physiological context. Latent TGFβ1 activation occurs upon proteolytic cleavage, by interaction with thrombospondin 1 and with the mannose-6-phosphate receptor (Annes et al., 2003; Jenkins, 2007). More recently, integrins αvβ5, αvβ6, and αvβ8, a yet unidentified β1 integrin and possibly αvβ3 integrin have been reported to participate in activating TGFβ1 (Sheppard, 2005; Wipff and Hinz, 2008). The knockout of integrin subunits which participate in TGFβ1 activation produces phenotypes that closely resemble that of a TGFβ1 knockout (Bader et al., 1998; Huang et al., 1996; Shull et al., 1992; Zhu et al., 2002). Importantly, the lungs of β6 knockout mice are protected from bleomycin-induced fibrosis (Munger et al., 1999) and inhibition of αvβ5 integrin in vitro reduces the fibrogenic character of fibroblastic cells (Asano et al., 2006). Hence, interfering with the integrins that are implicated in TGFβ1 activation is a possible therapeutic strategy to counteract the harmful activity of active TGFβ1 in a cell-specific manner, without impairing its beneficial effects on other cell types.

How can integrins activate a growth factor? Two distinct mechanisms are currently discussed which appear to co-exist. In the first mechanism that is sensitive to protease inhibitors, integrins are proposed to serve as a common docking point for latent TGFβ1 and its activating proteases (Jenkins, 2007; Sheppard, 2005; Wipff and Hinz, 2008). The second mechanism is independent from any proteolytic action and involves cell traction forces which are directly transmitted to the LLC via integrins. The resulting conformational change in the latent complex is suggested to liberate TGFβ1 and/or to present it to its receptor (Figure 3). It appears that at least four conditions need to be satisfied for this conformational change: (1) specific integrin binding to the LLC, (2) the presence of the contractile actin cytoskeleton to generate force and/or to provide mechanical resistance, (3) incorporation of latent TGFβ1 into the ECM as LLC, and (4) an ECM that mechanically resists the cellular traction forces exerted to the LLC (Figure 3).

First evidence that integrins can directly activate TGFβ1 independently from any proteolytic activity came from studies on the epithelial integrin αvβ6 (Annes et al., 2004; Jenkins et al., 2006; Munger et al., 1999). Very recently, we elucidated that integrin αvβ5 and possibly αvβ3 integrin can

