FIBROSIS

Therapy for Fibrotic Diseases: Nearing the Starting Line

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Fibrosis, or the accumulation of extracellular matrix molecules that make up scar tissue, is a common feature of chronic tissue injury. Pulmonary fibrosis, renal fibrosis, and hepatic cirrhosis are among the more common fibrotic diseases, which in aggregate represent a huge unmet clinical need. New appreciation of the common features of fibrosis that are conserved among tissues has led to a clearer understanding of how epithelial injury provokes dysregulation of cell differentiation, signaling, and protein secretion. At the same time, discovery of tissue-specific features of fibrogenesis, combined with insights about genetic regulation of fibrosis, has laid the groundwork for biomarker discovery and validation, and the rational identification of mechanism-based antifibrotic drugs. Together, these advances herald an era of sustained focus on translating the biology of fibrosis into meaningful improvements in quality and length of life in patients with chronic fibrosing diseases.

INTRODUCTION

Despite contributing to as much as 45% of deaths in the industrialized world (*1*), fibrotic diseases have been largely overlooked—until now. Over the past 25 years, our understanding of the pathogenesis of fibrosis has coalesced into a coherent view of how tissues accumulate collagen-rich extracellular matrix (ECM) (that is, scar tissue) in response to tissue injury. The vibrancy of a recent Keystone Symposium on Tissue Fibrosis (*2*) attests to the growing recognition that fibrotic diseases are becoming therapeutically tractable.

In this review, we convey our evolving understanding of how tissue injury and repair lead to fibrosis and present promising new approaches for diagnosis and treatment of fibrotic diseases that follow from that understanding. We emphasize features common to all forms of fibrosis, as well as tissue-specific pathways that may facilitate therapeutic development (*3*). We also highlight the emerging consensus about the sources of fibrogenic cells, relevant translational models, and obstacles to progress. Finally, we describe new treatments already in clinical trials that exemplify the range of strategies harnessed by several pharmaceutical and biotechnology companies.

COMMON FEATURES OF FIBROSIS ACROSS TISSUES

Excessive tissue scarring (that is, fibrosis) is a common feature of most chronic diseases. In epithelial organs, especially the lung, liver, skin, and kidney, the replacement of normal functional units of cells with collagen-rich scar tissue and the architectural distortion caused by scar retraction are major factors in progressive loss of organ function and eventual failure. Because each of these organs has a different purpose, is exposed to diverse environmental factors, and is composed of different cell types, there are unique features and consequences of tissue fibrosis among these organs. There are also, however, core features shared by pathologic fibrosis among multiple organs (Fig. 1).

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Epithelial injury and dysfunction

Recurrent or persistent epithelial injury is a core element that both initiates and sustains progressive fibrosis (Figs. 1 and 2). In most currently used animal models of fibrosis, the disease is provoked by severe initial injury to epithelium (for example, bleomycin and fluorescein isothiocyanate treatment or thoracic irradiation in lung, carbon tetrachloride or bile duct ligation in liver, and ureteral obstruction or cisplatin in kidney). Immunological attack of epithelial cells can also induce pathologic fibrosis. Examples include ligation of the transmembrane death receptor Fas by Fas ligand on epithelium in lung and liver (*4*, *5*) and immunological injury to skin and other organs in graft-versus-host disease (*6*, *7*) or scleroderma (*8*).

At least five responses to injury-induced functional or physical disruption of epithelial cells can provoke tissue fibrosis. One is cell death, which can occur as a consequence of injury through either apoptosis or necrosis (*9*). It remains uncertain if epithelial cell death directly initiates downstream fibrotic pathways in fibrogenic cells or is a marker indicating that injury has been severe enough to provoke surviving cells to initiate tissue fibrosis.

