Connective Tissue Research, 2012; 53(3): 187–196
Copyright © Informa Healthcare USA, Inc.
ISSN 0300-8207 print/1607-8438 online
DOI: 10.3109/03008207.2011.642035

informa
healthcare

REVIEW

Fibroproliferative Disorders and Their Mechanobiology

Chenyu Huang,[1,2] Rei Ogawa[1]

[1] *Department of Plastic, Reconstructive and Aesthetic Surgery, Nippon Medical School, Tokyo, Japan,* [2] *Department of Plastic Surgery, Meitan General Hospital, Beijing, PR China*

Downloaded from informahealthcare.com by National Library of Medicine on 07/10/12
For personal use only.
Connect Tissue Res

Abstract

Benign and malignant fibroproliferative disorders (FPDs) include idiopathic pulmonary fibrosis, hepatic cirrhosis, myelofibrosis, systemic sclerosis, Dupuytren's contracture, hypertrophic scars, and keloids. They are characterized by excessive connective tissue accumulation and slow but continuous tissue contraction that lead to progressive deterioration in the normal structure and function of affected organs. In recent years, research in diverse fields has increasingly highlighted the potential role of mechanobiology in the molecular mechanisms of fibroproliferation. Mechanobiology, the heart of which is mechanotransduction, is the process whereby cells sense mechanical forces and transduce them, thereby changing the intracellular biochemistry and gene expression. Understanding mechanosignaling may provide new insights into the convergent roles played by interrelated molecules and overlapping signaling pathways during the inflammatory, proliferative, and fibrotic cellular activities that are the hallmarks of fibroproliferation. The main cellular players in FPDs are fibroblasts and myofibroblasts. Consequently, this article discusses integrins and the roles they play in cellular–extracellular matrix interactions. Also described are the signaling pathways that are known to participate in mechanosignaling: these include the transforming growth factor-β/Smad, mitogen-activated protein kinase, RhoA/ROCK, Wnt/β-catenin, and tumor necrosis factor-α/nuclear factor kappa-light-chain-enhancer of activated B cells pathways. Also outlined is the progress in our understanding of the cellular–extracellular matrix interactions that are associated with fibroproliferative mechanosignaling through matricellular proteins. The tensegrity and tensional homeostasis models are also discussed. A better understanding of the mechanosignaling pathways in the FPD microenvironment will almost certainly lead to the development of novel interventions that can prevent, reduce, or even reverse FPD formation and/or progression.

Keywords: fibroproliferation, fibrosis, mechanical stimulation, mechanotransduction pathways, cell–extracellular matrix interactions

INTRODUCTION

Fibrotic change or scarring of tissues is a common consequence of inflammation and tissue damage. While this scarring is usually temporary and reversible, it can sometimes be irreversible and difficult to treat. For example, vocal fold fibrosis, which results from trauma, surgery, or strong inflammation, rarely reverts into normal tissue. Such abnormal tissue responses to injury affect many organs and occur during processes such as liver cirrhosis and lung fibrosis that are collectively termed fibroproliferative disorders (FPDs).

FPDs are classified as aggressive or benign types. Aggressive fibroproliferative diseases include idiopathic pulmonary fibrosis, hepatic cirrhosis, myelofibrosis, and systemic sclerosis, and their common characteristics include the accumulation of mesenchymal cells and their connective tissue products in critical anatomic locations [1]. This leads to progressive organ dysfunction and even death. Benign FPDs include Dupuytren's contracture, hypertrophic scars, and keloids, and their common features are the excessive and exaggerated deposition of extracellular matrix (ECM) that leads to loss of compliance, slow but sustained contracture, and impaired function in the affected area. Fibrosis is the common outcome of both aggressive and benign FPDs and indicates an imbalance between collagen synthesis and its degradation [2] in extent or duration. Although the etiology of FPDs is unclear, these disorders are

Address correspondence to Rei Ogawa, MD, PhD, Department of Plastic, Reconstructive and Aesthetic Surgery, Nippon Medical School, 1-1-5 Sendagi Bunkyo-ku, Tokyo 113-8603, Japan. E-mail: r.ogawa@nms.ac.jp

Received 4 May 2011; Revised 27 October 2011; Accepted 31 October 2011.

RIGHTSLINK

associated with aberrant tissue remodeling as a result of an orchestrated series of inflammatory, proliferative, and fibrotic events.

Evidence from numerous fields of study increasingly suggests that mechanobiology may play an important role in the common molecular mechanisms that underlie FPD formation and progression. Mechanobiology, the heart of which is mechanotransduction, is a process whereby cells sense mechanical forces and transduce them, thereby changing the intracellular biochemistry and gene expression [3,4]. All organs are subjected every day to mechanical loading due to autonomic activities such as respiration and pulse or physical stresses due to gravity and movement. However, the organs that are affected by FPDs are subjected to and have an inappropriate response to mechanical injury. In particular, slow but continuous tissue contraction is a dominant characteristic of FPDs; this is typified by scars. At the molecular level, interrelated molecules with overlapping functions are expressed during the inflammatory, proliferative, and fibrotic cellular activities that are the hallmarks of the fibroproliferative response. Understanding mechanosignaling is likely to provide key insights into the potentially convergent roles of these molecules. The identification of mechanosignaling pathways will also be indispensable for the development of targeted pharmacological interventions that could ultimately prevent, reduce, or reverse the formation or progression of FPDs.

To this end, we analyzed the literature and sought to identify the most common mechanobiological characteristics of FPDs in different organs/cells that are subjected to various mechanical microenvironments. The currently available models that are described in this article comprise a variety of dynamic mechanical processes and target cells. The multiplicity of these models reflects the fact that FPDs affect different organs and their cells and that these are exposed to distinct physiological or pathological mechanical stimuli; the frequencies and magnitudes of these stimuli also vary. For example, bone, cartilage, and muscles are often subjected to compression and tension forces, lungs are frequently exposed to stretching, and blood vessels are repeatedly subjected to hydrostatic pressure and shear forces. The main cellular players in FPDs are fibroblasts and myofibroblasts. This article focuses on these cells and the main mechanical stimuli to which these cells are exposed and discusses integrins and the roles they play in cellular–ECM interactions. Also described are the mechanosignaling pathways that appear to be involved in transducing mechanical stimuli, namely the transforming growth factor-β (TGF-β)/Smad, mitogen-activated protein kinase (MAPK), RhoA/Rho-associated protein kinase (ROCK), Wnt/β-catenin, and tumor necrosis factor-α (TNF-α)/nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathways.

THE MECHANORECEPTOR INTEGRINS AND THEIR ROLES IN FPDS

The shared characteristic of FPDs is the accumulation of mesenchymal cells and their connective tissue products, which indicates that there is an imbalance between collagen synthesis and degradation [2]. Integrins are well-known mechanoreceptors that span the cell membrane and thereby connect the cytoskeleton to the ECM. This location means that these molecules can actively participate in transmitting mechanical signals into the cell, which then evokes cellular behavior such as fibroblast proliferation, myofibroblast differentiation, and collagen contraction that characterize FPDs.

The transmembrane integrins are composed of α and β subunits that make up at least 24 heterodimers. The main integrins that actively participate in fibroblast proliferation [5], collagen contraction [6], and myofibroblast differentiation [7] include α1β1, α2β1, and α11β1. α2β1 is the key integrin that is involved in the ability of a fibroblast to bind and contract [6] three-dimensional collagen lattices [8] during wound remodeling [9] and to mediate the induction of collagenase [10]. The α2β1 integrin can also affect the mechanotransduction of both slowly and rapidly adapting cutaneous mechanoreceptors in hairy rat skin, thus modulating the cutaneous response to compressive indentation [11]. Moreover, the α1β1 integrin is believed to be specifically involved in the flow-induced differentiation of fibroblasts but not required for their ECM adhesion or contraction since α1β1 integrin-blocking antibodies completely inhibit the flow-induced expression of α-smooth muscle actin (α-SMA) but do not interfere with the fibroblast-mediated contraction of a gel [12]. In addition, the lack of α1β1 integrin predisposes mice to excessive glomerulosclerosis after nonimmunologically induced renal injury. Such a response is mediated by both the loss of direct interactions of α1β1 with its ligand collagen IV and the increased production of reactive oxygen species [13]. Finally, α11β1 integrin, which is also a collagen receptor on fibroblasts, can be induced in a mechanosensitive manner and regulates myofibroblast differentiation [7]. Other integrins, such as αvβ5, were also found to participate in myofibroblast differentiation [14].

