REVIEW

Mechanical Feed-Forward Loops Contribute to Idiopathic Pulmonary Fibrosis

Margaret A.T. Freeberg,^{*} Apostolos Perelas,^{*} Jane K. Rebman,^{*} Richard P. Phipps,[†] Thomas H. Thatcher,^{*} and Patricia J. Sime^{*}

From the Division of Pulmonary Disease and Critical Care Medicine,^{*} Virginia Commonwealth University, Richmond; and Independent Scholar,[†] Henrico, Virginia

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Address correspondence to
Patricia J. Sime, M.D., Virginia
Commonwealth University,
1101 East Marshall St., Sanger
Hall 1-030, Richmond,
VA 23298. E-mail: [patricia.sime@vcuhealth.org](mailto:sime@vcuhealth.org).

Idiopathic pulmonary fibrosis is a progressive scarring disease characterized by extracellular matrix accumulation and altered mechanical properties of lung tissue. Recent studies support the hypothesis that these compositional and mechanical changes create a progressive feed-forward loop in which enhanced matrix deposition and tissue stiffening contribute to fibroblast and myofibroblast differentiation and activation, which further perpetuates matrix production and stiffening. The biomechanical properties of tissues are sensed and responded to by mechanotransduction pathways that facilitate sensing of changes in mechanical cues by tissue resident cells and convert the mechanical signals into downstream biochemical signals. Although our understanding of mechanotransduction pathways associated with pulmonary fibrosis remains incomplete, recent progress has allowed us to begin to elucidate the specific mechanisms supporting fibrotic feed-forward loops. The mechanosensors discussed here include integrins, Piezo channels, transient receptor potential channels, and nonselective ion channels. Also discussed are downstream transcription factors, including myocardin-related transcription factor and Yes-associated protein/transcriptional coactivator with PDZ-binding motif. This review describes mechanosensors and mechanotransduction pathways associated with fibrosis progression and highlights promising therapeutic insights. (*Am J Pathol* 2021, 191: 18–25; <https://doi.org/10.1016/j.ajpath.2020.09.008>)

Idiopathic pulmonary fibrosis (IPF) is a progressive scarring disease of the lung with a life expectancy shorter than that of lung cancer.^{1–3} It is characterized in part by the accumulation of extracellular matrix (ECM) proteins in the interstitial space and impaired gas exchange, thereby decreasing lung function. The ECM composition in the lung plays an important role in regulating cell behavior and function.

Wolff's law, proposed in 1892, states⁴: “Every change in the form and function of bone or of their function alone is followed by certain definite changes in their internal architecture, and equally definite alteration in their external conformation, in accordance with mathematical laws.” The law defines that bone adapts and optimizes strength in response to the loads under which it is placed. Although the principle of mechanical adaptation is most obvious with muscle and bones,⁵ all tissues adapt to the mechanical loads they are under, including the lung.⁶

Structure–function relationships can be observed by differences in ECM architecture and are accompanied by distinct mechanical properties in anatomic compartments of the lung.^{7–9} It experiences several principal mechanical forces. It is under pressure from inhaled air, and it is subjected to cyclic tensile stretching with respiration cycles, as well as to the stiffness of the underlying ECM. These forces are sensed by fibroblasts that synthesize and modify the ECM to structurally stabilize the tissue and shield other cells

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from mechanical stimulation.¹⁰ In healthy lung tissue, this is a homeostatic process involving synthesis, modification, and degradation of matrix, collectively called matrix remodeling. In pathologic conditions, an imbalance in this process can lead to many pathologies, including increased ECM stiffness (fibrosis), decreased elasticity (emphysema), and airway remodeling (asthma). The imbalanced matrix buildup in fibrosis creates a self-fulfilling feed-forward loop of enhanced matrix deposition and stiffening.¹¹

Recent and ongoing work in IPF supports the hypothesis that diseased matrix is both a consequence and a driver of fibrosis.^{12–14} IPF is characterized by accumulation of fibroblasts and myofibroblasts within fibroblastic foci, deposition and cross-linking of the ECM, and dysregulation of proteases. Cumulatively, this action results in a buildup of ECM, increased stiffness (loss of compliance), thickened alveolar septae, and impairment of gas exchange, leading to progressive dyspnea, supplemental oxygen dependence, and eventually death. This stiffened matrix changes the forces sensed by fibroblasts, which drive myofibroblast differentiation, expression and cross-linking of ECM proteins, and other profibrotic effector functions, leading to a progressive feed-forward loop centered on the matrix mechanics.^{11,13}