A second common epithelial response to injury is the dysregulation of metabolic pathways, which results in cell stress and activation. Cell and tissue injury alter local microperfusion, oxygen nutrient delivery, maintenance of pH, and toxin removal. Cells respond to this hostile environment with a multitude of stress responses, which collectively promote cell survival (*10*). These cell responses are frequently insufficient, and the result is cell death. In chronic diseases, cellular stress responses can be persistently activated as a survival adaptation. Such stressed cells are the source of proinflammatory cytokines, chemokines, and other factors that can stimulate myofibroblasts and their precursors.

The unfolded protein response, or endoplasmic reticulum (ER) stress, is a stereotypical protective reaction that can mitigate the consequences of accumulated unfolded or poorly folded proteins (*11*). Certain secretory cells, such as type 2 alveolar epithelial cells, pancreatic β cells, and hepatocytes, may be especially prone to ER stress because of the large amount of protein transiting the ER in these cells. For example, in families with dominantly inherited pulmonary fibrosis, mutations in two major secretory products of alveolar epithelial cells, surfactant proteins A (*12*) and C (*13*, *14*), cause disease. These mutations impair protein folding and induce ER stress (*13*, *15*). Similarly,

markers of ER stress have been identified in alveolar epithelial cells from patients with sporadic pulmonary fibrosis (*16*), and ER stress occurs in livers of patients with nonalcoholic and alcoholic fatty liver disease, as well as viral hepatitis and α_1-antitrypsin deficiency (*17*, *18*).