The ECM is increasingly being seen as a dynamic, mobile, and multifunctional regulator of cell behavior rather than merely a scaffold for cells and a storehouse for cytokines [15]. This perception is reflected in two integrin-related models of the active cell–ECM interactions that explain the force equilibrium in normal healthy tissue. The first of these models is the tensegrity model. Tensegrity is the tensional integrity that is used by cells to stabilize their form under mechanical stimuli. It depends on the architecture of the cytoskeleton and the level of its prestress, and it allows the cell to respond to mechanical stimuli that are sensed by the

Connect Tissue Res Downloaded from informahealthcare.com by National Library of Medicine on 07/10/12
For personal use only.

RIGHTSLINK

clustered integrins and actin-associated molecules that physically interconnect the ECM to intracellular actin microfilaments at focal adhesion complexes [16,17], thus maintaining the shape stability even if they undergo continuous dynamic remodeling at the molecular level [18]. The second model, the tensional homeostasis model, postulates that the cellular forces generated after the sensation of mechanical stimuli by integrins can modify the degree of matrix tension, leading to the reorganization of the matrix. This could lead to matrix stiffening and thereby induce tissue rigidity, such as that seen in fibrosis. Thus, matrix stiffness can be linked to integrin-mediated cytoskeletal tension [19]. In line with these models, it has been shown that the tractional forces generated by fibroblasts during their migration on a compliant collagen substratum can reorganize collagen matrices [20]. And the loss of such homeostasis or equilibrium could promote the progression of tissue fibrosis [21].

Integrins also actively crosstalk with various signaling pathways. The activation of integrins and ECM binding result in the early activation of phosphoinositol 3-OH-kinase (PI3K), which stimulates a cytoplasmic signaling pathway. Moreover, the mechanical stretch stimulation of c-Jun NH_2-terminal kinase (JNK) is dependent on new integrin binding to the ECM [22]. In addition, the activation of the Ras-extracellular signal-related kinase MAPK (Ras-ERK MAPK) cascade is dependent on Shc and focal adhesion kinase (FAK) since Shc is responsible for the initial high-level activation of ERK that occurs after cell adhesion, and the more slowly activated FAK appears to sustain the activation of ERK [23]. Furthermore, interactions between Thy-1 and αvβ5 integrin were found to inhibit the contraction-induced activation of latent TGF-β1 in lung fibroblasts; they also inhibited the TGF-β1-dependent differentiation of lung fibroblasts into lung myofibroblasts [14]. In addition, when gingival fibroblasts were cultured on collagen-coated plates and treated with TGF-β1, their α2 and β1 integrin content increased by 50%, whereas these integrin subunits were undetectable in TGF-β-treated gingival fibroblasts that were cultured on the more compliant floating gels [24]. Finally, there is now a growing recognition of the role epithelial–mesenchymal transition (EMT) plays in FPDs since it has been shown that epithelial cells can undergo phenotypic conversion and give rise to the matrix-producing fibroblasts and myofibroblasts that participate in fibrogenesis. It has been shown that EMT transcription is activated by β-catenin activation, which is induced by a convergence of TGF-β, integrin-linked kinase, and Wnt signals. These insights are nicely illustrated by the review of Liu [25].

MECHANOSIGNALING PATHWAYS RELATED TO THE FIBROPROLIFERATIVE REACTION

To date, research on FPDs has focused primarily on several imbalances. Some are imbalances in the cellular interactions between fibroblasts and other cell types, which include endothelial cells in idiopathic pulmonary fibrosis [26] and keratinocytes in hypertrophic scarring [27]. Other imbalances relate to the pro- and anti-cytokines involved in fibrinolysis, inflammation and angiogenesis, and the accumulation/degradation of ECM. Studies on the mechanosignaling pathways that relate to these imbalances have concentrated on the TGF-β/Smad, MAPK, RhoA/ROCK, Wnt/β-catenin, and TNF-α/NF-κB pathways (Figure 1).

TGF-β/Smad Signaling

TGF-β is a multifunctional growth factor that plays a critical role as a potent modulator of connective tissue remodeling and ECM gene expression [28]. To date, three mammalian isoforms, TGF-β1, TGF-β2, and TGF-β3, have been identified. TGF-β signals through a heteromeric receptor complex of type I and II receptor serine/threonine kinases [29] and propagates its signal by using Smads [30]. The Smad family includes three classes, namely receptor-regulated Smads, the single common-mediator Smad (Smad 4), and inhibitory Smads [31]. Smad2 and Smad3 are receptor-regulated Smads and TGF-β receptor I substrates [30]. After the Smad anchor for receptor activation protein facilitates the recruitment of Smad2 and Smad3 to activated receptors, Smad2 and Smad3 associate as heterodimeric complexes with Smad4. The complexes are then transported into the nucleus, where they bind to DNA and initiate target gene transactivation [30,32].

TGF-β plays a significant role in the mechanotransduction processes that occur during fibroproliferation. Extensive evidence shows that subjecting a variety of cells from diverse origins to mechanical stimuli induces TGF-β mRNA expression and TGF-β protein activation, synthesis, and secretion. For example, the latent TGF-β1 in the ECM is directly activated by the external stretching of rat lung myofibroblast cultures as well as by increased myofibroblast intracellular tension. Moreover, corresponding in vivo data reveal that stressed wound granulation tissues exhibit significantly higher Smad2/3 activation than relaxed tissues; this is indicative of enhanced TGF-β1 signaling [33]. Similarly, subjecting cultured rat renal fibroblasts to cyclic tensile stretching for 48 hr increases their expression of TGF-β and ECM components; this expression can be attenuated by a COX-2 inhibitor [34]. Moreover, cultured human tendon fibroblasts increase their secretion of TGF-β1 when they undergo cyclic biaxial mechanical stretching [35]. In addition, rat glomerular mesangial cells exposed to repeated stretching/relaxation cycles show increased expression of TGF-β1 mRNA [36]. Mechanical stimuli other than stretching have similar effects. When keloid-derived fibroblasts are subjected to equibiaxial strain, they exhibit more TGF-β1 and TGF-β2 transcription than normal skin-derived fibroblasts; this trend correlates with their increased production of TGF-β1 and TGF-β2 protein. However, this differential

Connect Tissue Res Downloaded from informahealthcare.com by National Library of Medicine on 07/10/12
For personal use only.

© 2012 Informa Healthcare USA, Inc.

