Extracellular matrix as a driver of progressive fibrosis

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The extracellular matrix (ECM) is dynamically tuned to optimize physiological function. Its major properties, including composition and mechanics, profoundly influence cell biology. Cell-ECM interactions operate through an integrated set of sensor and effector circuits that use several classes of receptors and signal transduction pathways. At the single-cell level, the ECM governs differentiation, metabolism, motility, orientation, proliferation, and survival. At the cell population level, the ECM provides higher-order guidance that is essential for physiological function. When pathological changes in the ECM lead to impairment of organ function, we use the term "fibrosis." In this Review, we differentiate fibrosis initiation from progression and focus primarily on progressive lung fibrosis impairing organ function. We present a working model to explain how the altered ECM is not only a consequence but also a driver of fibrosis. Additionally, we advance the concept that fibrosis progression occurs in a fibrogenic niche that is composed of a fibrogenic ECM that nurtures fibrogenic mesenchymal progenitor cells and their fibrogenic progeny.

Introduction

As a group, fibrotic disorders of the heart, blood vessels, lungs, kidney, liver, and other organs account for more than a third of the annual death rate in industrialized countries (1). Fibroproliferation is integral to host defense. Rapid closure of an integumentary or visceral wound is essential for restoration of tissue and organ integrity. Control of resistant intracellular pathogen infections is accomplished by formation of a dense circumferential scar. When fibroproliferation ensues after a single discrete injury, the process can be reversible or result in a durable scar comprising highly cross-linked collagens and other extracellular matrix (ECM) components. Examples of reversibility include many instances of integumentary wounding (2) and acute lung injury when the cause is controlled (3, 4). Durable fibrosis follows myocardial infarction (5) and tuberculosis (6). Sustained fibroproliferation, commonly designated as aberrant wound healing, frequently occurs after repetitive or persistent injurious stimuli. Examples include alcoholic cirrhosis, hypertension-induced kidney fibrosis, autoimmune disease, and uncontrolled chronic infection. In each of these circumstances, injury triggers a canonical coagulation/innate/adaptive immune response (7). Fibroproliferation ceases or regresses when the injurious stimulus is successfully mitigated or terminated or when the immune response is pharmacologically modulated or spontaneously abates. However, studies of idiopathic pulmonary fibrosis (IPF), a relentlessly progressive disorder, have revealed mechanisms of fibrosis progression that can be self-sustaining once established (8, 9). This indicates that fibrosis initiation and progression can be uncoupled. As a relatively new concept in the field of fibrosis, this Review will primarily focus on self-sustaining aspects of progressive fibrosis, emphasizing studies of the lung with examples from other organ systems.

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The ECM as a driver of fibrosis progression

Progressive fibrosis remains one of the most vexing problems in modern medicine. Its seeming intractability does not result from a lack of scientific attention. Instead, we suspect that the formulation guiding even the best studies may be incomplete. Most experimental work has been guided by inferences from the advanced state of knowledge about fibrosis that follows a discrete injury, not by studies focused on self-sustaining progressive fibrosis as a discrete entity. As is the case for cancer biology, conceptually separating fibrosis initiation from fibrosis progression may enable the field to move forward. An extensive body of work across several organs implicates parenchymal cell injury with activation of the TGF-β pathway in disease initiation (10–12). This line of investigation continues to provide increasingly precise information about the molecular mechanisms leading to parenchymal cell attrition, setting the stage for fibroproliferation (13, 14).

In parallel with our increased understanding of fibrosis initiation, we have learned that fibrosis progression involves both cell-intrinsic/autonomous and ECM-driven mechanisms. Cell-autonomous fibrogenicity was initially identified in studies using primary mesenchymal cells from fibrotic tissue and organs (15–20), corroborated in zebrafish and mouse xenograft models (21, 22), and verified in mouse lineage tracing studies (23). The discovery of fibrogenic mesenchymal progenitor cells (MPCs) in the lungs of patients with IPF provided definitive proof in humans (9, 24). A role for individual ECM components and fragments as drivers of fibrosis progression has been firmly established for several decades. Fragments of fibrin, fibronectin, and hyaluronan are all potentially fibrogenic (25). More recent studies have provided new insights into ECM-mediated positive-feedback loops using decellularized lung ECM from patients with IPF (8, 26). In the absence of exogenous cytokines, IPF ECM alone induces normal lung fibroblasts to become activated myofibroblasts and to downregulate microRNA-29 (miR-29), a master negative regulator of stromal genes. Once formed, IPF ECM sets up a profibrotic feedback loop that is capable of sustaining progressive fibrosis (Figure

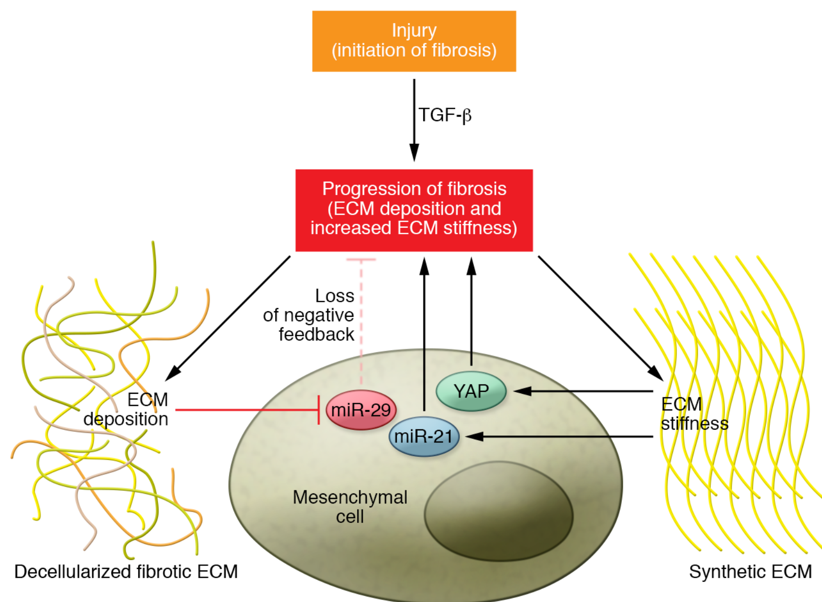

Figure 1. ECM-mediated feedback loops during fibrosis initiation and progression. (Upper) Tissue injury leads to TGF-β activation and downstream canonical and noncanonical signals that initiate fibrosis. Once initiated, fibrosis can progress in the absence of the initial stimulus. (Lower) The fibrotic ECM can suppress miR-29, a master negative regulator of stromal genes. This results in increased ribosome recruitment to hundreds of stromal genes and sustained deposition of ECM, thus constituting a positive-feedback loop. Increased matrix stiffness activates the Hippo pathway effector Yes-associated protein 1 (YAP), which can drive ECM deposition and matrix stiffening, constituting another positive-feedback loop. Mesenchymal progenitor cell mechanical memory of substratum stiffness is mediated by miR-21, allowing these progenitors to stably maintain their fibrogenic phenotype and further stiffen the ECM.

1). Increased substratum stiffness activates the mechanosensitive Hippo pathway effector Yes-associated protein 1 (YAP). Active YAP, in turn, upregulates ECM deposition and stiffness, constituting another positive-feedback loop (27). In MPCs, increased stiffness mediates acquisition of mechanical memory by causing a sustained increase of miR-21, a positive regulator of ECM deposition (28). Thus, although the evidence across all organs is incomplete, available data support the idea that progressive fibrosis in the absence of ongoing injury occurs in a *fibrogenic niche* comprising fibrogenic progenitors and their fibrogenic progeny residing in an ECM that is itself fibrogenic.