Current therapeutic options slow the progression of IPF but do not halt or reverse the scarring in the lung.¹⁵ Given the role of the ECM in the initiation and progression of fibrosis, therapeutic strategies that target the matrix or cellular mechanical sensing may be an essential target to combine with current therapeutic options. The current review describes how lung fibroblasts sense and respond to mechanical stresses, as well as how altered mechanical properties of the fibrotic lung drive the progression of fibrosis. Possible therapeutic insights are also discussed.

The ECM of the Lung and Mechanical Changes in IPF

The matrix is bioactive and plays a role in the progression of IPF as a regulator of cellular phenotype and behavior. In the lung, the ECM is composed of a basement membrane with thin sheets of glycoproteins and the interstitial matrix, creating a fiber network that provides structure and the bulk of the mechanical integrity of the tissue.¹⁶ The ECM contains >300 different proteins, including collagens, proteoglycans, and glycoproteins.¹⁶ There is interest in identifying the physical aspects, including ECM composition and mechanical properties, that contribute to cellular activation and disease initiation and progression. Examples include barrier function and activation of endothelial and epithelial cells in response to stretch and shear, as well as fibroblast activation in response to stiffness and stretch of the ECM.

The biomechanical properties of lung tissue are strongly influenced by the biochemical composition of the ECM. The ECM in IPF is compositionally different from that in healthy lung tissue, with expanded collagens and

glycosaminoglycans.^{17–19} Immunohistochemical analysis shows that fibroblastic foci are rich in collagen and EDA-fibronectin.¹⁹ Periostin is a glycoprotein that is secreted by monocytes and fibrocytes, is overexpressed in IPF, and promotes fibrogenesis.²⁰ The accumulation of additional ECM is due to enhanced myofibroblast activation and production of ECM, coupled with inhibited ECM turnover and degradation.^{21–23} Furthermore, posttranslational modification such as enzymatic and chemical cross-linking are altered in IPF and contribute to tissue stiffening. Increased mechanical forces within tissues activate latent transforming growth factor- β (TGF- β) through integrin-dependent mechanisms,^{24–26} driving profibrotic effector functions, including ECM deposition and cross-linking that increase tissue stiffness and thereby further activates TGF- β (Figure 1). Cross-linking enzymes include lysyl oxidase (LOX), lysyl oxidase-like (LOXL), and transglutaminases (TGs). Pathologic cross-linking due to up-regulation of LOXL and TG enzyme activity enhances fibroblast accumulation and increases resistance to ECM turnover.²⁷ TG2 knockout mice are protected from bleomycin-induced pulmonary fibrosis. Serum LOXL2 levels are elevated in patients with IPF and are associated with increased risk of disease progression.²⁸ Despite promising preclinical studies,²⁹ a Phase II clinical trial with the LOXL2 inhibitor simtuzumab showed no effect on progression-free survival for patients with IPF.³⁰ More in-depth details on the ECM of the lung in healthy and diseased states have been provided in several previously published review articles.^{16,31,32}

Multiple mechanical forces contribute to pathologic tissue remodeling and fibrosis. Prior studies of lung tissue mechanics (in the normal and diseased states) have been limited to the characterization of matrix stiffness.^{11,17,26,33} Tissue stiffness, measured in kilopascals (kPa), measures the force required for deformation. Normal lung tissue has a stiffness in the range of 0.5 to 5 kPa, whereas fibrotic lung tissue is in the range of 15 to 100 kPa.^{11,17,26,33} Lung fibroblasts also experience mechanical forces from stretching, as the lungs expand and contract during breathing. Unlike either normal or fibrotic lung tissue, solid plastic tissue culture dishes have a substrate stiffness of approximately 100,000 kPa, and there is evidence that these forces induce nonphysiological cellular responses to mechanical strain.^{34–36}

