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Biomechanical regulation of mesenchymal cell function

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Abstract

Purpose of review—Cells of mesenchymal origin are strongly influenced by their biomechanical environment. They also help to shape tissue architecture and reciprocally influence tissue mechanical environments through their capacity to deposit, remodel, and resorb extracellular matrix and to promote tissue vascularization. Although mechanical regulation of cell function and tissue remodeling has long been appreciated in other contexts, the purpose of this review is to highlight the increasing appreciation of its importance in fibrosis and hypertrophic scarring.

Recent findings—Experiments in both animal and cellular model systems have demonstrated pivotal roles for the biomechanical environment in regulating myofibroblast differentiation and contraction, endothelial barrier function and angiogenesis, and mesenchymal stem cell fate decisions. Through these studies, a better understanding of the molecular mechanisms transducing the biomechanical environment is emerging, with prominent and interacting roles recently identified for key network components including transforming growth factor- β /SMAD, focal adhesion kinase, MRTFs, Wnt/ β -catenin and YAP/TAZ signaling pathways.

Summary—Progress in understanding biomechanical regulation of mesenchymal cell function is leading to novel approaches for improving clinical outcomes in fibrotic diseases and wound healing. These approaches include interventions aimed at modifying the tissue biomechanical environment, and efforts to target mesenchymal cell activation by, and reciprocal interactions with, the mechanical environment.

Keywords

extracellular matrix; fibrosis; mechanotransduction; stiffness

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Conflicts of interest

There are no conflicts of interest.

INTRODUCTION

Cellular responses to mechanical cues are important in normal development and homeostasis, as well as in multiple disease conditions. The ability of cells to convert mechanical energy into chemical energy, also known as mechanotransduction, is widely conserved across cells and tissues. Mesenchymal cells are strongly influenced by their mechanical environment, and in turn shape their environment through their capacity to organize tissues by extracellular matrix deposition, remodeling, resorption and vascularization. Although the concept that mechanical forces shape tissue remodeling has been long appreciated in orthopedic and cardiovascular systems, its importance in fibrosis, wound healing, and hypertrophic scarring is undergoing significant re-evaluation and expansion. Recent efforts have led to remarkable progress in understanding key molecular links that orchestrate cellular and tissue level responses to mechanical cues, including both dynamic cues such as stretch, and tonic cues such as matrix tension or rigidity. Although precise molecular mechanisms of mechanotransduction remain to be entirely elucidated, the recent progress made in this field sets the stage for a fuller appreciation of mesenchymal responses to the biomechanical environment. This review focuses on recent progress made in understanding mesenchymal cell responses to biomechanical cues, the network of molecular pathways linking biomechanical cues to cellular activation, and the clinical implications that emerge from our increased understanding of the mechanical influence on mesenchymal cell function in contexts of fibrosis, wound healing and hypertrophic scarring.

CELL AND TISSUE RESPONSES TO THE BIOMECHANICAL ENVIRONMENT

Mesenchymal cells are highly responsive to the mechanical environment. These cells alter their functional behaviors in stereotypical fashion in response to mechanical influences including stretch and changes in matrix tension and rigidity. This concept is probably best understood for fibroblasts, and recent progress on their responses to mechanical influences will be detailed below. We will also touch, however, on other mesenchyme-derived cell types and our developing understanding of their key functional responses to biomechanical cues.

Myofibroblast differentiation and function

Fibroblasts help to establish tissue architecture and determine tissue mechanical properties through regulated deposition and maintenance of extracellular matrix. In addition, these cells also deform the matrix through cell-generated forces, and respond to mechanical cues, including exogenously applied stretch, and matrix tension and rigidity sensed through internally generated forces. Although multiple markers of diverse fibroblast phenotypes have been described, the best-studied phenotypic conversion is between fibroblast and myofibroblast states, typically associated with acquisition of α -smooth muscle actin (ASMA) expression and increased contractile function. Recent work by several groups has shown that fibroblast function and myofibroblast differentiation are tightly linked to the deformability (rigidity or stiffness) of the extracellular matrix [1,2,3,4,5,6,7]. Intriguingly, several of these studies [1,2,5,6] indicate that the effects of matrix stiffness may be independent of profibrotic factors, such as transforming growth factor-beta (TGF- β), or may selectively promote the effects of these factors. The effect of matrix stiffness on

contractile function of fibroblasts, which has been inferred from substrate wrinkling and three-dimensional gel compaction, has recently been quantified in a rigorous fashion [8■]. These measurements revealed that cell-generated forces vary dramatically across matrix stiffness conditions, and confirmed that matrix stiffness alters forces over a range associated with contractile integrin-mediated liberation of active TGF- β (see section below on TGF- β). The discovery that matrix stiffening by itself promotes enhanced matrix deposition [2] has potentially profound implications for understanding progressive fibrosis. This discovery suggests that fibroblasts by themselves are not mechanically homeostatic, but rather that in the absence of external controls and in the presence of a permissive environment, these cells are intrinsically programmed for matrix production, contraction, and stiffening. Interestingly, other cell types also appear to be capable of expressing mesenchymal markers through an epithelial mesenchymal transition that is also dependent on matrix rigidity [9■, 10] and stretch [11], furthering amplifying the potential implications of matrix stiffening and mechanical forces for fibrotic pathologies.