In the text that follows, we consider the ECM in progressive fibrosis as part of a fibrogenic niche and focus on ECM characteristics that may generate and sustain fibrogenic mesenchymal progenitors and their progeny. The ECM characteristics we consider are *composition* and *mechanical properties*, both of which can have profound effects on cell biology. Our goal is to help illuminate a way forward toward a more complete understanding of self-sustaining progressive fibrosis. The reader is referred to other Reviews in this series for more details about the cell of origin in fibrotic disorders (29). We also refer the reader to the many excellent reviews of fibrosis initiation and fibrosis progression in the wake of a sustained or repetitive injury; and to reviews detailing the important emerging field of fibrosis reversibility (30, 31), where studies of liver fibrosis reversal after eradication of hepatitis C virus are leading the way.

ECM composition in progressive organ fibrosis

The ECM microenvironment provides cells with physical support for adhesion and cues that regulate position, cell cycle, metabolism, and differentiated state (32). The ECM is a major source of biochemical and biomechanical signals that are transduced and integrated to determine tissue organization and function. In general, excessive ECM production and aberrant ECM turnover characterize progressive organ fibrosis. In addition, dysregulation of ECM remodeling enzymes causes disorganization of ECM structure and ECM fragmentation, resulting in pathological cellular functions that contribute to fibrosis progression (33). Since ECM deposition and remodeling are dynamic processes during fibrosis progression, a comprehensive understanding of the spatial and temporal alterations in ECM composition is essential to fully elucidate the mechanisms underlying the progression of organ fibrosis. To illustrate, we will focus on the spatial-temporal heterogeneity of the ECM and cells characterizing the fibroblastic focus in IPF.

Myofibroblast core. Progressive fibrosis often has a characteristic pattern in each organ (34–37). We will focus on the pattern and polarity of fibrosis in the progressive fibrotic lung disease IPF, where recent studies provide insight into mechanism. In IPF, mesenchymal cells and their ECM products expand the alveolar wall, resulting in distortion and loss of the gas-exchange surface. The process begins at the bases and subpleural regions of the lungs and advances centripetally and apically (38). Studies of the IPF matrisome using mass spectrometry indicate that the IPF ECM is enriched for hyaluronan, latent TGF-β–binding protein 1, periostin, versican, fibulin, fibrillin, and a variety of collagens (26). However, how these alterations in ECM composition vary both regionally and temporally as IPF progresses remains incompletely understood.

Current understanding of the spatial-temporal heterogeneity in the IPF lung has been inferred from immunohistochemical analyses, which have been pivotal in elucidating regional differences in ECM composition and organization. More than 25 years ago, a seminal morphological investigation using a monoclonal antibody specific for pro–collagen I revealed that the myofibroblast core of the fibroblastic focus is the site of active ECM deposition (39). The myofibroblast core in IPF is enriched with fibrillar type I and III collagens, extra domain A fibronectin (EDA fibronectin), and fibrin; whereas the presence of type IV collagen varies (40). The myofibroblast core is also enriched with type VI collagen (41), the migratory moieties fascin and tenascin C (42), hyaluronan (43), and TGF-β (44).

Building on this foundation, recent work clarifies some of the molecular details in IPF. Indeed, the fibroblastic focus is a polar-

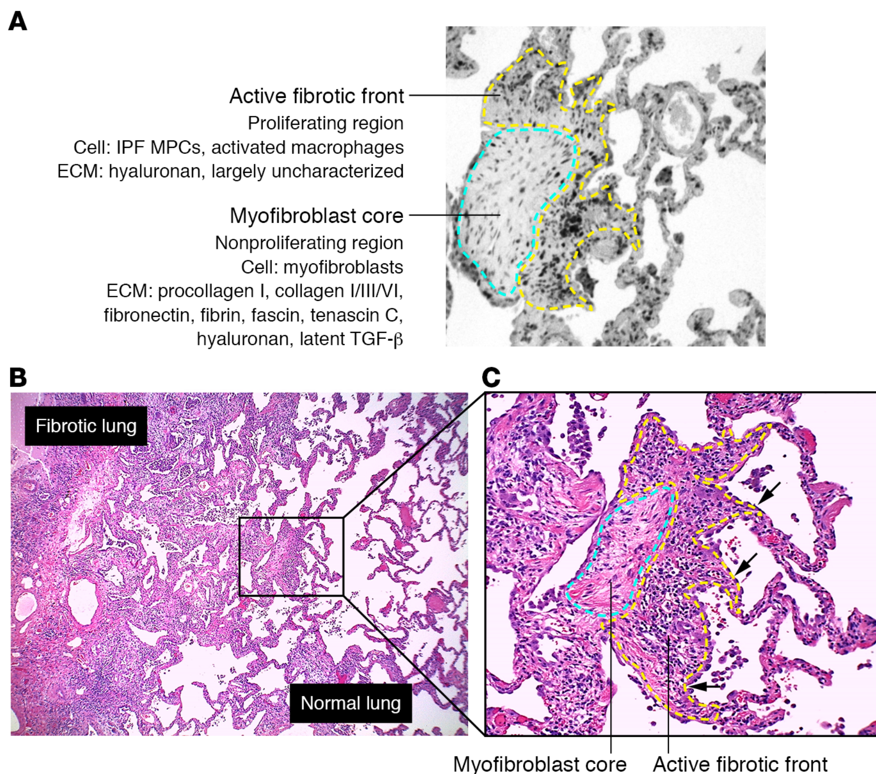

Figure 2. Polarity of the IPF Fibroblastic Focus. (**A**) The fibroblastic focus in IPF is polarized. It contains an active fibrotic front, which is a highly cellular region composed of proliferating fibrogenic MPCs, and activated macrophages embedded in a hyaluronan-rich ECM. The myofibroblast core contains noncycling myofibroblasts actively synthesizing collagen embedded in an ECM rich in collagen I/III/VI, fibronectin, fibrin, fascin, tenascin C, hyaluronan, and latent TGF-β. (**B**) An example of what is likely a newly developing fibroblastic focus (boxed region) at the advancing fibrotic front at the interface between fibrotic lung on the left and relatively uninvolved lung on the right. (**C**) Higher-power image of the boxed region in panel **B** showing the myofibroblast core and the active fibrotic front. At the periphery of the focus, thickened alveolar walls are juxtaposed between the active fibrotic front and morphologically preserved thin alveolar structures (indicated by arrows). This appearance supports a model of fibrosis progression in which cells in the active fibrotic front invade into contiguous morphologically preserved alveolar structures, causing progressive fibrotic destruction of the gas-exchange surface. The mesenchymal cells behind the fibrotic front (the progeny of IPF MPCs) differentiate into myofibroblasts that constitute the fibrotic core. Images adapted from Xia et al. (9).

ized structure composed of a mitotically active fibrotic front containing MPCs and their progeny, as well as a myofibroblast core region containing noncycling myofibroblasts actively synthesizing type I collagen (Figure 2 and ref. 9). The mechanism underlying the excessive collagen deposition by myofibroblasts involves fibrotic ECM-mediated suppression of miR-29, a master negative regulator of ECM genes (8). Thus, once formed, fibrotic ECM sets up a positive profibrotic feedback loop that stimulates further ECM production. TGF-β is expressed within the myofibroblast core (39, 44), and TGF-β stimulates the expression of EDA fibronectin, which promotes myofibroblast differentiation (45). In addition to fibrillar collagen and EDA fibronectin, glycosaminoglycans (GAGs) and versican are also abundant within the myofibroblast core (43). Versican is a chondroitin sulfate proteoglycan that promotes a variety of fibrogenic cellular functions, including proliferation, motility, and invasion (46). Importantly, the deposition of proteoglycans and GAGs alters the viscoelastic properties of the lung ECM, which in turn are capable of regulating fibroblast biology (47).