To better recapitulate the native physiological environment, researchers have developed *in vitro* model systems using silicone membranes or polyacrylamide gels with tunable substrate stiffness.^{37,38} There are now commercially available systems for tunable stiffness (Advanced BioMatrix, Carlsbad, CA), as well as application of cyclic stretch (Flexcell International, Burlington, NC; Strex, San Diego, CA). Using custom and commercially available systems, investigators have begun to elucidate the effects of mechanical cues on fibroblast activation as well as mechanotransduction mechanisms. Increased stiffness both enhances recruitment of fibroblasts (durotaxis)³⁹ and myofibroblast differentiation.^{11,25,32,40} Excitingly, reducing stiffness can

Figure 1 Mechanoreceptors and signal transduction pathways activating profibrotic gene expression. Fibroblasts sense mechanical cues such as cellular stretch and extracellular matrix stiffening through stretch-activated ion channels, G protein–coupled receptors, and integrins. RGD (arginine-glycine-aspartate) integrin–linked focal adhesion complexes can activate Rho signaling leading to actin polymerization increasing α -smooth muscle actin (α SMA) and cellular contraction. Sensing of mechanical cues is followed by downstream activation of biochemical transduction pathways that converge on and regulate the nuclear translocation of myocardin-related transcription factor (MRTF) and Yes-associated protein (YAP)/transcriptional coactivator with PDZ-binding motif (TAZ). This leads to subsequent transcriptional regulation of profibrotic gene expression such as myofibroblast differentiation, extracellular matrix (ECM) deposition, and cellular contraction, further altering the mechanical microenvironment. In addition, application of externally applied force to the latency-associated peptide and latent transforming growth factor β (TGF- β)–binding protein 1 (LTBP1) activates TGF- β and canonical TGF- β /SMAD signaling. FAK, focal adhesion kinase; LINC, linker of nucleoskeleton and cytoskeleton; TGF- β R, TGF- β receptor; TRP, transient receptor potential.

partially reverse a stiffness-induced myofibroblast phenotype.^{36,41,42} This action supports the hypothesis that restoration of healthy ECM will result in cells responding and reverting from their diseased phenotype to restore homeostasis. Lung tissue, as with other tissues in the body, has more complex mechanical properties, as they are viscoelastic, exhibiting stress–strain relationships dependent on time and history. This presents an opportunity to more comprehensively analyze the mechanical properties of lung tissue in healthy and diseased states, and then implement more complex *in vitro* systems to model the mechanical microenvironment.

IPF results in heterogeneous distribution of fibrotic lesions adjacent to normal-appearing areas within the lung. The boundary between the presumed healthy tissue and the fibrotic lesion introduces stress concentrations in which abrupt changes of mechanical properties are present, leading to high cellular and matrix strains at the transition. Furthermore, new techniques have revealed that the normal-appearing tissue adjacent to fibrotic lesions is not actually normal. Second harmonic generation imaging has shown that the fibrillar collagen microstructure of the “normal”-appearing tissue adjacent to fibrotic foci matches the second harmonic generation signature within the fibroblastic foci themselves.⁴³ A similar phenomenon has been observed in liver fibrosis in

which there is an observed increase in tissue stiffness before fibrotic matrix deposition.⁴⁴ These data indicate that the ECM microstructure at the leading edge of a fibrotic lesion is altered before the alveolus is destroyed. The mechanical properties at the leading edge will be particularly important to characterize with extreme cellular mechanical forces experienced by it. There is a prime need to understand how lung cells, particularly fibroblasts, sense and respond to these mechanical signals to determine cellular responses within the transition region.

Mechanotransduction

Mechanosensors

The matrix is bioactive and communicates with cells. Cells sense changes in matrix mechanical properties through force-induced conformation change of mechanically sensitive molecules; this leads to the activation of downstream biochemical signaling pathways, a process defined as mechanotransduction.^{45,46} Mechanosensors reside on the cell membrane and include integrins, stretch-activated ion channels, and G protein coupled–receptors (Figure 1).