Externally applied forces also provoke diverse fibroblast signaling responses, including activation of mitogen-activated protein (MAP) kinases [12], Akt [13], and focal adhesion kinase (FAK). Interestingly, stretch has been shown to augment TGF- β release and signaling [14■,15], and promote myofibroblast and profibrotic phenotypes [16], but has also been shown to suppress myofibroblastic differentiation in lung fibroblasts [17■]. These differences may reflect differences in loading specifics (cyclic vs. static), cell of origin (lung vs. dermal), or both. Most of the work on matrix rigidity and stretch responses originated in two-dimensional planar systems, and while early results suggest reasonable predictive power when translated into three-dimensional systems, and ultimately back into physiological contexts, more work is needed. In addition, it has only recently become possible to separately tune both matrix rigidity and stretch within the same system [18■], and the possibilities for investigating these mechanical cues in tandem to enhance physiological relevance is appealing.

Endothelial barrier function and angiogenesis

Endothelial cells have long been recognized to sense and integrate biomechanical cues encoded in vascular flow and shear stress patterns. More recent work has focused on matrix rigidity as a key regulator of integrated endothelial functions. For example, Mammoto *et al.* [19] described a unique transcriptional program that regulates capillary network formation through the Rho inhibitor p190RhoGAP and the angiogenic growth factor receptor VEGFR2. The activity of this transcriptional program was shown to be sensitive to extracellular matrix elasticity, resulting in optimal angiogenesis and capillary network formation in a relatively narrow range of matrix stiffness. Subsequent work by Rao *et al.* [20] combined endothelial cells with mesenchymal stem cells to study vascular network formation in three-dimensional matrices. These experiments identified an inverse relationship between matrix stiffness and network formation. Further investigation using a matrix cross-linker to vary stiffness independently of matrix density confirmed the inhibitory effect of increasing matrix stiffness on vascular network formation. Together, these findings suggest that tissue stiffness must be within a permissible range to promote vascularization, which may have interesting implications for angiogenesis in wound healing and fibrosis.

Work from three groups [21■,22,23] has shown that matrix rigidity also importantly regulates endothelial barrier function, and influences the transmigration of leukocytes across endothelial monolayers. Based on alterations in vessel wall stiffness with aging, such functional effects on endothelial barrier function could have important implications for the development of atherosclerosis. Given the importance of restoring endothelial barrier function during repair and resolution of injury, such biomechanical influences could importantly impact on the divergence between successful resolution of injury and persistent dysfunction leading to aberrant wound healing or fibrosis.

Mesenchymal stem cell fate decisions

Several groups have recently followed up on the seminal findings of Engler *et al.* [24] that indicated that extracellular matrix rigidity influences mesenchymal stem cell (MSC) fate decisions. Dupont *et al.* [25■] showed that MSC differentiation along adipogenic vs. osteogenic lineages as determined by matrix rigidity was under the control of the transcriptional co-activators YAP and TAZ, and that exogenous control of these factors could override matrix stiffness cues. Park *et al.* [26] provided more context for this issue by showing that MSCs on stiff substrates express smooth muscle cell markers ASMA and calponin-1, whereas MSCs on soft substrates express both chondrogenic and adipogenic markers. They further examined the influence of TGF- β , which increased smooth muscle cell marker expression on stiff substrates, while selectively increasing chondrogenic marker expression and suppressing adipogenic marker expression on soft substrates. These data suggest that in addition to direct effects of matrix rigidity on cell fate, mechanical cues also strongly influence cellular responses to biochemical stimuli. Recently, Guvendiren and Burdick [27■] developed a novel dynamic matrix system, which allows stiffness to be changed while cells are resident. They provided evidence for relatively early cell commitment to fate decisions under the influence of mechanical cues, consistent with other recent findings [28].

MECHANORESPONSIVE PATHWAYS OF CELLULAR ACTIVATION

Several pathways are emerging as key regulators of biomechanical responses in mesenchymal cells. Interestingly, cross-talk among these pathways is pervasive (Fig. 1), suggesting the presence of a complex network of interactions that are mechanically tuned and serve to broadly influence cell fate decisions and functional programs.

Transforming growth factor- β

TGF- β is synthesized and secreted as part of a latent complex that can be anchored to the extracellular matrix [29]. The latent complex includes a peptide sequence recognized by integrins, and a pivotal study Munger *et al.* [30] demonstrated that integrin-mediated activation of TGF- β is critical to pulmonary fibrosis. Recent work has reinforced the concept that physical forces dramatically influence TGF- β bioavailability and signaling [14■], and have shown that cell-generated mechanical forces play a critical role in triggering bioavailability of active TGF- β 1 from its extracellular matrix-bound latent complex [31,32■,33■]. The recent solution of the latent TGF- β crystal structure identified the preferred orientation of force that is needed to unfasten the 'straitjacket' that encircles each

TGF- β monomer [33■], and Buscemi *et al.* [32■] recently estimated that ≈ 40 pN force is needed to release TGF- β from the latency-associated peptide. Forces in this range are consistent with the biophysical limits of individual integrin-matrix linkages [34]. Fibroblasts cultured across a range of matrix stiffness conditions generate tractions that increase dramatically with matrix stiffness, reaching local values as high as 6000 Pa (or 6000 pN/ μm^2) in isolated areas [8■]. Based on a recent estimate that the number of cell-substrate interacting structures is ≈ 170 per μm^2 within cell matrix adhesions [35], and assuming equivalent force distribution across 170 linkages, local tractions of 6000 Pa translates to 36 pN per molecule, similar to the estimate of force needed for TGF- β activation (40 pN). Consistently with these estimates, Wipff *et al.* [31] observed a modest matrix stiffness-dependent increase in fibroblast activation of TGF- β 1 of ≈ 1.3 -fold between matrices of 5 and 20 kPa stiffness. They also used thrombin stimulation to induce fibroblast contraction, and observed increased TGF- β 1 activation on 20 kPa matrices by ≈ 1.75 -fold, but found no effect on 6 kPa matrices [31]. Such findings are consistent with recent observations that exogenous stimuli selectively augment cell tractions on stiff, but not soft matrices [8■]. Taken together with the broad functional effects of TGF- β on matrix remodeling, these findings suggest the potential for an adverse feedback cycle of increased fibroblast contraction on stiff matrices, promoting TGF- β activation and further enhancing matrix deposition, with potentially important implications for fibrotic pathologies.