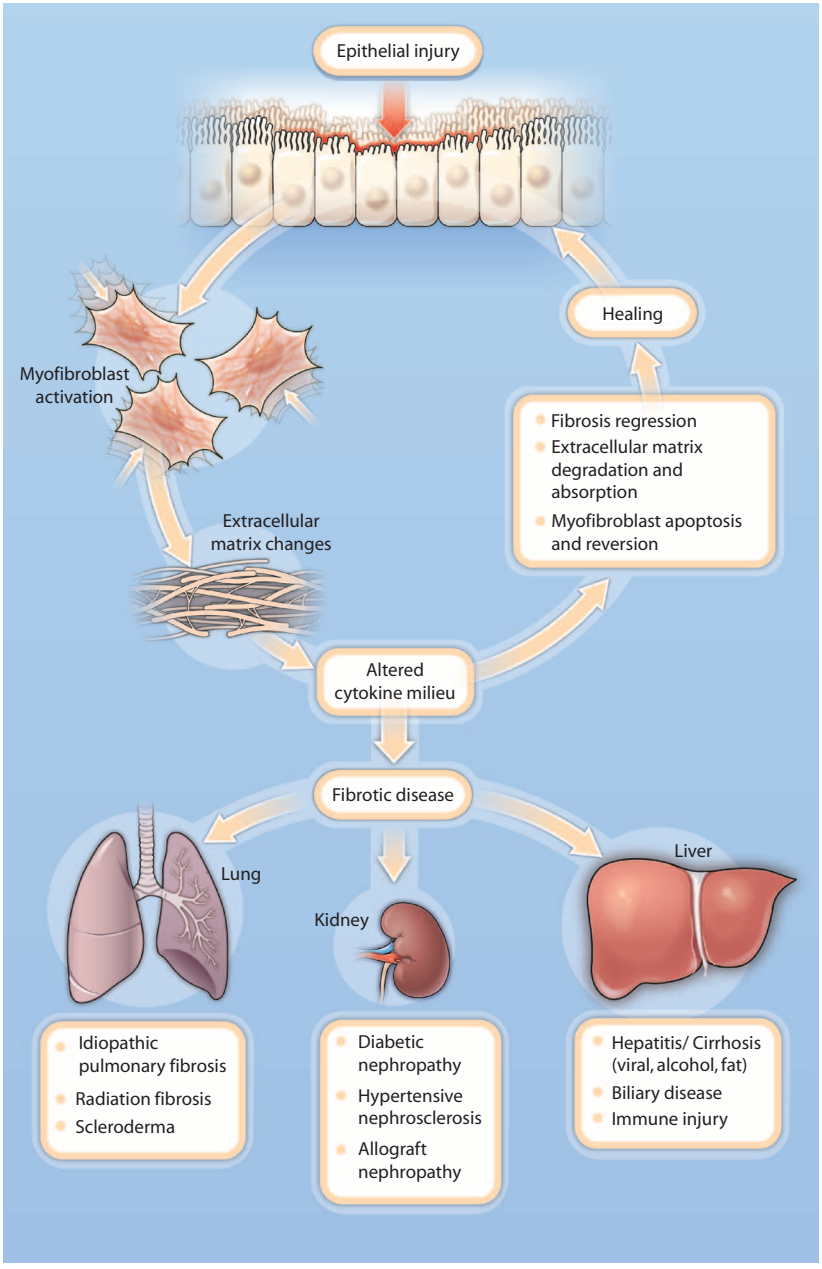

Fig. 1. Common events in fibrosis progression and regression across tissues. In tissues such as lung, kidney, and liver, fibrosis often occurs as a result of sustained injury to the epithelium, which causes the overproduction of cytokines and growth factors. These, in turn, promote the recruitment and activation of mesenchymal cell precursors to form myofibroblasts. The myofibroblasts secrete ECM proteins and promote an altered cytokine milieu that supports a fibrotic reaction. Under normal conditions, the fibrotic matrix is degraded, the myofibroblasts undergo either apoptosis or reversion to a nonactivated state, the epithelium undergoes repair, and the fibrosis resolves. In fibrotic disease, the normal repair and resolution processes are disrupted and the fibrotic reaction persists, leading to scarring and impaired organ function.

Also prominent among the cellular metabolic changes after injury is a marked change in adenosine 5′-triphosphate (ATP) generation. In harsh local environments, cells generate ATP from sources and by mechanisms that differ from those in healthy tissues. In particular, after injury, ATP is made through altered fatty acid metabolism, which leads to generation of damaging oxygen radicals. When fatty acids are oxidized through normal metabolic pathways regulated by the transcriptional regulators peroxisome proliferator–activated receptor α (PPARα) and PPARγ (*19*), cells are able to survive in the hostile environment of inflammation. However, cells that cannot generate sufficient ATP by fatty acid metabolism also generate increased levels of reactive oxygen species (ROS) through decoupling of mitochondria and a reduction in peroxisomal function. The ROS themselves stimulate the fibrotic process.

A third injury-triggered epithelial alteration is epithelial-to-mesenchymal transition (EMT), a discrete pattern of gene expression changes that parallels a similar process occurring normally during development (*20*, *21*). During both the pathologic and developmental versions of the EMT, there is decreased expression of key epithelial surface proteins, such as E-cadherin, and induction of the master regulatory transcription factors of EMT—Twist and Snail1. Although the EMT is no longer viewed as a significant source of fibrogenic cells (see 'SOURCES OF FIBROGENIC CELLS,' below), Twist- and Snail-driven cellular reprogramming contributes critically to tissue fibrosis through still poorly characterized mechanisms. For example, genetic loss of Snail1 within hepatocytes protects mice from carbon tetrachloride–induced liver fibrosis (*22*). Transforming growth factor–β (TGFβ), a major inducer of fibrosis, induces EMT markers, in part through induction of Twist and Snail expression in epithelial cells, and loss of TGFβ signaling in epithelial cells (by deletion of the TGFβ type II receptor) protects mice from bleomycin-induced pulmonary fibrosis (*23*). The transcriptional targets of Twist and Snail1 during development are well established (*24*), but it is not known whether these genes are relevant to fibrotic EMT.

A fourth common epithelial response to injury involves interactions between integrins and TGFβ. TGFβ is secreted by nearly all cells and organs in mammals and stored in large excess outside cells through chemical cross-links to the ECM (*25*). During its synthesis and secretion, the TGFβ gene product is cleaved and assembled as a double homodimer consisting of a disulfide-linked homodimer of the N-terminal cleavage fragment, called the latency-associated peptide (LAP), and a disulfide-linked homodimer of the shorter C-terminal fragment, which is the active cytokine. LAP is in turn disulfide-linked to members of another protein family (the latent TGFβ binding proteins), which are cross-linked to ECM proteins through the action of an extracellular enzyme, tissue transglutaminase (*25*). Upon injury, epithelial cells in the lung, kidney, and biliary tract express high levels of the integrin $\alpha_v\beta_6$ (*26*), which binds with high affinity to the LAP portion of latent TGFβ1 (*27*) and TGFβ3 (*28*, *29*).