RIGHTSLINK

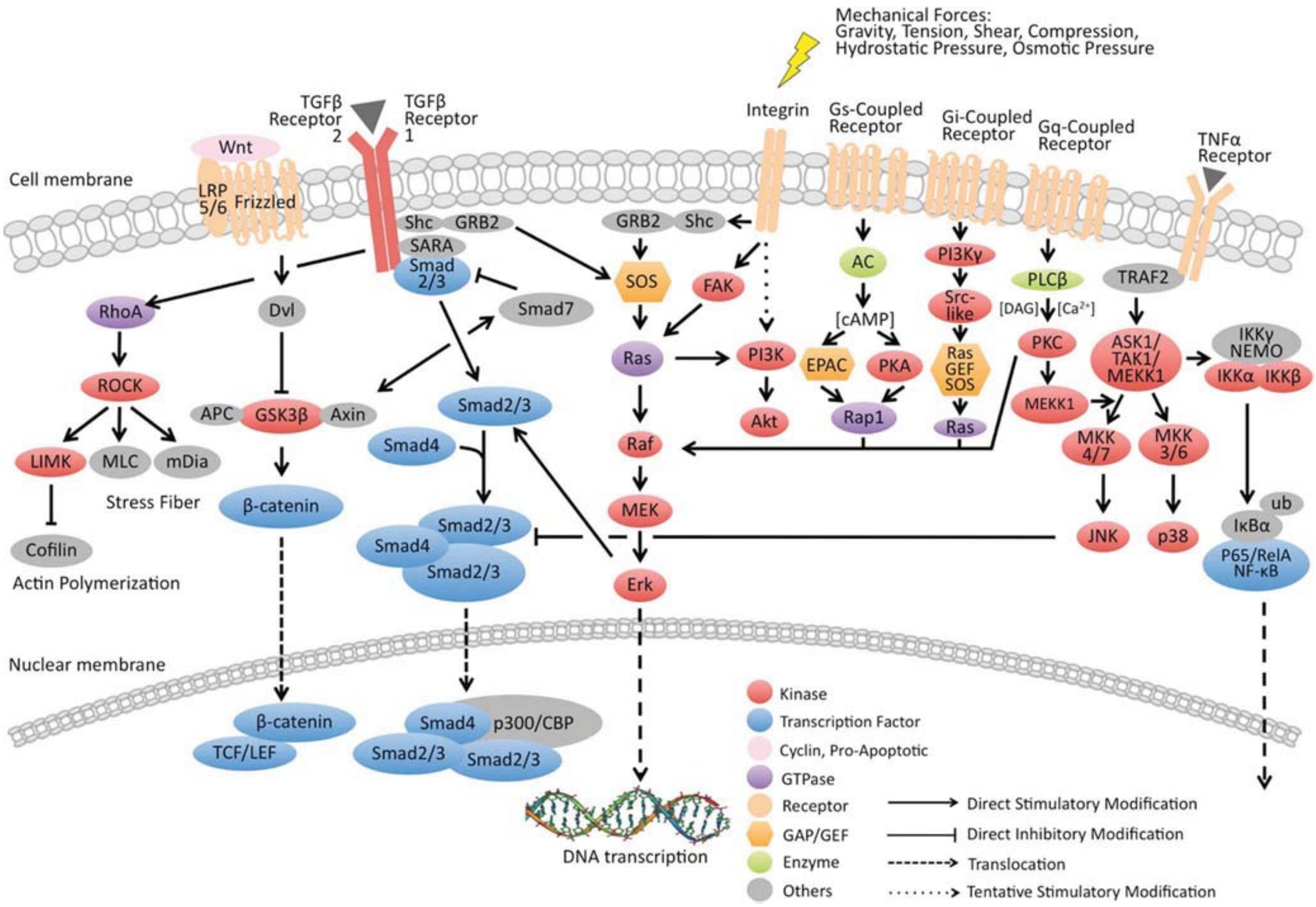

Figure 1. The FPD-related mechanosignaling of the TGF-β/Smad, MAPK, RhoA/ROCK, Wnt/β-catenin, and TNF-α/NF-κB pathways.

transcriptional response to mechanical strain could not be seen with regard to the TGF-β3 isoform. Moreover, the mechanoresponsive increase in TGF-β1 and TGF-β2 transcription can be blocked by an ERK inhibitor, which is an important molecule in the TGF-β signaling cascade [37]. Interestingly, TGF-β1 mRNA expression is even upregulated when murine fibroblasts cultured on a three-dimensional fibrin matrix are subjected to microdeformations produced by 125 mmHg suction [38]. Thus, the different mesenchymal cells that participate in FPD all exhibit active mechanoresponsiveness of TGF-β signaling molecules to various mechanical stimuli. This indicates the importance of the TGF-β signaling pathways in fibrosis-related mechanobiology.

TGF-β has recently attracted increasing interest because of its demonstrated ability to stimulate EMT, which may underlie the formation or progression of FPDs. Supporting this hypothesis is that the stimulation of human bronchial epithelial cells by TGF-β results in their transdifferentiation into myofibroblasts, the de novo expression of α-SMA, the increased formation of stress fibers, the loss of the epithelial marker E-cadherin, and increased collagen I expression [39]. Microarray analysis has also shown that Smad signaling downstream of TGF-β plays a critical role in the regulation of EMT target genes [40]. In fact, TGF-β is believed to regulate EMT progression via RhoA-dependent pathways. Supporting this is that it rapidly activates RhoA-dependent signaling pathways, which induce stress fiber formation and mesenchymal characteristics; moreover, the blockade of RhoA or its downstream target p160 ROCK inhibits TGF-β-mediated EMT [41].

TGF-β signaling also engages in crosstalk actively with other pathways. Connective tissue growth factor (CTGF) is commonly seen as a cofactor or a downstream mediator of TGF-β in enhancing fibrosis [42]. Mechanical tension can increase CTGF expression and gingival fibroblast proliferation via a TGF-β-dependent mechanism [43]. In fact, CTGF is required for the maintenance of skin fibrosis after the induction of fibrosis by TGF-β [44]. Besides, TGF-β signaling acts in a combinatorial manner with Wnt/β-catenin signaling, with Smad4 serving as a common mediator of the two pathways [45]. This crosstalk participates in the pathogenesis of hypertrophic scars and keloids [46]. Moreover, TGF-β can activate the ERK, JNK, and p38 MAPK pathways [47]. It has also been shown that α2β1 integrin plays a central role in TGF-β1-induced regulation of α-SMA and the maintenance of intracellular tension [24]. In addition, it has been suggested that TGF-β may also serve as a mechanoregulatory growth

Connect Tissue Res Downloaded from informahealthcare.com by National Library of Medicine on 07/10/12
For personal use only.

RIGHTSLINK

factor and that the alteration in cellular stress caused by this activity induces integrin expression [48].

MAPK Signaling

There are three main subgroups of MAPK pathways, namely those that involve ERK1/2, JNK, or p38 proteins. A typical signaling cascade involves the activation of an upstream mitogen-activated protein kinase kinase kinase (MAP3K) followed by MAPK cascades that are characterized by the sequential phosphorylation of the constituent MAP kinase kinase (MAP2K) and the MAPK [49].

MAPK signaling plays an important role in mechanobiology and can respond to various mechanical stimuli. For example, ERK1/2 in osteoblasts is phosphorylated by gravitational stress in a load dose-dependent manner, with maximum phosphorylation saturating at the mechanical loading level of 12*g* [50]. Moreover, bovine endothelial cells subjected to shear stress exhibit ERK, JNK [51], and p38MAPK [52] activation. Similarly, cyclic strain on pulmonary endothelial cells rapidly, albeit transiently, activates ERK, JNK, and p38MAPK, with their activation peaking 30 min after exposure to cyclic strain [53]. Cyclic stretching of rat fibroblasts induces both cell proliferation and MAPK activation, and the cell proliferation can be inhibited by MAPK inhibitors [54]. It has also been shown that the JNK, p38, and ERK phosphorylation and actin reorganization that are induced by cyclic stretching are driven by perturbations in cytoskeletal tension and are not mediated by FAK [55]. Similarly, the ERK1/2, JNK1/2/3, and p38 MAPKs in human lung fibroblasts can all be robustly activated by tonic stretching [56]. Moreover, p38 MAPK and ERK1/2 in dermal fibroblasts are significantly activated by high-frequency repetitive stretching but not by intermittent stretching; the frequency of the stretching also affects the signal activation profile [57]. In addition, the mechanical compression of bovine articular cartilage ex vivo stimulates the phosphorylation of ERK1/2, p38 MAPK, and stress-activated protein kinase/ERK kinase-1 (SEK-1) of the JNK pathway. The ERK phosphorylation exhibited a phased pattern where ERK1/2 phosphorylation was rapidly induced and peaked at 10 min, after which it quickly decayed to a level that was sustained for 24 hr. The phosphorylation of p38 MAPK was transient, with a peak being observed at 10 min. The SEK-1 phosphorylation was strongest, peaking at 1 hr [58].