Integrin-mediated mechanotransduction is the primary mechanism for cells to sense ECM mechanics. One critical mechanotransduction feed-forward loop in fibrosis is integrin-mediated mechanical activation of latent TGF- β ⁴⁷ (Figure 1). The integrins $\alpha_v\beta_3$, $\alpha_v\beta_5$, and $\alpha_v\beta_6$ have been shown to bind the RGD sequence (arginine-glycine-aspartate) in the latency-associated peptide of TGF- β 1 to activate latent TGF- β .^{48–50} Integrins are up-regulated in fibrotic disease.^{48,51,52} In addition, stiffness-mediated ligation of the $\alpha_v\beta_3$ integrin leads to myofibroblast activation.⁵³ Ligation is also enhanced by low extracellular pH,⁵⁴ implicating potential cross-talk with another feed-forward fibrotic loop of dysregulated cellular metabolism in fibrosis.^{55,56} Recent studies have shown therapeutic efficacy in reducing kidney fibrosis with inhibition of RGD-binding integrins ($\alpha_v\beta_1$, $\alpha_v\beta_3$, and $\alpha_v\beta_6$) with CWHM-12.⁵⁷ A monoclonal antibody targeted against integrin $\alpha_v\beta_6$ attenuated the development of bleomycin-induced fibrosis in mice.⁵¹ However, the clinical trial testing the monoclonal antibody BG0001 (formally STX-100) was terminated early because of safety concerns (<https://www.clinicaltrials.gov>; NCT01371035).

Mechanosensitive ion channels such as Piezo channels and the family of transient receptor potential (TRP) channels can also mediate the progression of fibrosis (Figure 1); however, the direct mechanisms are still being elucidated. TRP vanilloid 4 (TRPV4) is a nonselective cation channel, with high permeability to Ca²⁺, that opens in response to mechanical stresses.^{58–60} Inhibition of TRPV4 attenuated stiffness-induced cardio-myofibroblast differentiation.⁶¹ Furthermore, TRPV4 is essential for fibroblast to myofibroblast differentiation *in vitro*, and TRPV4 knockout mice are protected from bleomycin-induced pulmonary fibrosis, supporting its therapeutic potential.⁶² Piezo 1 and 2 are mechanically activated calcium ion channels.^{63–66} It is unclear whether Piezo channels play a role in fibrosis but are down-regulated in both small cell and non-small cell lung cancers.⁶⁷ Loss of Piezo 1 and 2 promotes *in vitro* cell migration and *in vivo* tumor growth,^{68,69} but it remains unclear if it has implications in fibrosis.

Transcription Factors

Mechanical cues that are first sensed via receptors on the cell membrane are converted to chemical signals via mechanotransduction pathways that regulate many aspects of cell behavior, including motility, proliferation, morphology, and survival. Downstream signaling following activation of the mechanosensors discussed earlier often converges on several common transcription factors.

Yes-associated protein (YAP) is a transcriptional coactivator of the hippo signaling pathway. There are multiple upstream signals that regulate the hippo pathway, including G protein-coupled receptor signaling,⁷⁰ Wnt signaling,⁷¹ and mechanical stress.⁷² YAP interacts with its paralog transcriptional coactivator with PDZ-binding motif (TAZ). Both YAP and TAZ increase expression in response to substrates of

higher stiffness.^{72,73} YAP and TAZ are expressed in fibrotic but not healthy lung tissue.^{73,74} Furthermore, the profibrotic effects of YAP/TAZ can in part be attributed to activating plasminogen activator inhibitor-1, independent of TGF- β signaling.⁷³ Plasminogen activator inhibitor-1 contributes to fibrosis by suppressing matrix remodeling^{73,75–77} and altered cell cycle pathways through mammalian target of rapamycin/phosphatidylinositol 3-kinase.^{78,79} This creates another mechanotransduction feed-forward loop increasing matrix stiffness, hippo–YAP/TAZ signaling, myofibroblast activation, and fibrosis progression with increasing tissue stiffness (Figure 1). In addition, up-regulation in TAZ has been shown to promote the epithelial–mesenchymal transition, a process that contributes to an expanded myofibroblast population in tissues in fibrotic pathologies.⁸⁰ Excitingly, pharmacologic inhibition of YAP/TAZ with G α_s -coupled dopamine receptor D1 reverses bleomycin-induced fibrosis in mice when administered starting 10 days after bleomycin injury.⁸¹ Systemic YAP/TAZ inhibition with the receptor agonist ACT-333679 also illustrates the antifibrotic effects *in vitro*.⁸²