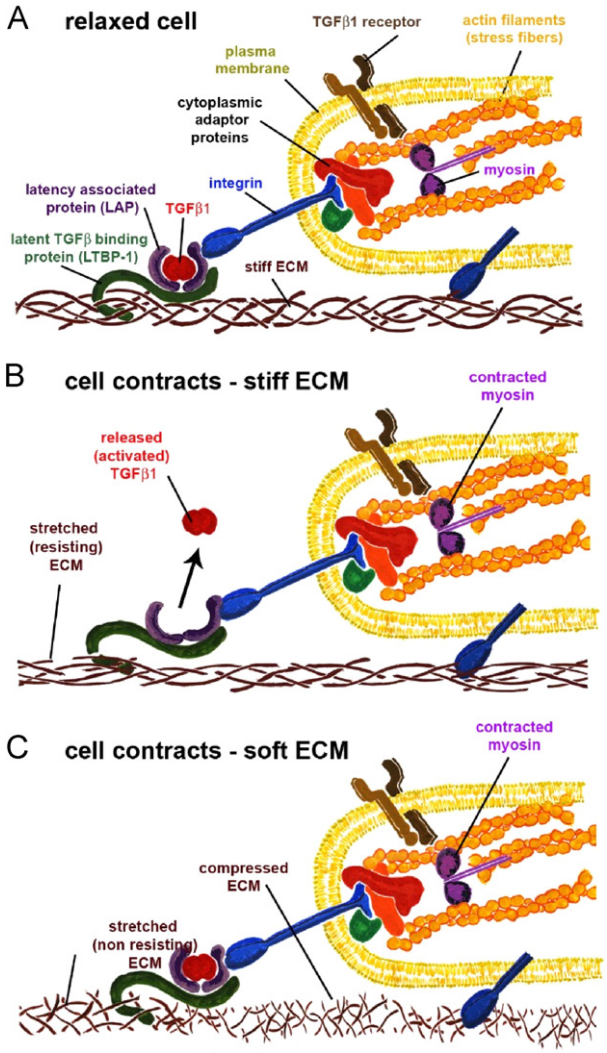

Figure 3 An artistic view on TGFβ1 activation by integrin-mediated myofibroblast contraction. (A) TGFβ1 is biologically latent when binding to the latency-associated protein (LAP). Association of TGFβ1/LAP with the latent TGFβ1 binding protein (LTBP-1) builds the large latent complex (LLC) which is part of the ECM. The contractile activity of myofibroblast stress fibers is transmitted via integrins to specific binding sites in the LAP protein. (B) When the LLC is anchored in a well-organized and stiff ECM, myofibroblast contraction can induce allosteric changes in the complex, leading to liberation of biologically active TGFβ1. Active TGFβ1 will feed back to the activating cell or to cells in close vicinity by binding to its receptor. (C) In the context of a compliant ECM, the LLC is dragged toward the pulling cell due to the lack of mechanical resistance; no conformation change in the LLC occurs and TGFβ1 remains latent.

directly activate TGFβ1 in myofibroblasts (Wipff et al., 2007). All integrins that contribute to the activation of TGFβ1, regardless of the underlying mechanism, physically interact with the LAP portion of the LLC (Sheppard, 2005). Interfering with the respective binding sequence abolishes TGFβ1 activation in cultured epithelial cells (Munger et al., 1999) and in myofibroblasts (Wipff et al., 2007). Knockout of this binding site in mice results in defects in vasculogenesis, a deficient immune system and enhanced inflammation, similar to a TGFβ1 knockout (Yang et al., 2007).

Incubating purified integrins with latent TGFβ1 alone is not sufficient to activate the LLC and association of integrins with the cytoskeleton appears mandatory for this action. Both disruption of actin filaments with cytochalasin D and truncation of the αvβ6 integrin cytoplasmic tail prevent TGFβ1 activation (Munger et al., 1999; Sheppard, 2005). Subsequent studies carried out with β6 integrin-transfected fibroblasts, overexpressing either constitutively active or dominant negative forms of the small GTPase RhoA, consolidated the role of the cytoskeleton in the activation process (Jenkins et al., 2006). Activation of RhoA is a central element in promoting actin-myosin contraction in these cells and the level of RhoA activity correlates with their level of TGFβ1 activation (Jenkins et al., 2006). First direct evidence for a contribution of mechanical stress in TGFβ1 activation was recently provided by experiments with myofibroblasts that activate TGFβ1 as a function of their contractile activity (Wipff et al., 2007). Inducing myofibroblast contraction with thrombin, angiotensin-II and endothelin-1 increases TGFβ1 activation; this effect depends on integrin binding to LAP (Wipff et al., 2007).

The third mandatory element for traction-mediated activation of TGFβ1 by integrins is incorporation of the LLC into the ECM. The connection between LLC–ECM binding and integrin-mediated release of active TGFβ1 was recently elucidated by identifying the pivotal ECM binding motif in the LTBP-1 protein (Annes et al., 2004). An engineered construct comprising only the LAP-binding domain and the N-terminal ECM-binding hinge region of LTBP-1 is sufficient to substitute for this function of the full-length LTBP-1 (Annes et al., 2004). Deletion of this ECM-binding hinge region from LTBP-1 abolishes αvβ6 integrin-mediated activation of TGFβ1 (Annes et al., 2004).

The physiological relevance of the relationship between binding of the LLC to the ECM and TGFβ1 activation is understandable in the context of connective tissue repair and development progressive fibrosis. In these situations, ECM stiffness gradually increases with ongoing fibroblast remodeling activity (Hinz and Gabbiani, 2003). To induce a conformational change in the latent TGFβ1 complex by integrin-mediated pulling on the LAP, the ECM must provide mechanical resistance

(Figure 3). Indeed, myofibroblasts only activate TGF-β1 by integrin-mediated contraction when cultured on silicone substrates with a stiffness that corresponds to that of contracting fibrotic and granulation tissue but not when grown on substrates exhibiting the compliance of normal connective tissue (Wipff et al., 2007). We propose that linking the level of active TGFβ1 release with the degree of ECM stiffness restricts generation and autocrine maintenance of the myofibroblast phenotype to the appropriate (contractible) mechanical microenvironment (Figure 3).