The active fibrotic front. The active fibrotic front is found at the perimeter of the myofibroblast core in a highly cellular area between the myofibroblast core and relatively preserved alveolar structures. It contains proliferating, fibrogenic MPCs and their progeny together with activated macrophages (9, 48). Hyaluronan is abundantly expressed in the IPF lung (41), including the progenitor-rich perimeter region, and is known to be an important component of the stem/progenitor cell niche in health and disease. It is tempting to speculate that in the IPF active fibrotic front, the presence of hyaluronan might support the fibrogenic MPC pool, thus serving as an integral component of the fibrogenic niche. Expansion of self-renewing MPCs and their transit-amplifying progeny, together with recruitment of immune-modulatory cells, might lead to enlargement of adjacent alveolar walls en route to forming another myofibroblast core. In addition, hyaluronan stimulates invasion and overexpression of hyaluronic acid synthase 2 by mesenchymal cells, which confers them with an invasive phenotype (49). Disruption of CD44, a major hyaluronan receptor, inhibits mesenchymal cell invasiveness. These data support a model of fibrosis progression in which hyaluronan nurtures MPCs and stimulates mesenchymal cells within the active fibrotic front to invade adjacent uninvolved alveolar walls and mediate progressive fibrotic lung destruction. As a group, these studies highlight how regional differences in ECM composition can create a niche that regulates cellular phenotype and drives fibrotic progression.

Although the ECM can direct cell biology in a manner that either facilitates tissue repair or drives pathological remodeling, there is also evidence that during fibrosis progression, mesenchymal cells with autonomous behavior can emerge (50). Autonomous functions include the ability to elude physiological signals that mediate negative feedback provided by the ECM. For example, during physiological tissue repair, polymerized type I collagen ligates the $\alpha_2\beta_1$ integrin to activate the antimitotic tumor suppressor phosphatase PTEN to limit cell proliferation (51). In contrast, in IPF, altered integrin signaling fails to increase PTEN, allowing unfettered activation of the PI3K/Akt signaling pathway. This permits IPF lung mesenchymal cells to escape the proliferation-suppressive effects of polymerized collagen that normally terminate collagen deposition by fibroblasts (17, 51) and activate apoptosis (52, 53). These data indicate that at some point during the course of fibrosis progression, mesenchymal cells can acquire a distinct and durable fibrogenic phenotype such that they no longer respond to the ECM cues that would typically terminate a fibrotic

response. Most of the molecular details of how a durable fibrogenic phenotype is acquired remain to be elucidated; however, the acquisition of fibrogenic mechanical memory in response to ECM stiffness in a miR-21–dependent manner is one mechanism (28). Thus, once in motion, fibrosis progression is biologically robust. Fibrogenic ECM corrupts the miR-29 and miR-21 axes in cells still dependent on exogenous signals, and cell-autonomous fibrogenic MPCs produce fibrogenic daughter cells that differentiate to become activated myofibroblasts.

There are intriguing parallels between the ECM compositions of the invasive cancer front, the active fibrotic front, and the myofibroblast core in IPF. Like in IPF, hyaluronan accumulates in cancer tissues, where it supports cancer stem cell function (54) and cancer cell proliferation, motility, and viability (55). Hyaluronan cross-links with a variety of other ECM molecules, including versican, which itself cross-links with type I collagen, fibulin, fibrillin, and fibronectin to facilitate tumor cell invasion (56). The ECM of the invasive breast cancer front is characterized by collagen deposition, linearization, and thickening, together with an abundance of activated macrophages and high TGF-β activity (57). These findings support the view that during breast cancer progression, collagen deposition and linearization are linked to immune cell infiltration and activation of TGF-β. The similarities between the ECM of the invasive cancer front and the active fibrotic front of IPF align with the idea that common alterations in ECM composition and structure underlie the progressive nature of these diseases. Parallels with cancer remain to be explored in IPF and other organs undergoing progressive fibrosis.

A possible fibrogenic niche in cirrhosis. The pattern of cirrhosis following liver injury depends on the causative agent. Fibrosis begins in the portal tracts in chronic hepatitis C virus (HCV) infection and around the central vein in alcoholic liver disease. In chronic HCV infection, fibrosis progression manifests as fibrotic septa extending from portal tracts that connect to create bridging fibrosis. Each bridge is composed of fibrotic ribbons that interconnect branches of portal tracts. A proteomics approach applied to human HCV-infected liver has defined the ECM composition of bridging fibrosis, the phase of the disease that characterizes the transition from moderate to severe fibrosis (58). In cirrhosis, the space of Disse (the perisinusoidal region containing plasma and hepatic stellate cells [HSCs]) and portal tracts are sites of myofibroblast accumulation and ECM deposition. These myofibroblast-rich regions are replete with fibrillar type I and III collagens (59), fibronectin (60), and type V and VI collagen (61). Type IV collagen expression is variable and codistributes with laminin (62, 63). Increased expression of the migratory marker tenascin C has been found within the space of Disse and portal ducts (64). Hyaluronan is codistributed with α-smooth muscle actin–expressing cells within portal tracts (65), and TGF-β is expressed within fibrous septa (66). These studies highlight similarities of ECM composition within the myofibroblast-rich regions in lung and liver fibrosis, consistent with the idea of some shared underlying mechanisms.

Although speculative, it is possible to extend the concept of the fibrogenic niche to cirrhosis. Fate-tracing experiments suggest that HSCs may be a source of fibrogenic fibroblasts in some liver fibrosis models (67), although the evidence remains inconclusive (68, 69). Although not considered true progenitors, HSCs share properties with mesenchymal progenitors, including the capacity for tri-lineage differentiation (70, 71). HSCs reside in a perisinusoidal location in the space of Disse (72, 73), which, as a working hypothesis, can be conceptualized as a putative fibrogenic niche. Activated HSCs appear in increased numbers in regions of perisinusoidal fibrosis and bridging fibrosis. Like IPF MPCs, HSCs undergo differentiation to activated myofibroblasts in response to cytokines (74) and increased ECM stiffness (35, 36).

ECM mechanical properties

A hallmark of fibrotic tissue is an increase in its elastic modulus (stiffness). In human fibrosis of the lung (26, 27), liver (75, 76), kidney (77, 78), and vasculature (79), the ECM on average becomes stiffer than normal. Mechanotransduction of ECM stiffness plays important biological roles. Mechanotransduction pathways impact such critical cellular functions as proliferation, differentiation, and migration (32). Here we focus specifically on how mechanotransduction of ECM cues might influence fibrosis progression. The reader is referred to another Review in this series for a more comprehensive consideration of mechanotransduction in fibrosis (80).