G proteins are closely interlinked with MAPK signaling. For example, Gs-cAMP-PKA-mediated activation of Rap-1 is involved in the inhibition of the C-Raf-containing ERK molecule in fibroblast cells [59,60]. Moreover, Gi can stimulate ERK1/2 signaling in a Ras-dependent manner that involves its $\beta\gamma$ subunits [61]. In addition, Gq can stimulate ERK1/2 through PKC-Raf signaling [59,62] and can signal to JNK via MAPK/ERK kinase kinase-1 [63]. Furthermore, the synchronous activation of the ERK pathway and PI3K is essential for the increased collagen type I and III production of keloid fibroblasts [64].

RhoA/ROCK Signaling

The Rho GTPase family has been divided into eight subfamilies. Of these, Rho, Rac, and Cdc42 are of primary importance. The Rho subfamily comprises three members, namely RhoA, RhoB, and RhoC. Rho GTPases regulate the organization of the actin cytoskeleton by promoting the assembly of focal adhesions and actin stress fibers [65]. RhoA is the best characterized Rho GTPase and is associated with FPDs. ROCK is a direct effector of RhoA [66]. The two isoforms of ROCK, ROCKI and ROCKII, are collectively referred to as Rho kinases. The many downstream targets of ROCK include versatile cytoskeleton-related proteins such as LIM kinase (LIMK) [67] and myosin light chain [68].

The RhoA/ROCK pathway plays an important role in those FPDs that share the common features of connective tissue accumulation and slow but continuous contraction. In hepatic fibrosis, hepatic stellate cells are the target treatment and it has been shown that they are affected by Rho kinase through various pathways, including ERK1/2, JNK, and p38 MAPKs [69]. Y-27632, a Rho kinase inhibitor, suppresses hepatic fibrosis at least in part by preventing the activation of hepatic stellate cells [70]. Y-27632 can also ameliorate tubulointerstitial fibrosis in mouse kidneys that have unilateral ureteral obstruction [71]. Similarly, fasudil, another Rho-kinase inhibitor, can attenuate interstitial fibrosis in the rat kidney [72]. Moreover, Rho plays a critical role in TGF-β1-induced cytoskeletal remodeling and α-SMA synthesis during EMT [73]. A complete and rapid reversal of EMT morphology and patterns of gene expression can be achieved within 24 hr by the combined inhibition of TGF-βRI and ROCK, with the inhibition of the former blocking mesenchymal gene expression and the inhibition of the latter stabilizing the epithelial structure [74].

Significantly, the RhoA-ROCK pathway can be activated directly by mechanical stress. Shear stress on bovine aortic endothelial cells was found to induce sequential signaling through the Rho-ROCK-LIMK-cofilin pathway [67]. Moreover, static tensile forces applied to rat cardiac fibroblasts induce actin assembly through the Rho-ROCK-LIMK-cofilin pathway. They also enhance SMA expression via megakaryocytic acute leukemia (MAL); this process marks the transition of cardiac fibroblasts to myofibroblasts in response to mechanical loading [75]. MAL [76], also known as myocardin-related transcription factor A (MRTFA) [77], megakaryoblastic leukemia 1 (MKL1) [78], or basic, SAP, and coiled-coil domain (BSAC) [79], is a serum response factor coactivator and can move from the cytoplasm to the nucleus in response to Rho-induced actin polymerization [80]. Thus, it links RhoA activation and actin dynamics to transcriptional

Connect Tissue Res Downloaded from informahealthcare.com by National Library of Medicine on 07/10/12
For personal use only.

© 2012 Informa Healthcare USA, Inc.

RIGHTSLINK

activation in the nucleus. The tenascin-C gene can also be triggered by mechanical stimuli via RhoA-dependent actin dynamics that result in the nuclear accumulation of MAL. For example, when mouse embryo fibroblasts are exposed to equibiaxial cyclic strain, tenascin-C mRNA is induced in this fashion [81]. Moreover, the tenascin-C mRNA expression in chick skin fibroblasts that is caused by cyclic strain is strongly attenuated when ROCK is inhibited [82].

Rho/ROCK signaling can also be in active communication with ECM-related molecules. For example, the maintenance of radiation-induced fibrogenic differentiation in intestinal mesenchymal cells requires Rho/ROCK/CTGF activation [83]. In intestinal radiation-induced fibrosis, blockade of p160 ROCK alters the actin network and decreases the constitutive expression of CTGF, most probably through the inhibition of NF-κB [84]. Moreover, RhoA-activating drugs in combination with cyclic strain causes a superinduction of tenascin-C mRNA, which is suppressed by ROCK inhibition [85].

TNF-α/NF-κB Signaling

TNF-α is a homotrimer composed of three 157 amino acid subunits and is a potent mediator of immunity and inflammation. It also participates in the control of cell proliferation, differentiation, and apoptosis [86] by interacting with two distinct cell surface receptors, TNFR1 and TNFR2 [87]. The binding of TNF to its receptors (especially TNFR1) triggers receptor trimerization and ultimately results in the activation of NF-κB, JNK, and p38 MAPK.

When hypertrophic scars are subjected in vitro to mechanical compression that resembles the clinical use of elastocompression, TNF-α secretion is diminished; in contrast, the compression does not alter the TNF-α release of normal scars [88]. Cyclic stretching of human lung fibroblasts also activates NF-κB via stretch-activated channels that induce the influx of Ca^{2+} [89,90]. Nevertheless, more convincing evidence showing that TNF and its subsequent signaling molecules respond directly to mechanical stimulation is needed.

The TNF-α/NF-κB signaling pathway also extensively crosstalks with other pathways. TNF-α can suppress TGF-β1-induced α-SMA expression; this suppression is mediated by the JNK signaling pathway, as shown by the fact that a JNK inhibitor, but not a p38 MAPK inhibitor, reversed the suppression. The TNF-α-mediated suppression of α-SMA expression is thought to occur via the inhibition of Smad3 phosphorylation [91]. Notably, TNF-α also enhances both the EMT and cell contraction of human alveolar epithelial cells induced by TGF-β1 [92]. Moreover, in human skin wound healing, TNF-α induces EMT via BMP-2 [93].

Wnt/β-Catenin Signaling

The Wnt/β-catenin pathway directly connects extracellular signals, gene transcription, and cell-cycle controls [94]. Canonical Wnt signals are transduced through Frizzled family receptors [95] and the low-density lipoprotein receptor-related proteins 5 and 6 (LRP5/LRP6) of the transmembrane proteins [96].

Wnt signaling through its effector β-catenin is thought to play essential roles in FPDs. It participates in the genesis or progression of FPDs, at the least as its targets and effectors. When Dupuytren's disease fibroblasts in three-dimensional collagen cultures are subjected to isometric tension, their β-catenin levels increase, whereas when isometric tension is reduced, their β-catenin levels decrease dramatically [97]. Similarly, fluid shear causes C57BL/6J osteoblasts to upregulate key Wnt signaling genes such as *Wnt1*, *Wnt3a*, *Lrp5*, *Axin*, and *LEF1*. Moreover, the fluid shear-induced upregulation of β-catenin mRNA and protein levels could be blocked completely by endostatin, a potent inhibitor of the canonical Wnt pathway [98]. Besides, mechanical stretching of the alveolar type II epithelial cells not only induces EMT but also significantly increases the expressions of wisp-1 (a transducer of the canonical Wnt pathway) and other Wnt-related mRNAs. The blockade of wisp-1 prevents the stretched cells from undergoing EMT [99]. However, although this active secondary participation of Wnt signaling in response to mechanics during fibrosis is too obvious to be ignored, direct evidence demonstrating its immediate activation by mechanical stimulation remains to be further illustrated.