The activation of the latent form of TGFβ is also regulated (Table 1) through the action of injury-induced danger signals from damaged cells, including phospholipids sphingosine 1-phosphate (*29*) and lysophosphatidic acid (LPA) (*30*), and the coagulation protease, thrombin (*31*). These mediators are released, or in the case of thrombin converted to their biologically active forms (that is, activated), with injury and subsequently bind to G protein (heterotrimeric guanine nucleotide–binding protein)–coupled receptors on the epithelial cells to induce actin/myosin-mediated contraction (*19*). Epithelial cell contraction exerts retractile force on the tethered latent TGFβ (transmitted through the bound $\alpha_v\beta_6$ integrin), inducing TGFβ activation and highly localized signaling (*29*).

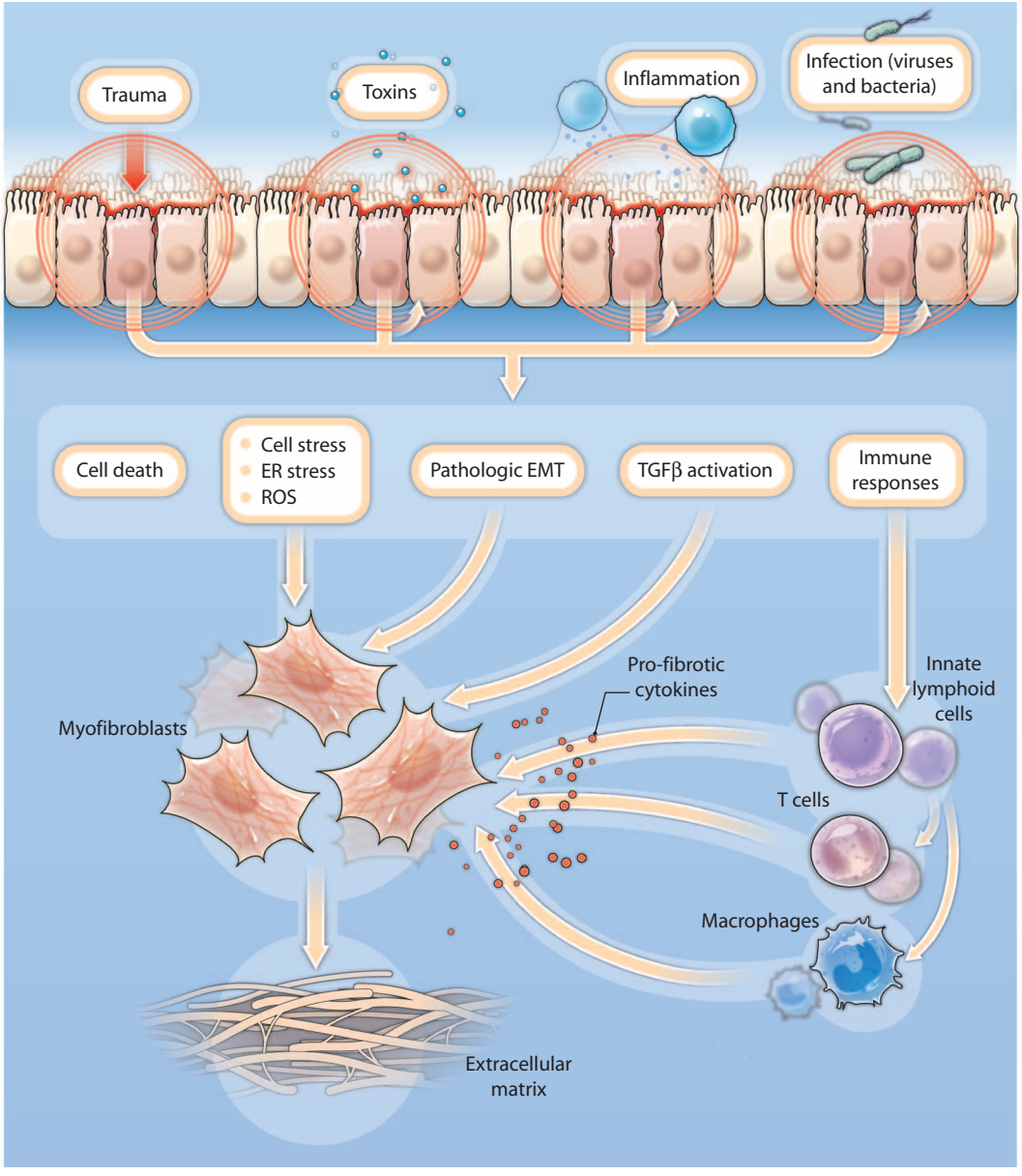

Fig. 2. Mechanisms by which epithelial injury can lead to organ fibrosis. A wide range of injurious stimuli initiate several distinct profibrotic programs in epithelial cells. In the most extreme case, injury induces cell death, which is sufficient to initiate fibrosis in a variety of fibrotic disease models. Injured epithelial cells also undergo dysregulated metabolism, leading to production of reactive oxygen species (ROS) and ER stress, or they can be reprogrammed toward a mesenchymal-like phenotype (pathologic EMT). In response to danger signals that are released or activated in response to injury, epithelial cells use cell-surface integrins to activate latent TGFβ, a well-characterized central mediator of tissue fibrosis. Finally, injured epithelial cells recruit and activate a variety of immune cells, which, in turn, release profibrotic cytokines, along with innate lymphoid cells, T cells and macrophages [for example, interleukin-13 (IL-13), IL-17, among many others]. Each of these perturbations of epithelial cells ultimately leads to expansion, recruitment, and/or activation of tissue myofibroblasts, the principal source of the pathologic ECM that characterizes organ fibrosis.