One prominent mechanism for mechanotransduction of ECM stiffness operates through the Hippo pathway effector YAP. This mechanism serves to illustrate some principles underlying mechanotransduction of ECM inputs. Cell-ECM and cell-cell interactions play key roles in regulating YAP activity. Cell-ECM interactions are mediated predominantly by integrins, proteoglycan receptors, and their ligands at sites of focal adhesions where a variety of sensor proteins (e.g., focal adhesion kinase [FAK], talin, vinculin, the non-receptor tyrosine kinase SRC) interpret ECM mechanics and elicit downstream signaling responses (81). Cell-cell interactions operating through E-cadherin at sites of adherens/tight junctions modulate a cascade of protein signaling components that integrate and interpret mechanical inputs (82). Increased ECM stiffness can drive fibroblast ECM production in a YAP-dependent manner (27), and YAP expression has been identified in fibrosis of the lung (27), liver (83), and polycystic kidney disease (84) in humans. Enforced YAP expression in experimental animal models of lung fibrosis drives fibrosis progression (27). In addition, treatment with a pharmacological inhibitor of YAP function inhibits fibrosis in a fibrogenic kidney injury model (85); and YAP suppression in liver injury models reduces fibrosis (83). In the context of cancer stroma, cancer-associated fibroblasts express active YAP, which is required for ECM stiffening and maintenance of their pathological functions (86). These studies indicate that mechanotransduction of ECM stiffness mediated through YAP has the ability to serve as an integral component of the molecular mechanism for fibrosis progression.

ECM spatial heterogeneity and mechanotransduction in fibrosis

On average, fibrotic tissue is stiffer than healthy tissue. However, reassessment of reported stiffness of human fibrotic tissue compared with healthy tissue reveals substantial overlaps in the distributions of elastic moduli; in some cases, these necessitate large sample sizes to discern average stiffness differences in lung (26)

and liver (87). One group reports nonsignificant elastic modulus differences comparing healthy and fibrotic human kidneys (88). In part, these data likely reflect the striking spatial heterogeneity of the ECM in fibrotic organs. This is relevant because current efforts to test the importance of increased ECM stiffness in fibrosis progression are hampered by the limited availability of regional mechanical data. We currently lack information about the mechanical properties of densely scarred regions versus the myofibroblast core versus the active fibrotic front versus relatively preserved areas in any form of organ fibrosis.

Cirrhosis assumes a variety of morphological patterns that are dependent on etiology: portal-portal, portal-central, and central-central septa (89). To date, few studies have used atomic force microscopy (AFM) to measure the elastic modulus of human liver tissue (90), and none have registered the mechanical properties of the ECM to specific morphological patterns in the cirrhotic liver. It will be important to understand the precise mechanical properties sensed by each type of cell within each region of each morphological pattern to develop the most clinically relevant models of fibrosis progression in an etiology- and pattern-specific manner.

IPF is composed of a multifocal network of fibroblastic foci that stand as independent structures ranging from 1.3×10^4 to 9.9×10^7 m^3 (91). Fibroblastic foci are polarized structures with a myofibroblast core that actively produce ECM adjacent to normal alveolar structures (9). It is therefore likely that there are stiffness gradients along the fibroblastic focus, a steep stiffness gradient between the focus and adjacent normal structures, and another gradient between the focus and regions dominated by dense collections of ECM. Available AFM data from the IPF lung indicate that stiffness gradients span very short distances (26); however, there are no published data systematically registering elastic modulus to specific morphological regions, the fibroblastic focus, transitional alveoli with the earliest signs of thickening and cellular infiltration, adjacent uninvolved alveoli, or mature scar. The prevailing assumption in the field is that the cells within the fibroblastic focus sense a pathologically stiff ECM that drives fibrosis progression (26, 27, 92, 93). A critical knowledge gap in the field of progressive fibrosis is the exact stiffness a cell senses in situ as fibrosis progresses and which receptors and transduction pathways are involved.

Modeling ECM mechanotransduction in fibrosis

A seminal study showed that MSCs cultured on a stiff 2-dimensional (2D) substratum activate YAP (94), spurring a line of investigation that used a variety of biomaterials with well-defined properties to model how cells respond to mechanical stimuli (95). In addition to stiffness, key ECM properties that can modulate cell biology include dimensionality, viscoelasticity, and cyclic stretch. Moreover, cell-cell interactions can influence the response to both static and dynamic mechanical properties. To focus the discussion, control of YAP will serve as the example.

Dimensionality. In MSCs on a 2D substratum, ECM stiffness drives YAP translocation from the cytoplasm (inactive YAP) to the nucleus (active YAP) (94). However, in most tissues and organs, the cells enveloped in a fibrotic ECM experience a 3D environment. To examine the impact of dimensionality, investigators created hydrogels with tunable stiffness and cultured MSCs either on a 2D planar surface or embedded within a 3D hydrogel (96). In the 2D system, increasing stiffness drove YAP activation, whereas in the 3D system, exactly the opposite occurred, with stiffness promoting YAP inactivation. This report demonstrates the important role played by substratum dimensionality in cellular mechanosensing of ECM stiffness.

Viscoelasticity. Not only do cells perceive the difference between a 2D and a 3D environment, but they also sense changes in ECM viscoelasticity (stress-relaxation), defined as the force exerted by the ECM to maintain its original structure followed by its relaxation. When MSCs are cultured in 2D or 3D hydrogels in which both stiffness and viscoelasticity can be tuned, increasing viscoelasticity inactivates YAP independent of elastic modulus and dimensionality (97, 98).

Cyclic stretch. All organs undergo cyclic stretch with the periodicity of pulsatile blood flow (1 to 3 Hz). The lungs have the superimposed periodicity of the respiratory cycle, which varies by nearly an order of magnitude comparing healthy lungs with late-stage fibrotic lungs (0.1 vs. 1 Hz at rest). Thus, another mechanical property to account for is the mechanical strain caused by stretch. For example, mammary epithelial cells activate YAP in response to cyclic stretch, which promotes their proliferation (99). Cyclic stretch-compression can also regulate miR-29 and thereby collagen expression in periodontal ligament cells (100).

Cell-cell interactions. A more complete model of how cells interact with their ECM will account for cell-cell interactions. To illustrate, a hyaluronic acid hydrogel system was developed that enabled the independent copresentation of the HAVDI adhesive motif from N-cadherin (simulating cell-cell interactions) and the RGD adhesive motif from fibronectin (simulating cell-ECM interactions) to MSCs across a physiological range of ECM stiffness (101). An increase in HAVDI ligation with RGD ligation held constant led to reduced nuclear YAP localization with resultant modulation of cell proliferation and differentiation. Mechanistically, there was a reduction in contractile force generation due to the ability of N-cadherin ligation to dampen the Rac1-GTP/myosin IIA/focal adhesion signaling axis triggered by integrin ligation (101).

Mechanotransduction of ECM stiffness is one important mechanical property in fibrosis progression. However, the studies cited above, which track YAP activity in the context of ECM stiffness, dimensionality, viscoelasticity, cyclic stretch, and cell-cell interactions, demonstrate the importance of accounting for multiple parameters when constructing a model of the in vivo biology of progressive fibrosis. It is important to note that other mechanosensitive pathways also intersect with YAP signaling networks. For instance, mechanotransduction through Notch generates signals that form a positive-feedback loop with YAP. This feedback loop can be inhibited by Wnt/β-catenin signaling (102). It will be important to determine how other mechanotransduction pathways (103), such as FAK, ROCK/RhoA, and actin cytoskeletal rearrangements, are altered by ECM cues. Thus, a clinically relevant model of ECM mechanosensing will need to account for a network of inputs and signaling pathways.