FOCAL ADHESION KINASE—‘Outside-in’ transmission of environmental mechanical forces to the cell, as well as ‘inside-out’ transmission of traction forces from the cell to its environment, both fundamentally involve the cytoskeleton [36]. Force transmission in both directions flows through focal adhesions, which include the proteins vinculin, paxillin, p130Cas and FAK, and link the cytoskeleton with the extracellular matrix through transmembrane integrin receptors. Focal adhesion assembly itself is greatly augmented both by forces applied externally to cells and by forces generated in cells internally by myosin-driven contraction [37]. Both externally applied and internally produced forces transmitted by the cytoskeleton generate biochemical signals by inducing focal adhesion protein conformational changes that activate FAK [37]. A requirement for FAK activation has been demonstrated for cells in culture to be able to respond to externally applied forces. FAK activation is primarily mediated by autophosphorylation of tyrosine 397 (Y397). The ability of externally applied forces to augment focal adhesion assembly in fibroblasts and to regulate the migration of these cells is abrogated in FAK-deficient or FAK-Y397F-expressing fibroblasts [38]. A requirement for FAK activation for mechanotransduction on the more complex level of tissues and organs has recently been demonstrated as well [39■]. Mechanical stress applied to healing cutaneous wounds in mice is sufficient to produce hypertrophic scars. Construction of transcriptome networks around mechanically regulated genes implicated FAK as a key mediator in this mechanical force model of hypertrophic scarring [40]. Studies with genetic deletion or pharmacologic inhibition of FAK in this hypertrophic scarring model have recently confirmed the central role of this kinase in fibroproliferative responses to injuries that are driven by mechanical forces. Scar area and matrix density were both significantly attenuated in this model in fibroblast-specific FAK deficient mice and in mice treated with a small molecule FAK inhibitor compared with control animals [39■].

MYOCARDIN-RELATED TRANSCRIPTION FACTORS—In addition to activating FAK as described above, the rearrangements of the cytoskeleton that are involved in force transmission activate transcriptional programs of force-responsive genes. The myocardin-related transcription factors (MRTFs), MRTF-A and MRTF-B, have been identified as important links between actin dynamics and gene expression [41]. Force transmission by the cytoskeleton involves polymerization of monomeric globular actin (G-actin) into filamentous actin (F-actin) fibers. The MRTFs are G-actin binding proteins, and their formation of stable complexes with G-actin results in their sequestration in the cytoplasm. Polymerization of G-actin into the F-actin filaments liberates MRTFs, which then translocate to the nucleus. In the nucleus, the MRTFs act as cofactors for serum response factor (SRF), augmenting the activity of this important nuclear transcription factor [42,43]. MRTF-A has recently been demonstrated to be required for matrix stiffness-induced fibroblast ASMA expression and myofibroblast differentiation, as stiffness failed to induce these processes in mouse lung fibroblasts deficient in MRTF-A [5■]. Signaling by the Rho GTPase RhoA, and its downstream Rho-associated coiled-coil forming kinases ROCK1 and ROCK2, regulate effector proteins that modulate the polymerization equilibrium of G-actin and F-actin, and consequently regulate MRTF nuclear translocation. As would therefore be expected, inhibition of RhoA/ROCK in MRTF-A-sufficient fibroblasts abrogated stiff matrix-induced actin cytoskeletal reorganization, MRTF-A nuclear translocation and myofibroblast differentiation [5■]. Conversely, MRTF-A overexpression in fibroblasts results in dramatic increases in ASMA expression and accumulation into highly organized stress fibers, a hallmark of myofibroblast activation [44]. Recent studies of MRTF-A-deficient mice in models of cardiac fibrosis have demonstrated the importance of this transcription factor for myofibroblast differentiation and accumulation *in vivo*. Genetic deletion of MRTF-A in mice resulted in reduced scar formation following myocardial infarction or angiotensin II treatment, associated with a reduced number of ASMA-positive myofibroblasts [44].

Wnt and β -catenin

The Wnt/ β -catenin pathway is an evolutionarily conserved pathway critical for development and increasingly recognized to play an important role in fibrosis [45,46■]. Recent evidence indicates that both exogenous stretch [11] and internal actomyosin-generated forces [47■] can lead to signaling through the Wnt/ β -catenin pathway. Though the mechanisms by which mechanical cues are transduced into activation of this signaling pathway remain to be delineated, the implications are potentially profound given the developmental and disease roles for Wnt signaling and the broad interconnectedness of Wnt with other important signaling pathways. For instance, β -catenin appears to interface with additional mechanically activated pathways, including a critical role in TGF- β -induced myofibroblast differentiation of aortic valve interstitial cells [6], and a collaborative role with MRTF in promoting TGF- β -dependent epithelial-mesenchymal transition [48].