FUTURE DIRECTIONS IN FPD RESEARCH

Although mechanobiological research has revealed a number of promising signaling pathways that participate in benign and malignant FPDs, much remains to be elucidated. First, the fact that all FPDs exhibit the excessive accumulation of ECM that then undergoes slow but continuous contraction indicates that time plays a key role in the mechanics-related progression of FPDs. This leads to the following questions: what are the key events that cause a normal wound-healing process to become aberrant (eventually resulting in fibrosis) and when do these events occur? Moreover, what orchestration of the signaling pathways causes them to maintain the activation needed to sustain the contraction of the ECM?

Second, depending on the FPD and the tissue/organ involved, the progression of fibrosis is sometimes, but not always, invasive. This leads to another question: in the case of noninvasive FPDs, which guiding signals confine the stiff connective tissue within a stiff-compliant boundary? For example, what causes a scar to remain within the dermis rather than breaking into adipose tissue, which shares a common mesenchymal origin with fibroblasts? An additional question is can mechanical signaling contribute to the different invasive properties of an FPD and an essential tumor?

Connect Tissue Res Downloaded from informahealthcare.com by National Library of Medicine on 07/10/12
For personal use only.

RIGHTSLINK

Third, mechanosignaling pathways not only crosstalk extensively between themselves but also crosstalk with pathways that occur in inflammation, angiogenesis, proliferation, and hypoxia. Can the activation of such crosstalking in pivotal locations be responsible for modulating fibrotic events in target tissues? For example, tension applied to integrins can activate the Rho-ROCK/mDia pathway [100], and the balance between the activities of mDia and ROCK can regulate the Skp2-p27 pathway and the following G1/S transition [101]. Given these observations, is it possible that external mechanics could regulate the transcription of proliferative genes?

In summary, the mechanosignaling pathways in FPDs include the TGF-β/Smad, MAPK, RhoA/ROCK, Wnt/β-catenin, and TNF-α/NF-κB signaling pathways. These intracellular signaling pathways, together with integrins and cell–ECM interactions, mediate the active responses of FPD-associated cells to mechanical stimuli. Further research into these fibrosis-related mechanosignaling pathways is required and is likely to reveal promising molecular markers that can be used for specialized and targeted pharmacological or clinical treatments.

In FPDs, intracellular mechanosignaling is activated by various mechanical stimuli such as stretching, tensile strain, compression, hydrostatic pressure, suction, and gravity. The signaling pathways include the TGF-β/Smad, MAPK, RhoA/ROCK, Wnt/β-catenin, and TNF-α/NF-κB pathways. Most of the research examining the role these pathways play in cellular responses to mechanical stimuli has focused on fibroblasts and myofibroblasts. This reflects the fact that these are the main and commonly shared cellular ingredients of the different FPDs, which are characterized by the accumulation of mesenchymal cells and their connective tissue products. The different pathways crosstalk extensively. However, at the center of these interactions lie the integrins, which are the mechanoreceptors that span the cell membrane and thereby connect the cytoskeleton to the ECM. Integrins interact with nearly every signaling pathway and actively participate in cell–ECM interactions.

***Declaration of interest*:** The authors report no conflicts of interest. The authors alone are responsible for the contents and writing of the article.

REFERENCES

[1] Hertz, M.I., Henke, C.A., Nakhleh, R.E., Harmon, K.R., Marinelli, W.A., Fox, J.M., Kubo, S.H., Shumway, S.J., Bolman, R.M., III, and Bitterman, P.B. (1992). Obliterative bronchiolitis after lung transplantation: A fibroproliferative disorder associated with platelet-derived growth factor. *Proc. Natl. Acad. Sci. U.S.A.* 89:10385–10389.

[2] Wynn, T.A. (2008). Cellular and molecular mechanisms of fibrosis. *J. Pathol.* 214:199–210.

[3] Ingber, D.E. (2008). Tensegrity-based mechanosensing from macro to micro. *Prog. Biophys. Mol. Biol.* 97:163–179.

[4] Ingber, D.E. (2003). Mechanobiology and diseases of mechanotransduction. *Ann. Med.* 35:564–577.

[5] Pozzi, A., Wary, K.K., Giancotti, F.G., and Gardner, H.A. (1998). Integrin alpha1beta1 mediates a unique collagen-dependent proliferation pathway in vivo. *J. Cell Biol.* 142: 587–594.

[6] Schiro, J.A., Chan, B.M., Roswit, W.T., Kassner, P.D., Pentland, A.P., Hemler, M.E., Eisen, A.Z., and Kupper, T.S. (1991). Integrin alpha 2 beta 1 (VLA-2) mediates reorganization and contraction of collagen matrices by human cells. *Cell* 67:403–410.

[7] Carracedo, S., Lu, N., Popova, S.N., Jonsson, R., Eckes, B., and Gullberg, D. (2010). The fibroblast integrin alpha11beta1 is induced in a mechanosensitive manner involving activin A and regulates myofibroblast differentiation. *J. Biol. Chem.* 285:10434–10445.

[8] Klein, C.E., Dressel, D., Steinmayer, T., Mauch, C., Eckes, B., Krieg, T., Bankert, R.B., and Weber, L. (1991). Integrin alpha 2 beta 1 is upregulated in fibroblasts and highly aggressive melanoma cells in three-dimensional collagen lattices and mediates the reorganization of collagen I fibrils. *J. Cell Biol.* 115:1427–1436.

[9] Noszczyk, B.H., Klein, E., Holtkoetter, O., Krieg, T., and Majewski, S. (2002). Integrin expression in the dermis during scar formation in humans. *Exp. Dermatol.* 11:311–318.

[10] Langholz, O., Röckel, D., Mauch, C., Kozlowska, E., Bank, I., Krieg, T., and Eckes, B. (1995). Collagen and collagenase gene expression in three-dimensional collagen lattices are differentially regulated by alpha 1 beta 1 and alpha 2 beta 1 integrins. *J. Cell Biol.* 131:1903–1915.

[11] Khalsa, P.S., Ge, W., Uddin, M.Z., and Hadjiargyrou, M. (2004). Integrin alpha2beta1 affects mechano-transduction in slowly and rapidly adapting cutaneous mechanoreceptors in rat hairy skin. *Neuroscience* 129:447–459.

[12] Ng, C.P., Hinz, B., and Swartz, M.A. (2005). Interstitial fluid flow induces myofibroblast differentiation and collagen alignment in vitro. *J. Cell. Sci.* 118:4731–4739.

[13] Chen, X., Moeckel, G., Morrow, J.D., Cosgrove, D., Harris, R.C., Fogo, A.B., Zent, R., and Pozzi, A. (2004). Lack of integrin alpha1beta1 leads to severe glomerulosclerosis after glomerular injury. *Am. J. Pathol.* 165:617–630.

[14] Zhou, Y., Hagood, J.S., Lu, B., Merryman, W.D., and Murphy-Ullrich, J.E. (2010). Thy-1-integrin alphav beta5 interactions inhibit lung fibroblast contraction-induced latent transforming growth factor-beta1 activation and myofibroblast differentiation. *J. Biol. Chem.* 285:22382–22393.

[15] Aszódi, A., Legate, K.R., Nakchbandi, I., and Fässler, R. (2006). What mouse mutants teach us about extracellular matrix function. *Annu. Rev. Cell Dev. Biol.* 22:591–621.

[16] Ingber, D.E. (1997). Tensegrity: The architectural basis of cellular mechanotransduction. *Annu. Rev. Physiol.* 59:575–599.

[17] Ingber, D.E. (2003). Tensegrity II. How structural networks influence cellular information processing networks. *J. Cell Sci.* 116:1397–1408.

[18] Wang, N., Tytell, J.D., and Ingber, D.E. (2009). Mechanotransduction at a distance: Mechanically coupling the extracellular matrix with the nucleus. *Nat. Rev. Mol. Cell Biol.* 10:75–82.