The myocardin-related transcription factors (MRTFs) A and B act as cofactors for serum response factor; they are mechanosensitive transcription factors that regulate genes encoding structural and regulatory effectors of actin dynamics. The biomechanical cellular environment regulates MRTF nuclear translocation because MRTF binds to G-actin. Investigation of human lung fibroblasts grown on polyacrylamide gels of physiologically healthy and fibrotic stiffness showed that MRTF-A is required for stiffness-induced myofibroblast differentiation.¹³ Furthermore, inhibition of MRTF-A signaling ameliorates bleomycin-induced fibrosis in mice.^{83,84} In addition, inhibition of MRTF signaling with CCG-203971 enhances fibroblast apoptosis in experimental models of fibrosis.⁸⁵ Deletion of MRTF-A also reduced fibrosis after myocardial infarction,⁸⁶ indicating potential overlapping mechanisms across multiple organ fibroses. However, MRTF-A/B and YAP/TAZ cross-talk with SMAD3 downstream of TGF- β ,^{87–89} indicating a number of redundant and confounding profibrotic pathways.

Therapeutic Implications

Targeting of the matrix and mechanotransduction pathways has emerged as a potential therapeutic approach for IPF. Cessation of the progressive stiffening of the ECM is likely to be a key component of halting the overall progression of disease. Previous clinical studies have investigated inhibiting the progressive stiffening caused by matrix cross-linking activity (LOXL2) (<https://www.clinicaltrials.gov>; NCT01769196) and mechanosensing by integrins (<https://www.clinicaltrials.gov>; NCT03573505). RAINIER [Study to Assess the Efficacy and Safety of Simtuzumab (GS-6624) in Adults with Idiopathic Pulmonary Fibrosis (IPF); <https://www.clinicaltrials.gov>; NCT01769196] was a Phase II randomized controlled trial investigating the potential role of simtuzumab, an antibody

targeting LOXL2, in patients with IPF.³⁰ Although the medication was safe, it failed to reach the primary or secondary outcomes, including mortality, disease-free progression, forced vital capacity (percent) decline, and 6-minute walk distance. It is not clear why this trial did not achieve the primary outcome. It is possible that the inhibitor was effective at reducing matrix cross-linking, but that it was insufficient to inhibit further myofibroblast differentiation and proliferation. Another possibility is that other matrix cross-linking enzymes may have compensated for the decreased LOXL2 activity to maintain a stable level of collagen cross-linking.

Despite the success of preclinical animal studies,⁵¹ the SPIRIT trial (An Efficacy and Safety Study of BG00011 in Participants With Idiopathic Pulmonary Fibrosis; <https://www.clinicaltrials.gov>; NCT03573505) investigating the role of a monoclonal antibody against integrin $\alpha v \beta 6$ was terminated early because of safety concerns.⁹⁰ However, there are several promising therapies currently in phase 2 clinical trials.⁹¹

There is mounting evidence that fibrosis is perpetuated by multiple overlapping feed-forward loops,⁹² of which mechanical stiffness is only one. Due to this redundancy, it may be unrealistic that a “magic bullet” on a sole target will have the ability to halt IPF progression. We envision that the future of IPF therapy will incorporate a fibroblast blocker (eg, nintedanib, pirfenidone) in combination with blockades of multiple accessory pathways such as matrix cross-linking (TG2, LOX-like enzymes), matrix sensing, Wnt signaling,⁹³ metabolism,^{56,94} and others to halt fibrosis by acting on major and accessory pathways involved in the disease. In this context, matrix stiffening may be one of the important targets, with other therapeutics focusing on growth factors, myofibroblasts, or damaged epithelium. Preventing lung fibroblasts from sensing the stiffness of their environment and responding to it by using sensor or transcription factor inhibitors might have more potential than blocking cross-linking enzymes, as that would reduce the profibrotic activities of fibroblasts in established fibrotic lesions as well as at the fibrotic front (fibrosis/normal interface). For example, targeting the sensing of altered mechanics (eg, integrins, TRP channels, Piezo channels) or the transcription factors that direct gene expression in response to stiffness (eg, YAP/TAZ, MRTF) is expected to down-regulate multiple important pathways, including matrix production and cross-linking by TG2, LOX, and LOXLs (Figure 1).