YAP/TAZ—Like Wnt/ β -catenin, the Hippo pathway is highly conserved in evolution and is critical in development and disease, controlling organ size, stem cell function and malignant transformation. YAP and TAZ are the downstream end effectors of the Hippo pathway, and serve as transcriptional co-activators driving gene expression and regulation of other transcriptional pathways. Recent work has demonstrated that biomechanical cues of matrix

stiffness and cell spreading regulate YAP and TAZ nuclear translocation and transcriptional activity independently of upstream Hippo pathway components [25■]. Stretch is also linked to Hippo pathway activation through the cytoskeleton-associated protein zyxin [49], and biomechanical activation of YAP and TAZ appears to be under the control of both F-actin [50■] and actin stress fibers [51]. The important effects of YAP and TAZ activation include prominent regulation of cell cycle [25■] and differentiation [52], and regulation of transcriptional targets, such as connective tissue growth factor [25■]. Although more investigation is needed to understand the mechanical regulation of this pathway, an intriguing aspect of its function is its intersection with the TGF- β and Wnt pathways. Cytoplasmic YAP and TAZ act as retention factors for TGF- β effectors SMAD2/3 in the cytoplasm, and in this location suppress TGF- β signaling. In contrast, nuclear YAP and TAZ are co-factors for SMAD signaling and enhance SMAD nuclear localization, enhancing TGF- β signaling [53–56]. Similarly, cytoplasmic and nuclear YAP and TAZ exert complex effects on the Wnt signaling effector β -catenin, influencing both its nuclear localization and transcriptional control of its target genes [57■,58].

BIOMECHANICAL CONTRIBUTIONS TO DISEASE PATHOGENESIS AND IMPLICATIONS FOR THERAPY

The increasing appreciation of biomechanical contributions to cell function and tissue remodeling through the pathways and cellular responses described above is opening new therapeutic avenues for treating fibrotic diseases and improving wound healing.

Matrix stiffness as a therapeutic target for fibrotic diseases

Through the mechanoresponsive pathways of cellular activation described above, increased matrix stiffness promotes fibroblast ASMA expression and differentiation into myofibroblasts [1,2,3■,4■,5■,6,7]. Pathologically increased matrix stiffness is a hallmark of essentially all fibrotic diseases, and myofibroblast differentiation and activation are central to their pathogenesis [59]. Therapeutic interventions designed to reduce matrix stiffness could therefore potentially benefit the entire class of fibrotic diseases, which when taken together account for enormous burdens of morbidity and mortality [60]. Tissue stiffness in fibrotic diseases is regulated by the cross-linking of matrix proteins, in addition to the amounts of these proteins that are deposited. In health, this protein cross-linking stabilizes and orients extracellular matrix assembly for correct function, and is facilitated by a series of enzymes including transglutaminases, lysyl oxidases, and prolyl hydroxylases [61]. In fibrotic disease, these enzymes represent potential therapeutic targets for reducing matrix stiffness. Lysyl oxidase-like-2 (LOXL2) is a cross-linking enzyme that demonstrates increased expression in human fibrotic lung and liver tissues compared with its limited expression in healthy tissues, and that has recently been targeted in animal fibrosis models. In commonly used mouse models of liver and lung fibrosis, an inhibitory monoclonal antibody to LOXL2 decreased the accumulation of tissue collagen and of ASMA-positive myofibroblasts, and decreased TGF- β pathway signaling [62]. Transglutaminase 2 (TG2), the most widely expressed member of the transglutaminase family of cross-linking enzymes, has also been shown to contribute to fibrosis in animal models. TG2-deficient mice are protected in commonly used mouse models of kidney [63] and lung fibrosis [64■]. In the

kidney model, both myofibroblast accumulation and TGF- β activation were reduced in the absence of TG2 expression [63]. In humans, TG2 expression and activity are increased in lung biopsy sections from pulmonary fibrosis patients compared with normal controls [64]. There now is substantial interest in translating the promising preclinical results of targeting matrix cross-linking enzymes in animal models to human fibrotic diseases. A phase I study of an inhibitory monoclonal antibody to LOXL2 in patients with pulmonary fibrosis is currently recruiting patients ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01362231) identifier: NCT01362231).

Tissue tension as a therapeutic target for hypertrophic scarring

Skin exhibits intrinsic tension, and mechanotransduction of this tension is central to normal healing of skin wounds [65]. Much like in fibrotic diseases, aberrant wound healing can result in hypertrophic scar formation that is associated with contractile fibroblasts [66] and mechanical signaling through FAK [39,67]. Recently developed experimental models have been used to show that exogenous application of mechanical tension to healing wounds is sufficient to produce hypertrophic scars in mice [40,68]. Based on this view of mechanical tension as central to exuberant scarring, stress-shielding silicone polymer sheets have been developed that can be prestrained and applied across cutaneous wounds [69]. The stress shielding devices effectively carry tensile loads normally borne by the skin, thereby modifying the mechanical environment by reducing tension across the wound bed. Preclinical studies in a swine model of cutaneous wound healing demonstrated that the stress-shielding approach resulted in reduced expression of profibrotic markers and reduced scarring [69]. In a phase I clinical trial ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00766727) identifier: NCT00766727), stress shielding after abdominal incisions demonstrated improved scar appearance relative to within patient controls.

Cell-matrix interactions as targets for discovery and screening

In addition to directly modifying biomechanical cues through devices or targeted alterations of matrix cross-linking, it is becoming possible to study cellular responses to defined biomechanical environments in new ways, which should provide both new insights and new targets for intervention. For instance, recent proteomics-based efforts to analyze the composition of focal adhesions have revealed myriad changes in their composition that depend on myosin contractility, indicating the profound effect mechanical loads have on signaling networks emanating from integrin based cell-matrix adhesions [70]. RNA interference-based screening has been used to elucidate gene targets that control matrix stiffness-dependent fibroblast polarization and traction force generation, important functions relevant to directed cell motility and tissue remodeling. Finally, the ability to control matrix mechanical properties in high-throughput platforms is leading to new insights into stem cell function [71], and opening the possibility for molecular screening within defined mechanical environments that mimic normal and pathologic tissue stiffness [72]. Such approaches will ultimately inform our understanding of how cells process cues from their mechanical environment, and allow us to test interventions aimed at altering this reciprocal interaction within relevant mechanical environments, with the ultimate goal of targeting the mechanobiological interface to improve clinical outcomes.