Conclusion

Myofibroblasts adapt their often harmful contractile activity to the level of stress in their surrounding tissue. To detect mechanical changes in the microenvironment, they utilize the same proteins that ultimately transmit stress fiber contraction to the ECM, namely integrins. To prevent formation of the myofibroblast, we propose to target these stress-sensors, in other words to render them blind for mechanical inputs. In addition, it becomes increasingly clear that activation of TGFβ1 is confined to cells expressing the appropriate integrin in the appropriate physiological context. Clearly, this renders local targeting of specific integrins a possible therapy to reduce the profibrotic effects of TGFβ1.

Acknowledgments

We thank Dr. J.-J. Meister (Laboratory of Cell Biophysics, EPFL, Lausanne, Switzerland) for his support and for providing laboratory facilities. The work of BH is supported by Grants from the Swiss National Science Foundation (#3100A0-102150/1 and #3100A0-113733/1), from the Gebert Rüf Stiftung, from the Service Académique, EPFL and from the Competence Centre for Materials Science and Technology (CCMX) of the ETH-Board, Switzerland.

References

Annes, J.P., Munger, J.S., Rifkin, D.B., 2003. Making sense of latent TGFbeta activation. Journal of Cell Science 116, 217–224.

Annes, J.P., Chen, Y., Munger, J.S., Rifkin, D.B., 2004. Integrin {alpha}V{beta}6-mediated activation of latent TGF-{beta} requires the latent TGF-{beta} binding protein-1. Journal of Cell Biology 165, 723–734.

Arora, P.D., Narani, N., McCulloch, C.A., 1999. The compliance of collagen gels regulates transforming growth factor-beta induction of alpha-smooth muscle actin in fibroblasts. American Journal of Pathology 154, 871–882.

Asano, Y., Ihn, H., Yamane, K., Jinnin, M., Tamaki, K., 2006. Increased expression of integrin alphavbeta5 induces the myofibroblastic differentiation of dermal fibroblasts. American Journal of Pathology 168, 499–510.

Bader, B.L., Rayburn, H., Crowley, D., Hynes, R.O., 1998. Extensive vasculogenesis, angiogenesis, and organogenesis precede lethality in mice lacking all alpha v integrins. Cell 95, 507–519.

Bershadsky, A., Kozlov, M., Geiger, B., 2006. Adhesion-mediated mechanosensitivity: a time to experiment, and a time to theorize. Current Opinion in Cell Biology 18, 472–481.

Carlson, M.A., Longaker, M.T., 2004. The fibroblast-populated collagen matrix as a model of wound healing: a review of the evidence. Wound Repair and Regeneration 12, 134–147.

Chen, C.S., Tan, J., Tien, J., 2004. Mechanotransduction at cell–matrix and cell–cell contacts. Annual Review of Biomedical Engineering 6, 275–302.

Chiappara, G., Gagliardo, R., Siena, A., Bonsignore, M.R., Bousquet, J., Bonsignore, G., Vignola, A.M., 2001. Airway remodelling in the pathogenesis of asthma. Current Opinion in Allergy and Clinical Immunology 1, 85–93.

Desmouliere, A., Darby, I.A., Gabbiani, G., 2003. Normal and pathologic soft tissue remodeling: role of the myofibroblast, with special emphasis on liver and kidney fibrosis. Laboratory Investigation 83, 1689–1707.

Desmouliere, A., Geinoz, A., Gabbiani, F., Gabbiani, G., 1993. Transforming growth factor-beta 1 induces alpha-smooth muscle actin expression in granulation tissue myofibroblasts and in quiescent and growing cultured fibroblasts. Journal of Cell Biology 122, 103–111.

Desmouliere, A., Guyot, C., Gabbiani, G., 2004. The stroma reaction myofibroblast: a key player in the control of tumor cell behavior. International Journal of Developmental Biology 48, 509–517.

Discher, D.E., Janmey, P., Wang, Y.L., 2005. Tissue cells feel and respond to the stiffness of their substrate. Science 310, 1139–1143.

Dugina, V., Fontao, L., Chaponnier, C., Vasiliev, J., Gabbiani, G., 2001. Focal adhesion features during myofibroblastic differentiation are controlled by intracellular and extracellular factors. Journal of Cell Science 114, 3285–3296.