Does ECM contribute to a fibrogenic niche in progressive fibrosis?

One provisional model for the stereotypical anatomic patterns of progressive fibrosis is that the actively developing lesions consti-

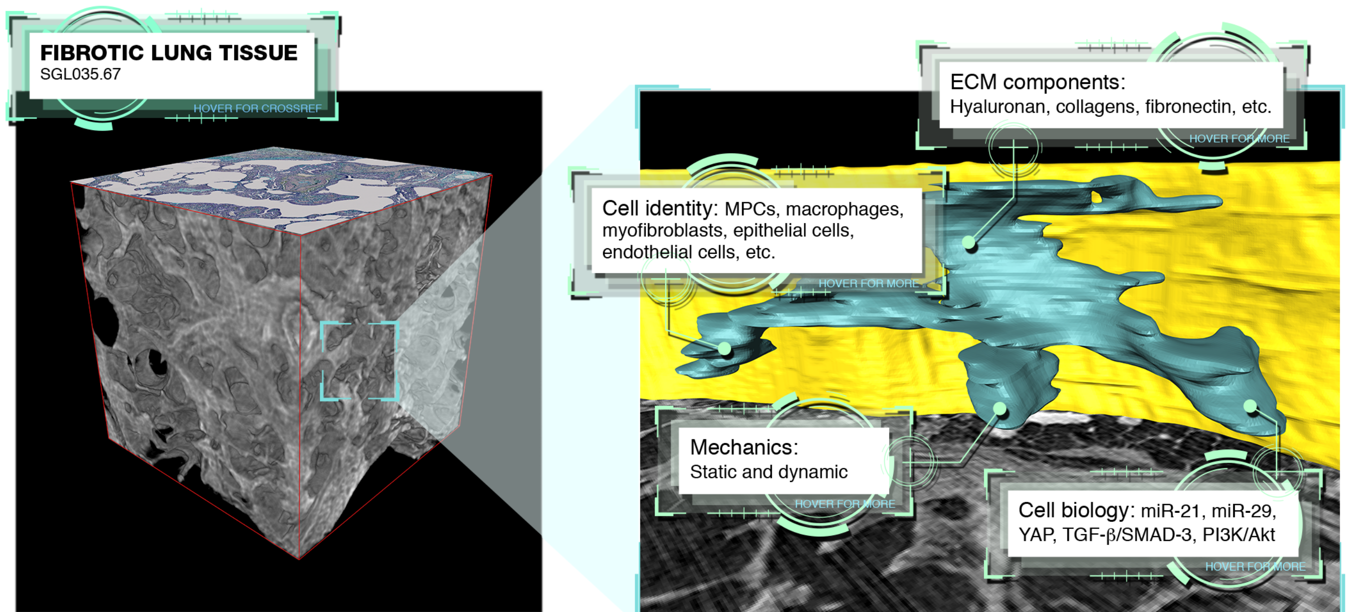

Figure 3. Tissue atlas: 3-D reconstruction of a fibrogenic niche coregistering mechanics, ECM composition, cell identity, and cell biology. Shown is a conceptual schematic of a tissue atlas using IPF as an example. Images adapted from Jones et al. (91). A comprehensive tissue atlas would combine – at both the micron and millimeter scale of resolution – static and dynamic mechanical measurements, data regarding ECM composition and organization, cell identity, cell differentiated state, and cell biology (e.g., proliferation markers, signaling footprints). These data would be registered region by region to key morphological features: myofibroblast core and active fibrotic front. With such a data set, investigators would be positioned to generate testable models that pinpoint targetable pathways critical to fibrosis progression based on (a) the precise mechanical properties a cell is sensing, (b) the ECM components a cell is interacting with, and (c) the resulting cell biology as a function of those inputs. Addition of MALDI-imaging mass spectrometry to the picture could provide unprecedented insights into progressive fibrosis (105, 106).

tute a fibrogenic niche. In our proposed model, these niches are to be distinguished from regions dominated by highly cross-linked type I collagen, and regions yet to be enveloped by fibrosis. The ECM in the fibrogenic niche is compartmentalized, with its composition and mechanical properties organized in a manner that nurtures, supports, and guides all of the cells that mediate fibrosis progression: self-renewing MPCs, actively proliferating transit-amplifying cells, and pathological myofibroblasts that persist and actively secrete ECM. This model has been applied and tested in IPF, in which all of these cell populations can be found in situ in distinct regions of highly polarized fibroblastic foci. However, a detailed analysis of the lung ECM in the niche compared with adjacent regions has not been conducted. In the liver, HSCs display some progenitor cell characteristics (70, 71). Whether all HSCs can serve this role or only a subpopulation can function as progenitors remains to be determined. Similarly, whether the properties of the ECM in the regions where HSCs undergo self-renewal differ from those where differentiation into activated myofibroblasts occurs is an open question. We currently lack fine-mapping data defining ECM composition, organization, and mechanics related to their fibrogenic properties. Thus, we regard the concept of a distinct fibrogenic niche with fibrogenic progenitors as the source of activated myofibroblasts in progressive lung and liver fibrosis not as fact, but as one working model to guide future experiments.

A key feature of progressive fibrosis in all of these settings is the robustness of the fibrotic process. Indeed, many of the forces that *initiate* fibrosis persist as fibrosis *progresses*. These include epithelial stress, activated TGF-β, activated macrophages, and growth factors for fibroblasts. What may distinguish self-limited fibrosis after injury (i.e., the canonical process of fibroproliferation that is critical for repair and regeneration) from progressive fibrosis in the apparent absence of ongoing injury is an inflection point or a singularity in an otherwise well-behaved negative-feedback system. At this point of departure from negative feedback, many or all of the canonical fibrosis drivers become dispensable. We refer to this as cell-autonomy in the same sense as cancer is cell-autonomous; the cells from these lesions are fibrogenic in fibrosis or tumorigenic in cancer. To extend the analogy, as in cancer, in vivo progression in fibrosis is a pernicious dance between the ECM and the intrinsically pathological cells. In both IPF and cancer, compelling data implicate cell-ECM collaboration as integral to the robustness of disease progression.

A tissue atlas as a possible way forward

There is compelling evidence that fibrotic ECM is chemically, topographically, and mechanically distinct from ECM in healthy tissue. Fibrosis varies dynamically in time and space and assumes stereotypical patterns of progression in each organ. Moreover, a variety of cell types across a spectrum of proliferative, differentiated, and activated states participate in fibrosis progression. What are lacking are data to integrate all of this disparate but critical information. To date, no study in any human fibrotic disorder has coregistered ECM mechanical properties, orientation, and composition with cell identity and relevant parameters of cell biology. Thus, despite advances in the field of fibrosis, we lack an agreed-upon model of fibrosis progression. In our opinion, the database

for such a model of fibrosis progression could take the form of a *tissue atlas*. A comprehensive tissue atlas would combine static and dynamic mechanical measurements, ECM composition and organization, cell identity, cell differentiated state, and cell biology (e.g., proliferation markers, signaling footprints) at both micron and millimeter resolution, registered region by region according to key morphological features in human fibrosis (Figure 3).