CONCLUSION

The past few years have seen rapid progress in understanding the molecular pathways controlling cellular responses to the mechanical environment. In parallel, the importance of biomechanical cues in controlling mesenchymal cell function has become apparent in both fibrosis and scarring. The intersection of these lines of investigation is leading to novel approaches aimed at controlling mechanical cues for therapeutic effect. As molecular pathways controlling cell reciprocal interactions with the mechanical environment become more fully elucidated, the potential for novel interventions will continue to grow, as will our understanding of the pathogenesis of these challenging clinical entities.

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REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- ■ of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 152–153).

1. Li Z, Dranoff JA, Chan EP, et al. Transforming growth factor-beta and substrate stiffness regulate portal fibroblast activation in culture. *Hepatology*. 2007; 46:1246–1256. [PubMed: 17625791]
2. Liu F, Mih JD, Shea BS, et al. Feedback amplification of fibrosis through matrix stiffening and COX-2 suppression. *J Cell Biol*. 2010; 190:693–706. [PubMed: 20733059]
3. Prager-Khoutorsky M, Lichtenstein A, Krishnan R, et al. Fibroblast polarization is a matrix-rigidity-dependent process controlled by focal adhesion mechanosensing. *Nat Cell Biol*. 2011; 13:1457–1465. [PubMed: 22081092] This is an interesting study of matrix-rigidity-dependent focal adhesion alignment and fibroblast polarization, with identification of protein tyrosine kinases essential for polarization.
4. Balestrini JL, Chaudhry S, Sarrazy V, et al. The mechanical memory of lung myofibroblasts. *Integr Biol (Camb)*. 2012; 4:410–421. [PubMed: 22410748] This study shows the priming and persistence effects of substrate stiffness on myofibroblast differentiation.
5. Huang X, Yang N, Fiore VF, et al. Matrix stiffness-induced myofibroblast differentiation is mediated by intrinsic mechanotransduction. *Am J Respir Cell Mol Biol*. 2012; 47:340–348. [PubMed: 22461426] Identifies MKL1 (MRTF-A) as a key actin-remodeling dependent transcriptional coactivator responsible for stiffness-dependent myofibroblast differentiation.
6. Chen JH, Chen WL, Sider KL, et al. beta-catenin mediates mechanically regulated, transforming growth factor-beta1-induced myofibroblast differentiation of aortic valve interstitial cells. *Arterioscler Thromb Vasc Biol*. 2011; 31:590–597. [PubMed: 21127288]
7. Galie PA, Westfall MV, Stegemann JP. Reduced serum content and increased matrix stiffness promote the cardiac myofibroblast transition in 3D collagen matrices. *Cardiovasc Pathol*. 2011; 20:325–333. [PubMed: 21306921]
8. Marinkovic A, Mih JD, Park JA, et al. Improved throughput traction microscopy reveals pivotal role for matrix stiffness in fibroblast contractility and TGF-beta responsiveness. *Am J Physiol Lung Cell Mol Physiol*. 2012; 00:000–000. Quantifies the effect of matrix stiffness across a pathophysiological range on contractile function of fibroblasts.