Eyden, B., 2005. The myofibroblast: a study of normal, reactive and neoplastic tissues, with an emphasis on ultrastructure. Part 1—normal and reactive cells. Journal of Submicroscopic Cytology Pathology 37, 109–204.

Gabbiani, G., 2003. The myofibroblast in wound healing and fibrocontractive diseases. Journal of Pathology 200, 500–503.

Geiger, B., Bershadsky, A., Pankov, R., Yamada, K.M., 2001. Transmembrane crosstalk between the extracellular matrix—cytoskeleton crosstalk. Nature Reviews Molecular Cell Biology 2, 793–805.

Giannone, G., Sheetz, M.P., 2006. Substrate rigidity and force define form through tyrosine phosphatase and kinase pathways. Trends in Cell Biology 16, 213–223.

Goffin, J.M., Pittet, P., Csucs, G., Lussi, J.W., Meister, J.J., Hinz, B., 2006. Focal adhesion size controls tension-dependent recruitment of alpha-smooth muscle actin to stress fibers. Journal of Cell Biology 172, 259–268.

Grinnell, F., 2003. Fibroblast biology in three-dimensional collagen matrices. Trends in Cell Biology 13, 264–269.

Hinz, B., 2006. Masters and servants of the force: the role of matrix adhesions in myofibroblast force perception and transmission. European Journal of Cell Biology 85, 175–181.

Hinz, B., 2007. Formation and function of the myofibroblast during tissue repair. Journal of Investigative Dermatology 127, 526–537.

Hinz, B., Gabbiani, G., 2003. Mechanisms of force generation and transmission by myofibroblasts. Current Opinion in Biotechnology 14, 538–546.

Hinz, B., Celetta, G., Tomasek, J.J., Gabbiani, G., Chaponnier, C., 2001a. Alpha-smooth muscle actin expression upregulates fibroblast contractile activity. Molecular Biology of the Cell 12, 2730–2741.

Hinz, B., Mastrangelo, D., Iselin, C.E., Chaponnier, C., Gabbiani, G., 2001b. Mechanical tension controls granulation tissue contractile activity and myofibroblast differentiation. American Journal of Pathology 159, 1009–1020.

Hinz, B., Dugina, V., Ballestrem, C., Wehrle-Haller, B., Chaponnier, C., 2003. Alpha-smooth muscle actin is crucial for focal adhesion maturation in myofibroblasts. Molecular Biology of the Cell 14, 2508–2519.

Huang, X.Z., Wu, J.F., Cass, D., Erle, D.J., Corry, D., Young, S.G., Farese Jr., R.V., Sheppard, D., 1996. Inactivation of the integrin beta 6 subunit gene reveals a role of epithelial integrins in regulating inflammation in the lung and skin. Journal of Cell Biology 133, 921–928.

Hynes, R.O., 2002. Integrins: bidirectional, allosteric signaling machines. Cell 110, 673–687.

Jenkins, G., 2007. The role of proteases in transforming growth factor-beta activation. International Journal of Biochemistry & Cell Biology (e-pub ahead of print).

Jenkins, R.G., Su, X., Su, G., Scotton, C.J., Camerer, E., Laurent, G.J., Davis, G.E., Chambers, R.C., Matthay, M.A., Sheppard, D., 2006. Ligation of protease-activated receptor 1 enhances alpha (v)beta6 integrin-dependent TGF-beta activation and promotes acute lung injury. The Journal of Clinical Investigation 116, 1606–1614.

Lan, H.Y., 2003. Tubular epithelial-myofibroblast transdifferentiation mechanisms in proximal tubule cells. Current Opinion in Nephrology and Hypertension 12, 25–29.

Luo, B.H., Carman, C.V., Springer, T.A., 2007. Structural basis of integrin regulation and signaling. Annual Review of Immunology 25, 619–647.

Munger, J.S., Huang, X., Kawakatsu, H., Griffiths, M.J., Dalton, S.L., Wu, J., Pittet, J.F., Kaminski, N., Garat, C., Matthay, M.A., Rifkin, D.B., Sheppard, D., 1999. The integrin alpha v beta 6 binds and activates latent TGF beta 1: a mechanism for regulating pulmonary inflammation and fibrosis. Cell 96, 319–328.

Phan, S.H., 2002. The myofibroblast in pulmonary fibrosis. Chest 122, 286S–289S.