Imaging technology and computing power have advanced to the point where it is feasible to tackle this undertaking. As an example, in studying the morphology of fibroblastic foci in IPF about a decade ago, investigators used early-generation 3D reconstruction algorithms to conclude that fibroblastic foci in IPF formed a large interconnected fibrotic reticulum (104). Recently, more advanced 3D reconstruction approaches revealed that fibroblastic foci are serpiginous independent structures, not interconnected — a fact with important mechanical implications (91). Adding cell biology and detailed ECM information to facilitate accurate modeling of these lesions is one path toward a much deeper mechanistic understanding of fibrotic progression. With such a data set, investigators would be positioned to generate testable models that pinpoint targetable pathways critical to fibrosis progression based on (a) the precise mechanical properties a cell is sensing; (b) the ECM components that the cell interacts with; and (c) the resulting cell biology as a function of these inputs. Such a study could pave the way forward for the creation of a model that recapitulates the in vivo biology and generate data-driven hypotheses aimed at unveiling the molecular mechanisms mediating fibrosis progression. We view this as one important step toward a strategic drug discovery program designed to interdict progressive fibrosis.

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1. Rockey DC, Bell PD, Hill JA. Fibrosis — a common pathway to organ injury and failure. *N Engl J Med*. 2015;373(1):96.
2. Desmoulière A, Badid C, Bochaton-Piallat ML, Gabbiani G. Apoptosis during wound healing, fibrocontractive diseases and vascular wall injury. *Int J Biochem Cell Biol*. 1997;29(1):19–30.
3. Kapanci Y, Weibel ER, Kaplan HP, Robinson FR. Pathogenesis and reversibility of the pulmonary lesions of oxygen toxicity in monkeys. II. Ultrastructural and morphometric studies. *Lab Invest*. 1969;20(1):101–118.
4. Polunovsky VA, et al. Role of mesenchymal cell death in lung remodeling after injury. *J Clin Invest*. 1993;92(1):388–397.
5. Prabhu SD, Frangogiannis NG. The biological basis for cardiac repair after myocardial infarction: from inflammation to fibrosis. *Circ Res*. 2016;119(1):91–112.
6. Hunter RL. Tuberculosis as a three-act play: a new paradigm for the pathogenesis of pulmonary tuberculosis. *Tuberculosis (Edinb)*. 2016;97:8–17.
7. Henson PM. Dampening inflammation. *Nat Immunol*. 2005;6(12):1179–1181.
8. Parker MW, et al. Fibrotic extracellular matrix activates a profibrotic positive feedback loop. *J Clin Invest*. 2014;124(4):1622–1635.
9. Xia H, et al. Calcium-binding protein S100A4 confers mesenchymal progenitor cell fibrogenicity in idiopathic pulmonary fibrosis. *J Clin Invest*. 2017;127(7):2586–2597.
10. Ahluwalia N, Shea BS, Tager AM. New therapeutic targets in idiopathic pulmonary fibrosis. Aiming to rein in runaway wound-healing responses. *Am J Respir Crit Care Med*. 2014;190(8):867–878.
11. Friedman SL, Sheppard D, Duffield JS, Violette S. Therapy for fibrotic diseases: nearing the starting line. *Sci Transl Med*. 2013;5(167):167sr1.
12. Henderson NC, et al. Targeting of αv integrin identifies a core molecular pathway that regulates fibrosis in several organs. *Nat Med*. 2013;19(12):1617–1624.
13. Young LR, et al. Epithelial-macrophage interactions determine pulmonary fibrosis susceptibility in Hermansky-Pudlak syndrome. *JCI Insight*. 2016;1(17):e88947.
14. Liang J, et al. Hyaluronan and TLR4 promote surfactant-protein-C-positive alveolar progenitor cell renewal and prevent severe pulmonary fibrosis in mice. *Nat Med*. 2016;22(11):1285–1293.
15. Diegelmann RF, Cohen IK, McCoy BJ. Growth kinetics and collagen synthesis of normal skin, normal scar and keloid fibroblasts in vitro. *J Cell Physiol*. 1979;98(2):341–346.
16. Wilborn J, Crofford LJ, Burdick MD, Kunkel SL, Strieter RM, Peters-Golden M. Cultured lung fibroblasts isolated from patients with idiopathic pulmonary fibrosis have a diminished capacity to synthesize prostaglandin E2 and to express cyclooxygenase-2. *J Clin Invest*. 1995;95(4):1861–1868.
17. Xia H, et al. Pathological integrin signaling enhances proliferation of primary lung fibroblasts from patients with idiopathic pulmonary fibrosis. *J Exp Med*. 2008;205(7):1659–1672.
18. Rodemann HP, Müller GA. Abnormal growth and clonal proliferation of fibroblasts derived from kidneys with interstitial fibrosis. *Proc Soc Exp Biol Med*. 1990;195(1):57–63.
19. Bhattacharyya S, Wei J, Varga J. Understanding fibrosis in systemic sclerosis: shifting paradigms, emerging opportunities. *Nat Rev Rheumatol*. 2011;8(1):42–54.
20. Galambos JT, Hollingsworth MA, Falek A, Warren WD, McCain JR. The rate of synthesis of glycosaminoglycans and collagen by fibroblasts cultured from adult human liver biopsies. *J Clin Invest*. 1977;60(1):107–114.
21. Pierce EM, et al. Therapeutic targeting of CC ligand 21 or CC chemokine receptor 7 abrogates pulmonary fibrosis induced by the adoptive transfer of human pulmonary fibroblasts to immunodeficient mice. *Am J Pathol*. 2007;170(4):1152–1164.
22. Benyumov AO, Hergert P, Herrera J, Peterson M, Henke C, Bitterman PB. A novel zebrafish embryo xenotransplantation model to study primary human fibroblast motility in health and disease. *Zebrafish*. 2012;9(1):38–43.
23. Rinkevich Y, et al. Skin fibrosis. Identification and isolation of a dermal lineage with intrinsic fibrogenic potential. *Science*. 2015;348(6232):aaa2151.
24. Xia H, et al. Identification of a cell-of-origin for fibroblasts comprising the fibrotic reticulum in idiopathic pulmonary fibrosis. *Am J Pathol*. 2014;184(5):1369–1383.
25. Genovese F, Karsdal MA. Protein degradation fragments as diagnostic and prognostic biomarkers of connective tissue diseases: understanding the extracellular matrix message and implication for current and future serological biomarkers. *Expert Rev Proteomics*. 2016;13(2):213–225.
26. Booth AJ, et al. Acellular normal and fibrotic human lung matrices as a culture system for in vitro investigation. *Am J Respir Crit Care Med*. 2012;186(9):866–876.
27. Liu F, et al. Mechanosignaling through YAP and TAZ drives fibroblast activation and fibrosis. *Am J Physiol Lung Cell Mol Physiol*. 2015;308(4):L344–L357.
28. Li CX, et al. MicroRNA-21 preserves the fibrotic mechanical memory of mesenchymal stem cells. *Nat Mater*. 2017;16(3):379–389.
29. Di Carlo SE, Peduto L. The perivascular origin of pathological fibroblasts. *J Clin Invest*. 2018;128(1):54–63.
30. Jun JI, Lau LF. Resolution of organ fibrosis. *J Clin Invest*. 2018;128(1):97–107.
31. Vinaixa C, Strasser SI, Berenguer M. Disease reversibility in patients with post-Hepatitis C cirrhosis: is the point of no return the same before and after liver transplantation? A review. *Transplantation*. 2017;101(5):916–923.
32. Bonnans C, Chou J, Werb Z. Remodelling the extracellular matrix in development and disease. *Nat Rev Mol Cell Biol*. 2014;15(12):786–801.
33. Mouw JK, Ou G, Weaver VM. Extracellular matrix assembly: a multiscale deconstruction. *Nat Rev Mol Cell Biol*. 2014;15(12):771–785.
34. Katzenstein AL, Myers JL. Idiopathic pulmo-