9. Leight JL, Wozniak MA, Chen S, et al. Matrix rigidity regulates a switch between TGF-beta1-induced apoptosis and epithelial-mesenchymal transition. *Mol Biol Cell*. 2012; 23:781–791. [PubMed: 22238361] Demonstrates that matrix rigidity switches cells between TGF-β1-induced apoptosis and epithelial-mesenchymal transition via effects on PI3K/Akt signaling.
10. Olsen AL, Bloomer SA, Chan EP, et al. Hepatic stellate cells require a stiff environment for myofibroblastic differentiation. *Am J Physiol Gastrointest Liver Physiol*. 2011; 301:G110–G118. [PubMed: 21527725]
11. Heise RL, Stober V, Cheluvvaraju C, et al. Mechanical stretch induces epithelial-mesenchymal transition in alveolar epithelia via hyaluronan activation of innate immunity. *J Biol Chem*. 2011; 286:17435–17444. [PubMed: 21398522]
12. Boudreault F, Tschumperlin DJ. Stretch-induced mitogen-activated protein kinase activation in lung fibroblasts is independent of receptor tyrosine kinases. *Am J Respir Cell Mol Biol*. 2010; 43:64–73. [PubMed: 19684308]
13. Paterno J, Vial IN, Wong VW, et al. Akt-mediated mechanotransduction in murine fibroblasts during hypertrophic scar formation. *Wound Repair Regen*. 2011; 19:49–58. [PubMed: 21134033]
14. Maeda T, Sakabe T, Sunaga A, et al. Conversion of mechanical force into TGF-beta-mediated biochemical signals. *Curr Biol*. 2011; 21:933–941. [PubMed: 21600772] This study shows that mechanical force regulates TGF-β/Smad2/3-mediated signaling in tenocytes.
15. Guo F, Carter DE, Leask A. Mechanical tension increases CCN2/CTGF expression and proliferation in gingival fibroblasts via a TGFbeta-dependent mechanism. *PLoS One*. 2011; 6:e19756. [PubMed: 21611193]
16. Lu F, Ogawa R, Nguyen DT, et al. Microdeformation of three-dimensional cultured fibroblasts induces gene expression and morphological changes. *Ann Plast Surg*. 2011; 66:296–300. [PubMed: 21233699]
17. Blaauw ME, Smit TH, Hanemaaijer R, et al. Cyclic mechanical stretch reduces myofibroblast differentiation of primary lung fibroblasts. *Biochem Biophys Res Commun*. 2011; 404:23–27. [PubMed: 21094632] Demonstrates an inhibitory effect of cyclic stretch on lung myofibroblast differentiation.
18. Throm Quinlan AM, Sierad LN, Capulli AK, et al. Combining dynamic stretch and tunable stiffness to probe cell mechanobiology *in vitro*. *PLoS One*. 2011; 6:e23272. [PubMed: 21858051] The first method to combine the effects of stretch and substrate stiffness to study fibroblast mechanobiology.
19. Mammoto A, Connor KM, Mammoto T, et al. A mechanosensitive transcriptional mechanism that controls angiogenesis. *Nature*. 2009; 457:1103–1108. [PubMed: 19242469]
20. Rao RR, Peterson AW, Ceccarelli J, et al. Matrix composition regulates three-dimensional network formation by endothelial cells and mesenchymal stem cells in collagen/fibrin materials. *Angiogenesis*. 2012; 15:253–264. [PubMed: 22382584]
21. Huynh J, Nishimura N, Rana K, et al. Age-related intimal stiffening enhances endothelial permeability and leukocyte transmigration. *Sci Transl Med*. 2011; 3:112ra122. This is first measurement of age-related subendothelial stiffening in mouse thoracic aorta, and study of stiffness effects on endothelial permeability.
22. Stroka KM, Aranda-Espinoza H. Endothelial cell substrate stiffness influences neutrophil transmigration via myosin light chain kinase-dependent cell contraction. *Blood*. 2011; 118:1632–1640. [PubMed: 21652678]
23. Krishnan R, Klumpers DD, Park CY, et al. Substrate stiffening promotes endothelial monolayer disruption through enhanced physical forces. *Am J Physiol Cell Physiol*. 2011; 300:C146–C154. [PubMed: 20861463]
24. Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. *Cell*. 2006; 126:677–689. [PubMed: 16923388]
25. Dupont S, Morsut L, Aragona M, et al. Role of YAP/TAZ in mechanotransduction. *Nature*. 2011; 474:179–183. [PubMed: 21654799] Demonstrates that matrix stiffness and cell spreading regulate YAP and TAZ nuclear translocation and transcriptional activity.

26. Park JS, Chu JS, Tsou AD, et al. The effect of matrix stiffness on the differentiation of mesenchymal stem cells in response to TGF-beta. *Biomaterials*. 2011; 32:3921–3930. [PubMed: 21397942]
27. Guvendiren M, Burdick JA. Stiffening hydrogels to probe short- and long-term cellular responses to dynamic mechanics. *Nat Commun*. 2012; 3:792. [PubMed: 22531177] A novel system for dynamic control of matrix stiffness is reported which allows stiffness to be changed from 1 to 10 kPa within a timescale of minutes to hours while cells are resident.
28. Fu J, Wang YK, Yang MT, et al. Mechanical regulation of cell function with geometrically modulated elastomeric substrates. *Nat Methods*. 2010; 7:733–736. [PubMed: 20676108]
29. Munger JS, Sheppard D. Cross talk among TGF-beta signaling pathways, integrins, and the extracellular matrix. *Cold Spring Harb Perspect Biol*. 2011; 3:a005017. [PubMed: 21900405]
30. Munger JS, Huang X, Kawakatsu H, et al. The integrin alpha v beta 6 binds and activates latent TGF beta 1: a mechanism for regulating pulmonary inflammation and fibrosis. *Cell*. 1999; 96:319–328. [PubMed: 10025398]
31. Wipff PJ, Rifkin DB, Meister JJ, Hinz B. Myofibroblast contraction activates latent TGF-beta1 from the extracellular matrix. *J Cell Biol*. 2007; 179:1311–1323. [PubMed: 18086923]
32. Buscemi L, Ramonet D, Klingberg F, et al. The single-molecule mechanics of the latent TGF-beta1 complex. *Curr Biol*. 2011; 21:2046–2054. [PubMed: 22169532] Provides direct evidence that a force of ~40 pN is needed to release active TGF-β1 from the latency-associated peptide.
33. Shi M, Zhu J, Wang R, et al. Latent TGF-beta structure and activation. *Nature*. 2011; 474:343–349. [PubMed: 21677751] Reports the crystal structure of latent TGF-β1, providing structural evidence of the long proposed force-dependent mechanism of integrin-mediated TGF-β1 activation.
34. Moore SW, Roca-Cusachs P, Sheetz MP. Stretchy proteins on stretchy substrates: the important elements of integrin-mediated rigidity sensing. *Dev Cell*. 2010; 19:194–206. [PubMed: 20708583]
35. Patla I, Volberg T, Elad N, et al. Dissecting the molecular architecture of integrin adhesion sites by cryo-electron tomography. *Nat Cell Biol*. 2010; 12:909–915. [PubMed: 20694000]
36. Eyckmans J, Boudou T, Yu X, Chen CS. A hitchhiker's guide to mechanobiology. *Dev Cell*. 2011; 21:35–47. [PubMed: 21763607]
37. Goldmann WH. Mechanotransduction and focal adhesions. *Cell Biol International*. 2012; 36:649–652.
38. Wang HB, Dembo M, Hanks SK, Wang Y. Focal adhesion kinase is involved in mechanosensing during fibroblast migration. *Proc Natl Acad Sci U S A*. 2001; 98:11295–11300. [PubMed: 11572981]
39. Wong VW, Rustad KC, Akaishi S, et al. Focal adhesion kinase links mechanical force to skin fibrosis via inflammatory signaling. *Nat Med*. 2012; 18:148–152. Demonstrates that mechanical force regulates hypertrophic scar formation via an inflammatory FAK-ERK-MCP-1 pathway.
40. Aarabi S, Bhatt KA, Shi Y, et al. Mechanical load initiates hypertrophic scar formation through decreased cellular apoptosis. *Faseb J*. 2007; 21:3250–3261. [PubMed: 17504973]
41. Olson EN, Nordheim A. Linking actin dynamics and gene transcription to drive cellular motile functions. *Nat Rev Mol Cell Biol*. 2010; 11:353–365. [PubMed: 20414257]
42. Sotiropoulos A, Gineitis D, Copeland J, Treisman R. Signal-regulated activation of serum response factor is mediated by changes in actin dynamics. *Cell*. 1999; 98:159–169. [PubMed: 10428028]
43. Miralles F, Posern G, Zaromytidou AI, Treisman R. Actin dynamics control SRF activity by regulation of its coactivator MAL. *Cell*. 2003; 113:329–342. [PubMed: 12732141]
44. Small EM, Thatcher JE, Sutherland LB, et al. Myocardin-related transcription factor-a controls myofibroblast activation and fibrosis in response to myocardial infarction. *Circ Res*. 2010; 107:294–304. [PubMed: 20558820]
45. Lam AP, Gottardi CJ. beta-catenin signaling: a novel mediator of fibrosis and potential therapeutic target. *Curr Opin Rheumatol*. 2011; 23:562–567. [PubMed: 21885974]
46. Beyer C, Schramm A, Akhmetshina A, et al. beta-catenin is a central mediator of pro-fibrotic Wnt signaling in systemic sclerosis. *Ann Rheum Dis*. 2012; 71:761–767. [PubMed: 22328737] Using mice with fibroblast-specific overexpression and deletion of β-catenin, this work identifies β-catenin as a key factor in fibroblast activation and tissue fibrosis in systemic sclerosis.