Rajkumar, V.S., Howell, K., Csiszar, K., Denton, C.P., Black, C.M., Abraham, D.J., 2005. Shared expression of phenotypic markers in systemic sclerosis indicates a convergence of pericytes and fibroblasts to a myofibroblast lineage in fibrosis. Arthritis Research and Therapy 7, R1113–R1123.

Ronnov-Jessen, L., Petersen, O.W., 1993. Induction of alpha-smooth muscle actin by transforming growth factor-beta 1 in quiescent human breast gland fibroblasts. Implications for myofibroblast generation in breast neoplasia. Laboratory Investigation 68, 696–707.

Sheppard, D., 2000. In vivo functions of integrins: lessons from null mutations in mice. Matrix Biology 19, 203–209.

Sheppard, D., 2005. Integrin-mediated activation of latent transforming growth factor beta. Cancer Metastasis Reviews 24, 395–402.

Shull, M.M., Ormsby, I., Kier, A.B., Pawlowski, S., Diebold, R.J., Yin, M., Allen, R., Sidman, C., Proetzel, G., Calvin, D., et al., 1992. Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. Nature 359, 693–699.

Tamariz, E., Grinnell, F., 2002. Modulation of fibroblast morphology and adhesion during collagen matrix remodeling. Molecular Biology of the Cell 13, 3915–3929.

Todorovic, V., Jurukovski, V., Chen, Y., Fontana, L., Dabovic, B., Rifkin, D.B., 2005. Latent TGF-beta binding proteins. International Journal of Biochemistry & Cell Biology 37, 38–41.

Tomasek, J.J., Vaughan, M.B., Haaksma, C.J., 1999. Cellular structure and biology of Dupuytren's disease. Hand Clinics 15, 21–34.

Tomasek, J.J., Gabbiani, G., Hinz, B., Chaponnier, C., Brown, R.A., 2002. Myofibroblasts and mechano-regulation of connective tissue remodelling. Nature Reviews Molecular Cell Biology 3, 349–363.

Virag, J.I., Murry, C.E., 2003. Myofibroblast and endothelial cell proliferation during murine myocardial infarct repair. American Journal of Pathology 163, 2433–2440.

Vogel, V., Sheetz, M., 2006. Local force and geometry sensing regulate cell functions. Nature Reviews Molecular Cell Biology 7, 265–275.

Wang, J., Zohar, R., McCulloch, C.A., 2006. Multiple roles of alpha-smooth muscle actin in mechanotransduction. Experimental Cell Research 312, 205–214.

Wells, R.G., 2005. The role of matrix stiffness in hepatic stellate cell activation and liver fibrosis. Journal of Clinical Gastroenterology 39, S158–S161.

Wipff, P.-J., Hinz, B., 2008. Integrins and the activation of latent transforming growth factor β1—an intimate relationship. European Journal of Cell Biology (e-pub ahead of print).

Wipff, P.J., Rifkin, D.B., Meister, J.J., Hinz, B., 2007. Myofibroblast contraction activates latent TGF-beta1 from the extracellular matrix. Journal of Cell Biology 179, 1311–1323.

Yang, Z., Mu, Z., Dabovic, B., Jurukovski, V., Yu, D., Sung, J., Xiong, X., Munger, J.S., 2007. Absence of integrin-mediated TGF{beta}1 activation in vivo recapitulates the phenotype of TGF{beta}1-null mice. Journal of Cell Biology 176, 787–793.

Yeung, T., Georges, P.C., Flanagan, L.A., Marg, B., Ortiz, M., Funaki, M., Zahir, N., Ming, W., Weaver, V., Janmey, P.A., 2005. Effects of substrate stiffness on cell morphology, cytoskeletal structure, and adhesion. Cell Motility and the Cytoskeleton 60, 24–34.

Zhong, C., Chrzanowska-Wodnicka, M., Brown, J., Shaub, A., Belkin, A.M., Burridge, K., 1998. Rho-mediated contractility exposes a cryptic site in fibronectin and induces fibronectin matrix assembly. Journal of Cell Biology 141, 539–551.

Zhu, J., Motejlek, K., Wang, D., Zang, K., Schmidt, A., Reichardt, L.F., 2002. Beta8 integrins are required for vascular morphogenesis in mouse embryos. Development 129, 2891–2903.