[19] Paszek, M.J., Zahir, N., Johnson, K.R., Lakins, J.N., Rozenberg, G.I., Gefen, A., Reinhart-King, C.A., Margulies, S.S., Dembo, M., Boettiger, D., Hammer, D.A., and Weaver, V.M. (2005). Tensional homeostasis and the malignant phenotype. *Cancer Cell* 8:241–254.

[20] Harris, A.K., Stopak, D., and Wild, P. (1981). Fibroblast traction as a mechanism for collagen morphogenesis. *Nature* 290:249–251.

© 2012 Informa Healthcare USA, Inc.

Connect Tissue Res Downloaded from informahealthcare.com by National Library of Medicine on 07/10/12
For personal use only.

RIGHTSLINK

[21] Butcher, D.T., Alliston, T., and Weaver, V.M. (2009). A tense situation: Forcing tumour progression. *Nat. Rev. Cancer* 9:108–122.

[22] Katsumi, A., Naoe, T., Matsushita, T., Kaibuchi, K., and Schwartz, M.A. (2005). Integrin activation and matrix binding mediate cellular responses to mechanical stretch. *J. Biol. Chem.* 280:16546–16549.

[23] Giancotti, F.G., and Ruoslahti, E. (1999). Integrin signaling. *Science* 285:1028–1032.

[24] Arora, P.D., Narani, N., and McCulloch, C.A. (1999). The compliance of collagen gels regulates transforming growth factor-beta induction of alpha-smooth muscle actin in fibroblasts. *Am. J. Pathol.* 154:871–882.

[25] Liu, Y. (2010). New insights into epithelial-mesenchymal transition in kidney fibrosis. *J. Am. Soc. Nephrol.* 21:212–222.

[26] Selman, M., and Pardo, A. (2002). Idiopathic pulmonary fibrosis: An epithelial/fibroblastic cross-talk disorder. *Respir. Res.* 3:3.

[27] Ghahary, A., and Ghaffari, A. (2007). Role of keratinocyte-fibroblast cross-talk in development of hypertrophic scar. *Wound Repair Regen.* 15:S46–S53.

[28] Quaglino, D., Jr, Nanney, L.B., Ditesheim, J.A., and Davidson, J.M. (1991). Transforming growth factor-beta stimulates wound healing and modulates extracellular matrix gene expression in pig skin: Incisional wound model. *J. Invest. Dermatol.* 97:34–42.

[29] Massagué, J., Attisano, L., and Wrana, J.L. (1994). The TGF-beta family and its composite receptors. *Trends Cell Biol.* 4:172–178.

[30] Massagué, J., and Wotton, D. (2000). Transcriptional control by the TGF-beta/Smad signaling system. *EMBO J.* 19: 1745–1754.

[31] Goumans, M.J., and Mummery, C. (2000). Functional analysis of the TGFbeta receptor/Smad pathway through gene ablation in mice. *Int. J. Dev. Biol.* 44:253–265.

[32] Tsukazaki, T., Chiang, T.A., Davison, A.F., Attisano, L., and Wrana, J.L. (1998). SARA, a FYVE domain protein that recruits Smad2 to the TGFbeta receptor. *Cell* 95:779–791.

[33] Wipff, P.J., Rifkin, D.B., Meister, J.J., and Hinz, B. (2007). Myofibroblast contraction activates latent TGF-beta1 from the extracellular matrix. *J. Cell Biol.* 179:1311–1323.

[34] El Chaar, M., Attia, E., Chen, J., Hannafin, J., Poppas, D.P., and Felsen, D. (2005). Cyclooxygenase-2 inhibitor decreases extracellular matrix synthesis in stretched renal fibroblasts. *Nephron. Exp. Nephrol.* 100:e150–e155.

[35] Skutek, M., van Griensven, M., Zeichen, J., Brauer, N., and Bosch, U. (2001). Cyclic mechanical stretching modulates secretion pattern of growth factors in human tendon fibroblasts. *Eur. J. Appl. Physiol.* 86:48–52.

[36] Yasuda, T., Kondo, S., Homma, T., and Harris, R.C. (1996). Regulation of extracellular matrix by mechanical stress in rat glomerular mesangial cells. *J. Clin. Invest.* 98:1991–2000.

[37] Wang, Z., Fong, K.D., Phan, T.T., Lim, I.J., Longaker, M.T., and Yang, G.P. (2006). Increased transcriptional response to mechanical strain in keloid fibroblasts due to increased focal adhesion complex formation. *J. Cell. Physiol.* 206:510–517.

[38] Lu, F., Ogawa, R., Nguyen, D.T., Chen, B., Guo, D., Helm, D.L., Zhan, Q., Murphy, G.F., and Orgill, D.P. (2011). Microdeformation of three-dimensional cultured fibroblasts induces gene expression and morphological changes. *Ann. Plast. Surg.* 66:296–300.

[39] Zhang, M., Zhang, Z., Pan, H.Y., Wang, D.X., Deng, Z.T., and Ye, X.L. (2009). TGF-beta1 induces human bronchial epithelial cell-to-mesenchymal transition in vitro. *Lung* 187:187–194.

[40] Valcourt, U., Kowanetz, M., Niimi, H., Heldin, C.H., and Moustakas, A. (2005). TGF-beta and the Smad signaling pathway support transcriptomic reprogramming during epithelial-mesenchymal cell transition. *Mol. Biol. Cell* 16:1987–2002.

[41] Bhowmick, N.A., Ghiassi, M., Bakin, A., Aakre, M., Lundquist, C.A., Engel, M.E., Arteaga, C.L., and Moses, H.L. (2001). Transforming growth factor-beta1 mediates epithelial to mesenchymal transdifferentiation through a RhoA-dependent mechanism. *Mol. Biol. Cell* 12(1):27–36.

[42] Grotendorst, G.R. (1997). Connective tissue growth factor: A mediator of TGF-beta action on fibroblasts. *Cytokine Growth Factor Rev.* 8:171–179.

[43] Guo, F., Carter, D.E., and Leask, A. (2011). Mechanical tension increases CCN2/CTGF expression and proliferation in gingival fibroblasts via a TGFβ-dependent mechanism. *PLoS One* 6:e19756.

[44] Mori, T., Kawara, S., Shinozaki, M., Hayashi, N., Kakinuma, T., Igarashi, A., Takigawa, M., Nakanishi, T., and Takehara, K. (1999). Role and interaction of connective tissue growth factor with transforming growth factor-beta in persistent fibrosis: A mouse fibrosis model. *J. Cell. Physiol.* 181:153–159.

[45] Letamendia, A., Labbé, E., and Attisano, L. (2001). Transcriptional regulation by Smads: Crosstalk between the TGF-beta and Wnt pathways. *J. Bone Joint Surg. Am.* 83:S31–S39.

[46] Sato, M. (2006). Upregulation of the Wnt/beta-catenin pathway induced by transforming growth factor-beta in hypertrophic scars and keloids. *Acta Derm. Venereol.* 86:300–307.

[47] Derynck, R., and Zhang, Y.E. (2003). Smad-dependent and Smad-independent pathways in TGF-beta family signalling. *Nature* 425:577–584.

[48] Brown, R.A., Sethi, K.K., Gwanmesia, I., Raemdonck, D., Eastwood, M., and Mudera, V. (2002). Enhanced fibroblast contraction of 3D collagen lattices and integrin expression by TGF-beta1 and -beta3: Mechanoregulatory growth factors? *Exp. Cell Res.* 274:310–322.

[49] Chang, L., and Karin, M. (2001). Mammalian MAP kinase signalling cascades. *Nature* 410:37–40.

[50] Hatton, J.P., Pooran, M., Li, C.F., Luzzio, C., and Hughes-Fulford, M. (2003). A short pulse of mechanical force induces gene expression and growth in MC3T3-E1 osteoblasts via an ERK 1/2 pathway. *J. Bone Miner. Res.* 18:58–66.