47. Samuel MS, Lopez JI, McGhee EJ, et al. Actomyosin-mediated cellular tension drives increased tissue stiffness and beta-catenin activation to induce epidermal hyperplasia and tumor growth. *Cancer Cell*. 2011; 19:776–791. [PubMed: 21665151] Demonstrates that β -catenin signaling is driven by tissue-stiffness-induced Rho-associated coiled-coil forming kinase activation.
48. Charbonney E, Speight P, Masszi A, et al. beta-catenin and Smad3 regulate the activity and stability of myocardin-related transcription factor during epithelial-myofibroblast transition. *Mol Biol Cell*. 2011; 22:4472–4485. [PubMed: 21965288]
49. Rauskolb C, Pan G, Reddy BV, et al. Zyxin links fat signaling to the hippo pathway. *PLoS Biol*. 2011; 9:e1000624. [PubMed: 21666802]
50. Sansores-Garcia L, Bossuyt W, Wada K, et al. Modulating F-actin organization induces organ growth by affecting the Hippo pathway. *EMBO J*. 2011; 30:2325–2335. [PubMed: 21556047] Modulation of F-actin through capping protein controls Hippo signaling and organ growth.
51. Wada K, Itoga K, Okano T, et al. Hippo pathway regulation by cell morphology and stress fibers. *Development*. 2011; 138:3907–3914. [PubMed: 21831922]
52. Hong JH, Hwang ES, McManus MT, et al. TAZ, a transcriptional modulator of mesenchymal stem cell differentiation. *Science*. 2005; 309:1074–1078. [PubMed: 16099986]
53. Varelas X, Sakuma R, Samavarchi-Tehrani P, et al. TAZ controls Smad nucleocytoplasmic shuttling and regulates human embryonic stem-cell self-renewal. *Nat Cell Biol*. 2008; 10:837–848. [PubMed: 18568018]
54. Varelas X, Samavarchi-Tehrani P, Narimatsu M, et al. The Crumbs complex couples cell density sensing to Hippo-dependent control of the TGF-beta-SMAD pathway. *Dev Cell*. 2010; 19:831–844. [PubMed: 21145499]
55. Aragon E, Goerner N, Zaromytidou AI, et al. A Smad action turnover switch operated by WW domain readers of a phosphoserine code. *Genes Dev*. 2011; 25:1275–1288. [PubMed: 21685363]
56. Alarcon C, Zaromytidou AI, Xi Q, et al. Nuclear CDKs drive Smad transcriptional activation and turnover in BMP and TGF-beta pathways. *Cell*. 2009; 139:757–769. [PubMed: 19914168]
57. Heallen T, Zhang M, Wang J, et al. Hippo pathway inhibits Wnt signaling to restrain cardiomyocyte proliferation and heart size. *Science*. 2011; 332:458–461. [PubMed: 21512031] Describes an interaction between Wnt and Hippo signaling suggesting a mechanism by which Hippo negatively modulates Wnt signaling in multiple contexts.
58. Varelas X, Miller BW, Sopko R, et al. The Hippo pathway regulates Wnt/beta-catenin signaling. *Dev Cell*. 2010; 18:579–591. [PubMed: 20412773]
59. Hinz B, Phan SH, Thannickal VJ, et al. Recent developments in myofibroblast biology: paradigms for connective tissue remodeling. *Am J Pathol*. 2012; 180:1340–1355. [PubMed: 22387320]
60. Wynn TA. Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. *J Clin Invest*. 2007; 117:524–529. [PubMed: 17332879]
61. Kolb MR, Gauldie J. Idiopathic pulmonary fibrosis: the matrix is the message. *Am J Respir Crit Care Med*. 2011; 184:627–629. [PubMed: 21920923]
62. Barry-Hamilton V, Spangler R, Marshall D, et al. Allosteric inhibition of lysyl oxidase-like-2 impedes the development of a pathologic microenvironment. *Nat Med*. 2010; 16:1009–1017. [PubMed: 20818376]
63. Shweke N, Boulous N, Jouanneau C, et al. Tissue transglutaminase contributes to interstitial renal fibrosis by favoring accumulation of fibrillar collagen through TGF-beta activation and cell infiltration. *Am J Pathol*. 2008; 173:631–642. [PubMed: 18688035]
64. Olsen KC, Sapinoro RE, Kottmann RM, et al. Transglutaminase 2 and its role in pulmonary fibrosis. *Am J Respir Crit Care Med*. 2011; 184:699–707. [PubMed: 21700912] Demonstrates that TG2 expression and activity are increased in fibrotic lungs and that TG2 plays a key role in fibroblast function.
65. Wong VW, Akaishi S, Longaker MT, Gurtner GC. Pushing back: wound mechanotransduction in repair and regeneration. *J Invest Dermatol*. 2011; 131:2186–2196. [PubMed: 21776006]
66. Bond JE, Ho TQ, Selim MA, et al. Temporal spatial expression and function of nonmuscle myosin II isoforms IIA and IIB in scar remodeling. *Lab Invest*. 2011; 91:499–508. [PubMed: 21102503]