Direct inhibition of cross-linking enzymes is not expected to reverse excess cross-links in established fibrotic areas. However, targeting mechanotransduction sensors and transcription factors has the benefit of blocking profibrotic stiffness signals, even in fibroblasts located in established fibrotic zones, and may allow the cell to return to a more homeostatic state. In addition, it may be worth re-evaluating candidates (eg, simtuzumab, integrin blocking) that reported efficacy in preclinical animal models but were not successful as monotherapies in human trials in new combinations with pirfenidone or nintedanib to see if they would be

effective in combination and possibly at lower doses that would present fewer safety issues.

Overall, complex pathologic conditions such as fibrosis will likely require multipronged therapeutic approaches targeting several pathways, similar to the way we treat many cancers. Current US Food and Drug Administration–approved therapies exhibit some efficacy in slowing the progression of fibrosis. However, the involvement of multiple pathways potentially requires targeting of these independent mechanisms to achieve the goal of halting disease progression. Interestingly, both therapies for IPF (pirfenidone and nintedanib) currently approved by the US Food and Drug Administration have multifactorial modes of action that are still being investigated. The potential of combining antifibrotic therapies has already been explored both *in vitro*⁹⁵ and *in vivo* in the INJOURNEY (Safety, Tolerability and PK of Nintedanib in Combination With Pirfenidone in IPF) trial.⁹⁶

Future Directions

There are several gaps in knowledge that, when filled, will advance our understanding of the role of matrix mechanics and mechanotransduction pathways in the progression of pulmonary fibrosis. One step will be to test the therapeutic benefit of inhibiting identified mechanics altering pathways, mechanosensors, and transcription factors in preclinical models of pulmonary fibrosis, discussed earlier in *Therapeutic Implications*. The overarching goal of targeting mechanotransduction mechanisms is to block the profibrotic mechanical signal and halt further progression of fibrosis, likely when combined with other options (eg, pirfenidone or nintedanib) currently approved by the US Food and Drug Administration. Researchers have yet to explore the potential synergistic effects of combining approved therapies with targeting mechanotransduction or mechanical changes. Moreover, identifying ways to selectively interfere with the fibrotic process in the lung, by either focusing on targets specific for the respiratory system or by using medication vehicles that ensure a localized delivery (ie, inhaled aerosols) may increase the safety of these interventions by limiting the systemic side effects. Another objective will be to further elucidate mechanotransduction pathways, not only in fibroblasts but also in epithelial cells. To date, the mechanical characterization of lung tissue has primarily been limited to the assessment of matrix stiffness. However, more complex mechanical properties alter cell behavior. To better understand the role of altered matrix mechanics, we need comprehensive mechanical characterization of all compartments of the lung, including viscoelastic properties of healthy and diseased lung. Following the identification of altered mechanical properties, there will be a need to systematically characterize how altered mechanics in disease progression drives cellular response of not just fibroblasts but all predominant cell types within the lung.

Conclusions

The importance of biomechanical signaling in disease pathogenesis is now accepted as a critical driver of disease progression. Increased tissue stiffness within the fibrotic foci is sensed and translated into profibrotic gene expression that further increases stiffness. Recent studies reviewed here help elucidate the role of mechanotransduction in the progression of IPF. It remains uncertain if and how mechanotransduction plays a role in the initiation of the disease. Targeting mechanotransduction pathways to treat IPF has the potential, when combined with other therapeutic interventions, to halt the progression of fibrosis. However, much work is still needed to elucidate pathologic mechanisms of applied mechanical forces independent of and in conjunction with the altered biochemical composition of the ECM. Beyond halting the expansion of the fibrotic matrix, long-term goals aim to degrade and regenerate the native lung environment to restore homeostatic cell–cell and cell–matrix interactions. Collectively, these advances in understanding mechanotransduction feed-forward loops in fibrosis have the potential to provide opportunities for identifying novel therapeutic targets for combination therapies to halt the progression of IPF.

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