[51] Li, S., Kim, M., Hu, Y.L., Jalali, S., Schlaepfer, D.D., Hunter, T., Chien, S., and Shyy, J.Y. (1997). Fluid shear stress activation of focal adhesion kinase. Linking to mitogen-activated protein kinases. *J. Biol. Chem.* 272:30455–30462.

[52] Azuma, N., Akasaka, N., Kito, H., Ikeda, M., Gahtan, V., Sasajima, T., and Sumpio, B.E. (2001). Role of p38 MAP kinase in endothelial cell alignment induced by fluid shear stress. *Am. J. Physiol. Heart Circ. Physiol.* 280:H189–H197.

[53] Kito, H., Chen, E.L., Wang, X., Ikeda, M., Azuma, N., Nakajima, N., Gahtan, V., and Sumpio, B.E. (2000). Role of mitogen-activated protein kinases in pulmonary endothelial cells exposed to cyclic strain. *J. Appl. Physiol.* 89:2391–2400.

[54] Wang, J.G., Miyazu, M., Xiang, P., Li, S.N., Sokabe, M., and Naruse, K. (2005). Stretch-induced cell proliferation is mediated by FAK-MAPK pathway. *Life Sci.* 76:2817–2825.

[55] Hsu, H.J., Lee, C.F., Locke, A., Vanderzyl, S.Q., and Kaunas, R. (2010). Stretch-induced stress fiber remodeling and the activations of JNK and ERK depend on mechanical strain rate, but not FAK. *PLoS One* 5:e12470.

[56] Boudreault, F., and Tschumperlin, D.J. (2010). Stretch-induced mitogen-activated protein kinase activation in lung fibroblasts is independent of receptor tyrosine kinases. *Am. J. Respir. Cell Mol. Biol.* 43:64–73.

[57] Nishimura, K., Blume, P., Ohgi, S., and Sumpio, B.E. (2007). Effect of different frequencies of tensile strain on human dermal fibroblast proliferation and survival. *Wound Repair Regen.* 15:646–656.

[58] Fanning, P.J., Emkey, G., Smith, R.J., Grodzinsky, A.J., Szasz, N., and Trippel, S.B. (2003). Mechanical regulation of mitogen-activated protein kinase signaling in articular cartilage. *J. Biol. Chem.* 278:50940–50948.

Connect Tissue Res Downloaded from informahealthcare.com by National Library of Medicine on 07/10/12
For personal use only.

RIGHTSLINK

Connect Tissue Res Downloaded from informahealthcare.com by National Library of Medicine on 07/10/12
For personal use only.

[59] Goldsmith, Z.G., and Dhanasekaran, D.N. (2007). G protein regulation of MAPK networks. *Oncogene* 26:3122–3142.

[60] Schmitt, J.M., and Stork, P.J. (2001). Cyclic AMP-mediated inhibition of cell growth requires the small G protein Rap1. *Mol. Cell. Biol.* 21:3671–3683.

[61] Crespo, P., Xu, N., Simonds, W.F., and Gutkind, J.S. (1994). Ras-dependent activation of MAP kinase pathway mediated by G-protein beta gamma subunits. *Nature* 369:418–420.

[62] Ueda, Y., Hirai, S., Osada, S., Suzuki, A., Mizuno, K., and Ohno, S. (1996). Protein kinase C activates the MEK-ERK pathway in a manner independent of Ras and dependent on Raf. *J. Biol. Chem.* 271:23512–23519.

[63] Minamino, T., Yujiri, T., Terada, N., Taffet, G.E., Michael, L.H., Johnson, G.L., and Schneider, M.D. (2002). MEKK1 is essential for cardiac hypertrophy and dysfunction induced by Gq. *Proc. Natl. Acad. Sci. U.S.A.* 99:3866–3871.

[64] Lim, I.J., Phan, T.T., Tan, E.K., Nguyen, T.T., Tran, E., Longaker, M.T., Song, C., Lee, S.T., and Huynh, H.T. (2003). Synchronous activation of ERK and phosphatidylinositol 3-kinase pathways is required for collagen and extracellular matrix production in keloids. *J. Biol. Chem.* 278:40851–40858.

[65] Ridley, A.J., and Hall, A. (1992). The small GTP-binding protein rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors. *Cell* 70:389–399.

[66] Leung, T., Chen, X.Q., Manser, E., and Lim, L. (1996). The p160 RhoA-binding kinase ROK alpha is a member of a kinase family and is involved in the reorganization of the cytoskeleton. *Mol. Cell. Biol.* 16:5313–5327.

[67] Lin, T., Zeng, L., Liu, Y., DeFea, K., Schwartz, M.A., Chien, S., and Shyy, J.Y. (2003). Rho-ROCK-LIMK-cofilin pathway regulates shear stress activation of sterol regulatory element binding proteins. *Circ. Res.* 92:1296–1304.

[68] Wong, C.C., Wong, C.M., Ko, F.C., Chan, L.K., Ching, Y.P., Yam, J.W., and Ng, I.O. (2008). Deleted in liver cancer 1 (DLC1) negatively regulates Rho/ROCK/MLC pathway in hepatocellular carcinoma. *PLoS One* 3:e2779.

[69] Fukushima, M., Nakamuta, M., Kohjima, M., Kotoh, K., Enjoji, M., Kobayashi, N., and Nawata, H. (2005). Fasudil hydrochloride hydrate, a Rho-kinase (ROCK) inhibitor, suppresses collagen production and enhances collagenase activity in hepatic stellate cells. *Liver Int.* 25:829–838.

[70] Tada, S., Iwamoto, H., Nakamuta, M., Sugimoto, R., Enjoji, M., Nakashima, Y., and Nawata, H. (2001). A selective ROCK inhibitor, Y27632, prevents dimethylnitrosamine-induced hepatic fibrosis in rats. *J. Hepatol.* 34:529–536.

[71] Nagatoya, K., Moriyama, T., Kawada, N., Takeji, M., Oseto, S., Murozono, T., Ando, A., Imai, E., and Hori, M. (2002). Y-27632 prevents tubulointerstitial fibrosis in mouse kidneys with unilateral ureteral obstruction. *Kidney Int.* 61:1684–1695.

[72] Satoh, S., Yamaguchi, T., Hitomi, A., Sato, N., Shiraiwa, K., Ikegaki, I., Asano, T., and Shimokawa, H. (2002). Fasudil attenuates interstitial fibrosis in rat kidneys with unilateral ureteral obstruction. *Eur. J. Pharmacol.* 455:169–174.

[73] Masszi, A., Di Ciano, C., Sirokmány, G., Arthur, W.T., Rotstein, O.D., Wang, J., McCulloch, C.A., Rosivall, L., Mucsi, I., and Kapus, A. (2003). Central role for Rho in TGF-beta1-induced alpha-smooth muscle actin expression during epithelial-mesenchymal transition. *Am. J. Physiol. Renal Physiol.* 284:F911–F924.

[74] Das, S., Becker, B.N., Hoffmann, F.M., and Mertz, J.E. (2009). Complete reversal of epithelial to mesenchymal transition requires inhibition of both ZEB expression and the Rho pathway. *BMC Cell Biol.* 10:94.

[75] Zhao, X.H., Laschinger, C., Arora, P., Szászi, K., Kapus, A., and McCulloch, C.A. (2007). Force activates smooth muscle alpha-actin promoter activity through the Rho signaling pathway. *J. Cell Sci.* 120:1801–1809.

[76] Mercher, T., Coniat, M.B., Monni, R., Mauchauffe, M., Nguyen Khac, F., Gressin, L., Mugneret, F., Leblanc, T., Dastugue, N., Berger, R., and Bernard, O.A. (2001). Involvement of a human gene related to the Drosophila spen gene in the recurrent t(1;22) translocation of acute megakaryocytic leukemia. *Proc. Natl. Acad. Sci. U.S.A.* 98:5776–5779.