67. Lagares D, Busnadiego O, Garcia-Fernandez RA, et al. Inhibition of focal adhesion kinase prevents experimental lung fibrosis and myofibroblast formation. *Arthritis Rheum.* 2012; 64:1653–1664. [PubMed: 22492165]
68. Wong VW, Paterno J, Sorkin M, et al. Mechanical force prolongs acute inflammation via T-cell-dependent pathways during scar formation. *FASEB J.* 2011; 25:4498–4510. [PubMed: 21911593] This article identifies mechanically regulated genes in a murine hypertrophic scar model.
69. Gurtner GC, Dauskardt RH, Wong VW, et al. Improving cutaneous scar formation by controlling the mechanical environment: large animal and phase I studies. *Ann Surg.* 2011; 254:217–225. [PubMed: 21606834] A preclinical/clinical report showing that a stress-shielding device significantly reduces cutaneous scar formation.
70. Kuo JC, Han X, Hsiao CT, et al. Analysis of the myosin-II-responsive focal adhesion proteome reveals a role for beta-Pix in negative regulation of focal adhesion maturation. *Nat Cell Biol.* 2011; 13:383–393. [PubMed: 21423176] The authors define the myosin II-responsive components of focal adhesions.
71. Gilbert PM, Havenstrite KL, Magnusson KE, et al. Substrate elasticity regulates skeletal muscle stem cell self-renewal in culture. *Science.* 2010; 329:1078–1081. [PubMed: 20647425]
72. Mih JD, Sharif AS, Liu F, et al. A multiwell platform for studying stiffness-dependent cell biology. *PLoS One.* 2011; 6:e19929. [PubMed: 21637769]

KEY POINTS

- The biomechanical environment is increasingly appreciated to play pivotal roles in regulating mesenchymal cell functions, including myofibroblast differentiation and contraction, endothelial barrier function and angiogenesis, and mesenchymal stem cell fate decisions.
- Several molecular pathways responsible for transducing the biomechanical environment are coming into focus, with prominent and interacting roles for key network components including TGF- β /SMAD, FAK, MRTFs, Wnt/ β -catenin and YAP/TAZ signaling pathways.
- The improved understanding of biomechanical control of mesenchymal cell function is leading to novel approaches for improving clinical outcomes in fibrotic diseases and wound healing, with interventions aimed at directly modifying the biomechanical environment and targeting the reciprocal relationship between cells and the mechanical environment.

FIGURE 1.

Mechanoresponsive pathways of cellular activation. Biomechanical cues, including externally applied forces as well as intracellular contractions, promote release of TGF- β from its latency-associated peptide (LAP), which binds to ECM via latent TGF- β -binding protein-1 (LTBP-1). These biomechanical cues also regulate YAP/TAZ nuclear translocation and transcriptional activity via F-actin and stress fibers. YAP/TAZ act as retention factors for SMAD2/3 in the cytoplasm, suppressing TGF- β signals, while serving as cofactors for SMAD transcriptional activity in the nucleus. Similarly, YAP/TAZ exerts complex effects on β -catenin, influencing both its nuclear localization and transcriptional control of its target genes. FAK activation plays key roles in both 'outside-in' and 'inside-out' transduction of physical forces. Rho/ROCK activation downstream of FAK is required for myosin II-driven intracellular contraction and is involved in β -catenin activation. Cytoskeleton rearrangements and actin polymerization, which reciprocally interact with the biomechanical environment, regulate MRTF nuclear translocation and co-activation of serum response factor (SRF)-dependent gene expression. LTBP-1, latent TGF- β -binding protein-1; LAP, latency associated peptide; ECM, extracellular matrix; LRP5/6, LDL receptor related protein 5 and 6; FZD, Frizzled; β -Cat, β -catenin; LEF, Lymphoid enhancer-binding factor; ROCK,

Rho-associated coiled-coil forming kinase; SRF, serum response factor; TCF, T-cell factor transcription factor; TEAD, TEA domain family member.

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