nary fibrosis: clinical relevance of pathologic classification. *Am J Respir Crit Care Med*. 1998;157(4 pt 1):1301–1315.
35. Hoofring A, Boitnott J, Torbenson M. Three-dimensional reconstruction of hepatic bridging fibrosis in chronic hepatitis C viral infection. *J Hepatol*. 2003;39(5):738–741.
36. Mauer SM et al. Structural-functional relationships in diabetic nephropathy. *J Clin Invest*. 1984;74(4):1143–1155.
37. Bataller R, Brenner DA. Liver fibrosis. *J Clin Invest*. 2005;115(2):209–218.
38. Raghu G, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Respir Crit Care Med*. 2011;183(6):788–824.
39. Kuhn C, McDonald JA. The roles of the myofibroblast in idiopathic pulmonary fibrosis. Ultrastructural and immunohistochemical features of sites of active extracellular matrix synthesis. *Am J Pathol*. 1991;138(5):1257–1265.
40. Kuhn C, Boldt J, King TE, Crouch E, Vartio T, McDonald JA. An immunohistochemical study of architectural remodeling and connective tissue synthesis in pulmonary fibrosis. *Am Rev Respir Dis*. 1989;140(6):1693–1703.
41. Specks U, Nerlich A, Colby TV, Wiest I, Timpl R. Increased expression of type VI collagen in lung fibrosis. *Am J Respir Crit Care Med*. 1995;151(6):1956–1964.
42. Chilosi M, et al. Migratory marker expression in fibroblast foci of idiopathic pulmonary fibrosis. *Respir Res*. 2006;7:95.
43. Bensadoun ES, Burke AK, Hogg JC, Roberts CR. Proteoglycan deposition in pulmonary fibrosis. *Am J Respir Crit Care Med*. 1996;154(6 pt 1):1819–1828.
44. Broekelmann TJ, Limper AH, Colby TV, McDonald JA. Transforming growth factor beta 1 is present at sites of extracellular matrix gene expression in human pulmonary fibrosis. *Proc Natl Acad Sci U S A*. 1991;88(15):6642–6646.
45. Serini G, et al. The fibronectin domain ED-A is crucial for myofibroblastic phenotype induction by transforming growth factor-beta1. *J Cell Biol*. 1998;142(3):873–881.
46. Ricciardelli C, Sakko AJ, Ween MP, Russell DL, Horsfall DJ. The biological role and regulation of versican levels in cancer. *Cancer Metastasis Rev*. 2009;28(1–2):233–245.
47. Venkatesan N, et al. Glycosyltransferases and glycosaminoglycans in bleomycin and transforming growth factor-β1-induced pulmonary fibrosis. *Am J Respir Cell Mol Biol*. 2014;50(3):583–594.
48. Yang L, et al. IL-8 mediates idiopathic pulmonary fibrosis mesenchymal progenitor cell fibrogenicity [published online ahead of print August 31, 2017]. *Am J Pathol*. https://doi.org/10.1152/ajplung.00200.2017.
49. Li Y, et al. Severe lung fibrosis requires an invasive fibroblast phenotype regulated by hyaluronan and CD44. *J Exp Med*. 2011;208(7):1459–1471.
50. Noble PW, Barkauskas CE, Jiang D. Pulmonary fibrosis: patterns and perpetrators. *J Clin Invest*. 2012;122(8):2756–2762.
51. Xia H, et al. Low α(2)β(1) integrin function enhances the proliferation of fibroblasts from patients with idiopathic pulmonary fibrosis by activation of the β-catenin pathway. *Am J Pathol*. 2012;181(1):222–233.
52. Nho RS, Hergert P. IPF fibroblasts are desensitized to type I collagen matrix-induced cell death by suppressing low autophagy via aberrant Akt/mTOR kinases. *PLoS One*. 2014;9(4):e94616.
53. Nho RS, Peterson M, Hergert P, Henke CA. FoxO3a (Forkhead Box O3a) deficiency protects idiopathic pulmonary fibrosis (IPF) fibroblasts from type I polymerized collagen matrix-induced apoptosis via caveolin-1 (cav-1) and Fas. *PLoS One*. 2013;8(4):e61017.
54. Borovski T, De Sousa E Melo F, Vermeulen L, Medema JP. Cancer stem cell niche: the place to be. *Cancer Res*. 2011;71(3):634–639.
55. Schwertfeger KL, Cowman MK, Telmer PG, Turley EA, McCarthy JB. Hyaluronan, inflammation, and breast cancer progression. *Front Immunol*. 2015;6:236.
56. Varga I, et al. Brevican, neurocan, tenascin-C and versican are mainly responsible for the invasiveness of low-grade astrocytoma. *Pathol Oncol Res*. 2012;18(2):413–420.
57. Acerbi I, et al. Human breast cancer invasion and aggression correlates with ECM stiffening and immune cell infiltration. *Integr Biol (Camb)*. 2015;7(10):1120–1134.
58. Baiocchini A, et al. Extracellular matrix molecular remodeling in human liver fibrosis evolution. *PLoS One*. 2016;11(3):e0151736.
59. Grimaud JA, Druguet M, Peyrol S, Chevalier O, Herbage D, El Badrawy N. Collagen immunotyping in human liver: light and electron microscope study. *J Histochem Cytochem*. 1980;28(11):1145–1156.
60. Hahn E, Wick G, Pencev D, Timpl R. Distribution of basement membrane proteins in normal and fibrotic human liver: collagen type IV, laminin, and fibronectin. *Gut*. 1980;21(1):63–71.
61. Mak K. Codistribution of collagens V and VI with collagens I and III in hepatic fibrosis of elderly cadavers. *FASEB J*. 2015;29(1 Suppl):544.4.
62. Mak KM, Chu E, Lau KH, Kwong AJ. Liver fibrosis in elderly cadavers: localization of collagen types I, III, and IV, α-smooth muscle actin, and elastic fibers. *Anat Rec (Hoboken)*. 2012;295(7):1159–1167.
63. Mak KM, Chen LL, Lee TF. Codistribution of collagen type IV and laminin in liver fibrosis of elderly cadavers: immunohistochemical marker of perisinusoidal basement membrane formation. *Anat Rec (Hoboken)*. 2013;296(6):953–964.
64. Van Eyken P, Sciot R, Desmet VJ. Expression of the novel extracellular matrix component tenascin in normal and diseased human liver. An immunohistochemical study. *J Hepatol*. 1990;11(1):43–52.
65. Ichida T, et al. Localization of hyaluronan in human liver sinusoids: a histochemical study using hyaluronan-binding protein. *Liver*. 1996;16(6):365–371.
66. Bedossa P, Peltier E, Terris B, Franco D, Poynard T. Transforming growth factor-β1 (TGF-β1) and TGF-β1 receptors in normal, cirrhotic, and neoplastic human livers. *Hepatology*. 1995;21(3):760–766.
67. Mederacke I, et al. Fate tracing reveals hepatic stellate cells as dominant contributors to liver fibrosis independent of its aetiology. *Nat Commun*. 2013;4:2823.
68. Tarlow BD, Finegold MJ, Grompe M. Clonal tracing of Sox9$^+$ liver progenitors in mouse oval cell injury. *Hepatology*. 2014;60(1):278–289.
69. Schaub JR, Malato Y, Gormond C, Willenbring H. Evidence against a stem cell origin of new hepatocytes in a common mouse model of chronic liver injury. *Cell Rep*. 2014;8(4):933–939.
70. Kordes C, Sawitza I, Götze S, Herebian D, Häussinger D. Hepatic stellate cells contribute to progenitor cells and liver regeneration. *J Clin Invest*. 2014;124(12):5503–5515.
71. Kordes C, Sawitza I, Götze S, Häussinger D. Hepatic stellate cells support hematopoiesis and are liver-resident mesenchymal stem cells. *Cell Physiol Biochem*. 2013;31(2–3):290–304.
72. Olsen AL, et al. Hepatic stellate cells require a stiff environment for myofibroblastic differentiation. *Am J Physiol Gastrointest Liver Physiol*. 2011;301(1):G110–G118.
73. Guvendiren M, Perepelyuk M, Wells RG, Burdick JA. Hydrogels with differential and patterned mechanics to study stiffness-mediated myofibroblastic differentiation of hepatic stellate cells. *J Mech Behav Biomed Mater*. 2014;38:198–208.
74. Mews P, et al. Pancreatic stellate cells respond to inflammatory cytokines: potential role in chronic pancreatitis. *Gut*. 2002;50(4):535–541.
75. Mueller S, Sandrin L. Liver stiffness: a novel parameter for the diagnosis of liver disease. *Hepat Med*. 2010;2:49–67.
76. Zhao G, et al. Mechanical stiffness of liver tissues in relation to integrin β1 expression may influence the development of hepatic cirrhosis and hepatocellular carcinoma. *J Surg Oncol*. 2010;102(5):482–489.
77. Stock KF, et al. ARFI-based tissue elasticity quantification in comparison to histology for the diagnosis of renal transplant fibrosis. *Clin Hemorheol Microcirc*. 2010;46(2–3):139–148.
78. Arndt R, et al. Noninvasive evaluation of renal allograft fibrosis by transient elastography — a pilot study. *Transpl Int*. 2010;23(9):871–877.
79. Briones AM, Arribas SM, Salaices M. Role of extracellular matrix in vascular remodeling of hypertension. *Curr Opin Nephrol Hypertens*. 2010;19(2):187–194.
80. Tschumperlin DJ, Ligresti G, Hilscher MB, Shah VH. Mechanosensing and fibrosis. *J Clin Invest*. 2018;128(1):74–84.
81. DuFort CC, Paszek MJ, Weaver VM. Balancing forces: architectural control of mechanotransduction. *Nat Rev Mol Cell Biol*. 2011;12(5):308–319.
82. Harvey KF, Zhang X, Thomas DM. The Hippo pathway and human cancer. *Nat Rev Cancer*. 2013;13(4):246–257.
83. Mannaerts I, et al. The Hippo pathway effector YAP controls mouse hepatic stellate cell activation. *J Hepatol*. 2015;63(3):679–688.
84. Happé H, et al. Altered Hippo signalling in polycystic kidney disease. *J Pathol*. 2011;224(1):133–142.
85. Szeto SG, et al. YAP/TAZ are mechanoregulators of TGF-β-Smad signaling and renal fibrogenesis. *J Am Soc Nephrol*. 2016;27(10):3117–3128.
86. Calvo F, et al. Mechanotransduction and YAP-dependent matrix remodelling is required for the generation and maintenance of cancer-associated fibroblasts. *Nat Cell Biol*. 2013;15(6):637–646.
87. Coco B, et al. Transient elastography: a new surrogate marker of liver fibrosis influenced by