[77] Wang, D.Z., Li, S., Hockemeyer, D., Sutherland, L., Wang, Z., Schratt, G., Richardson, J.A., Nordheim, A., and Olson, E.N. (2002). Potentiation of serum response factor activity by a family of myocardin-related transcription factors. *Proc. Natl. Acad. Sci. U.S.A.* 99:14855–14860.

[78] Ma, Z., Morris, S.W., Valentine, V., Li, M., Herbrick, J.A., Cui, X., Bouman, D., Li, Y., Mehta, P.K., Nizetic, D., Kaneko, Y., Chan, G.C., Chan, L.C., Squire, J., Scherer, S.W., and Hitzler, J.K. (2001). Fusion of two novel genes, RBM15 and MKL1, in the t(1;22)(p13;q13) of acute megakaryoblastic leukemia. *Nat. Genet.* 28:220–221.

[79] Sasazuki, T., Sawada, T., Sakon, S., Kitamura, T., Kishi, T., Okazaki, T., Katano, M., Tanaka, M., Watanabe, M., Yagita, H., Okumura, K., and Nakano, H. (2002). Identification of a novel transcriptional activator, BSAC, by a functional cloning to inhibit tumor necrosis factor-induced cell death. *J. Biol. Chem.* 277:28853–28860.

[80] Miralles, F., Posern, G., Zaromytidou, A.I., and Treisman, R. (2003). Actin dynamics control SRF activity by regulation of its coactivator MAL. *Cell* 113:329–342.

[81] Lutz, R., Sakai, T., and Chiquet, M. (2010). Pericellular fibronectin is required for RhoA-dependent responses to cyclic strain in fibroblasts. *J. Cell Sci.* 123:1511–1521.

[82] Chiquet, M., Sarasa-Renedo, A., and Tunç-Civelek, V. (2004). Induction of tenascin-C by cyclic tensile strain versus growth factors: Distinct contributions by Rho/ROCK and MAPK signaling pathways. *Biochim. Biophys. Acta* 1693:193–204.

[83] Haydont, V., Bourgier, C., and Vozenin-Brotons, M.C. (2007). Rho/ROCK pathway as a molecular target for modulation of intestinal radiation-induced toxicity. *Br. J. Radiol.* 80:S32–S40.

[84] Bourgier, C., Haydont, V., Milliat, F., François, A., Holler, V., Lasser, P., Bourhis, J., Mathé, D., and Vozenin-Brotons, M.C. (2005). Inhibition of Rho kinase modulates radiation induced fibrogenic phenotype in intestinal smooth muscle cells through alteration of the cytoskeleton and connective tissue growth factor expression. *Gut* 54:336–343.

[85] Sarasa-Renedo, A., Tunç-Civelek, V., and Chiquet, M. (2006). Role of RhoA/ROCK-dependent actin contractility in the induction of tenascin-C by cyclic tensile strain. *Exp. Cell Res.* 312:1361–1370.

[86] Baud, V., and Karin, M. (2001). Signal transduction by tumor necrosis factor and its relatives. *Trends Cell Biol.* 11:372–377.

[87] Tartaglia, L.A., and Goeddel, D.V. (1992). Two TNF receptors. *Immunol. Today* 13:151–153.

[88] Renò, F., Sabbatini, M., Lombardi, F., Stella, M., Pezzuto, C., Magliacani, G., and Cannas, M. (2003). In vitro mechanical compression induces apoptosis and regulates cytokines release in hypertrophic scars. *Wound Repair Regen.* 11:331–336.

[89] Inoh, H., Ishiguro, N., Sawazaki, S., Amma, H., Miyazu, M., Iwata, H., Sokabe, M., and Naruse, K. (2002). Uniaxial cyclic stretch induces the activation of transcription factor nuclear factor kappaB in human fibroblast cells. *FASEB J.* 16:405–407.

[90] Amma, H., Naruse, K., Ishiguro, N., and Sokabe, M. (2005). Involvement of reactive oxygen species in cyclic stretch-induced NF-kappaB activation in human fibroblast cells. *Br. J. Pharmacol.* 145:364–373.

[91] Goldberg, M.T., Han, Y.P., Yan, C., Shaw, M.C., and Garner, W.L. (2007). TNF-alpha suppresses alpha-smooth muscle actin expression in human dermal fibroblasts: An implication for abnormal wound healing. *J. Invest. Dermatol.* 127(11):2645–2655.

[92] Yamauchi, Y., Kohyama, T., Takizawa, H., Kamitani, S., Desaki, M., Takami, K., Kawasaki, S., Kato, J., and Nagase, T. (2010). Tumor necrosis factor-alpha enhances both

© 2012 Informa Healthcare USA, Inc.

RIGHTSLINK

epithelial-mesenchymal transition and cell contraction induced in A549 human alveolar epithelial cells by transforming growth factor-beta1. *Exp. Lung Res.* 36:12–24.

[93] Yan, C., Grimm, W.A., Garner, W.L., Qin, L., Travis, T., Tan, N., and Han, Y.P. (2010). Epithelial to mesenchymal transition in human skin wound healing is induced by tumor necrosis factor-alpha through bone morphogenic protein-2. *Am. J. Pathol.* 176:2247–2258.

[94] Bowley, E., O'Gorman, D.B., and Gan, B.S. (2007). Beta-catenin signaling in fibroproliferative disease. *J. Surg. Res.* 138:141–150.

[95] Bhanot, P., Brink, M., Samos, C.H., Hsieh, J.C., Wang, Y., Macke, J.P., Andrew, D., Nathans, J., and Nusse, R. (1996). A new member of the frizzled family from Drosophila functions as a Wingless receptor. *Nature* 382:225–230.

[96] Wehrli, M., Dougan, S.T., Caldwell, K., O'Keefe, L., Schwartz, S., Vaizel-Ohayon, D., Schejter, E., Tomlinson, A., and DiNardo, S. (2000). Arrow encodes an LDL-receptor-related protein essential for Wingless signalling. *Nature* 407:527–530.

[97] Howard, J.C., Varallo, V.M., Ross, D.C., Roth, J.H., Faber, K.J., Alman, B., and Gan, B.S. (2003). Elevated levels of beta-catenin and fibronectin in three-dimensional collagen cultures of Dupuytren's disease cells are regulated by tension in vitro. *BMC Musculoskelet. Disord.* 4:16.

[98] Lau, K.H., Kapur, S., Kesavan, C., and Baylink, D.J. (2006). Up-regulation of the Wnt, estrogen receptor, insulin-like growth factor-I, and bone morphogenetic protein pathways in C57BL/6J osteoblasts as opposed to C3H/HeJ osteoblasts in part contributes to the differential anabolic response to fluid shear. *J. Biol. Chem.* 281:9576–9588.

[99] Heise, R.L., Stober, V., Cheluvaraju, C., Hollingsworth, J.W., and Garantziotis, S. (2011). Mechanical stretch induces epithelial-mesenchymal transition in alveolar epithelia via hyaluronan activation of innate immunity. *J. Biol. Chem.* 286:17435–17444.

[100] Riveline, D., Zamir, E., Balaban, N.Q., Schwarz, U.S., Ishizaki, T., Narumiya, S., Kam, Z., Geiger, B., and Bershadsky, A.D. (2001). Focal contacts as mechanosensors: Externally applied local mechanical force induces growth of focal contacts by an mDia1-dependent and ROCK-independent mechanism. *J. Cell Biol.* 153:1175–1186.

[101] Mammoto, A., Huang, S., Moore, K., Oh, P., and Ingber, D.E. (2004). Role of RhoA, mDia, and ROCK in cell shape-dependent control of the Skp2-p27kip1 pathway and the G1/S transition. *J. Biol. Chem.* 279:26323–26330.

Connect Tissue Res Downloaded from informahealthcare.com by National Library of Medicine on 07/10/12
For personal use only.

RIGHTSLINK