major changes of transaminases. *J Viral Hepat*. 2007;14(5):360–369.

88. Syversveen T, Midtvedt K, Berstad AE, Brabrand K, Strøm EH, Abildgaard A. Tissue elasticity estimated by acoustic radiation force impulse quantification depends on the applied transducer force: an experimental study in kidney transplant patients. *Eur Radiol*. 2012;22(10):2130–2137.
89. Karsdal MA, et al. Novel insights into the function and dynamics of extracellular matrix in liver fibrosis. *Am J Physiol Gastrointest Liver Physiol*. 2015;308(10):G807–G830.
90. Saneyasu T, Akhtar R, Sakai T. Molecular cues guiding matrix stiffness in liver fibrosis. *Biomed Res Int*. 2016;2016:2646212.
91. Jones MG, et al. Three-dimensional characterization of fibroblast foci in idiopathic pulmonary fibrosis. *JCI Insight*. 2016;1(5):e86375.
92. Marinković A, Liu F, Tschumperlin DJ. Matrices of physiologic stiffness potently inactivate idiopathic pulmonary fibrosis fibroblasts. *Am J Respir Cell Mol Biol*. 2013;48(4):422–430.
93. Huang X, et al. Matrix stiffness-induced myofibroblast differentiation is mediated by intrinsic mechanotransduction. *Am J Respir Cell Mol Biol*. 2012;47(3):340–348.
94. Dupont S, et al. Role of YAP/TAZ in mechanotransduction. *Nature*. 2011;474(7350):179–183.
95. Caliari SR, Burdick JA. A practical guide to hydrogels for cell culture. *Nat Methods*. 2016;13(5):405–414.
96. Caliari SR, Vega SL, Kwon M, Soulas EM, Burdick JA. Dimensionality and spreading influence MSC YAP/TAZ signaling in hydrogel environments. *Biomaterials*. 2016;103:314–323.
97. Chaudhuri O, et al. Hydrogels with tunable stress relaxation regulate stem cell fate and activity. *Nat Mater*. 2016;15(3):326–334.
98. Chaudhuri O, et al. Substrate stress relaxation regulates cell spreading. *Nat Commun*. 2015;6:6364.
99. Codelia VA, Sun G, Irvine KD. Regulation of YAP by mechanical strain through Jnk and Hippo signaling. *Curr Biol*. 2014;24(17):2012–2017.
100. Chen Y, et al. Cyclic stretch and compression forces alter microRNA-29 expression of human periodontal ligament cells. *Gene*. 2015;566(1):13–17.
101. Cosgrove BD, et al. N-cadherin adhesive interactions modulate matrix mechanosensing and fate commitment of mesenchymal stem cells. *Nat Mater*. 2016;15(12):1297–1306.
102. Kim W, et al. Hippo signaling interactions with Wnt/β-catenin and Notch signaling repress liver tumorigenesis. *J Clin Invest*. 2017;127(1):137–152.
103. Duscher D, et al. Mechanotransduction and fibrosis. *J Biomech*. 2014;47(9):1997–2005.
104. Cool CD, Groshong SD, Rai PR, Henson PM, Stewart JS, Brown KK. Fibroblast foci are not discrete sites of lung injury or repair: the fibroblast reticulum. *Am J Respir Crit Care Med*. 2006;174(6):654–658.
105. Cornett DS, Reyzer ML, Chaurand P, Caprioli RM. MALDI imaging mass spectrometry: molecular snapshots of biochemical systems. *Nat Methods*. 2007;4(10):828–833.
106. Aichler M, Walch A. MALDI imaging mass spectrometry: current frontiers and perspectives in pathology research and practice. *Lab Invest*. 2015;95(4):422–431.