Through this process, epithelial cells at sites of injury expose neighboring cells to active TGFβ, initiating epithelial cell reprogramming or stimulating matrix production in adjacent fibroblasts (see below). TGFβ itself is a potent inducer of $\alpha_v\beta_6$ expression on epithelial cells (*32*, *33*), generating a critical positive feedback loop that accelerates the pace of subsequent fibrosis. In liver, the $\alpha_v\beta_6$ integrin is not expressed on hepatocytes but is present (and functionally important) on cholangiocytes (*34*, *35*), suggesting that a similar system may underlie fibrotic disorders of the bile ducts (for example, primary biliary cirrhosis or sclerosing cholangitis) but not of the hepatocytes. It is therefore less likely that this process contributes to parenchymal liver fibrosis, which makes up most of the chronic liver disease worldwide (a result of viruses, alcohol, or obesity).

Finally, in multiple models of fibrosis, injured epithelial cells initiate both innate and adaptive immune responses. As the first responders to environmental insults, epithelial cells secrete proinflammatory chemokines that can recruit neutrophils, monocytes, and lymphocytes into the injured organ to drive tissue fibrosis. Each of these cell types has been implicated in experimental models of organ fibrosis, but their roles in specific chronic fibrotic diseases in humans are less clear. The use of anti-inflammatory drugs in suppressing or reversing fibrosis

in the livers of patients with chronic hepatitis, and in the lungs of some patients with systemic inflammatory diseases such as rheumatoid arthritis, suggests that inflammatory cells contribute to fibrosis in an ongoing fashion in these clinical settings. However, the clear lack of benefit of anti-inflammatory drugs in idiopathic pulmonary fibrosis (IPF) suggests that other responses to epithelial injury are more prominent in this illness.

There is also an important local interaction between injured epithelial cells and distinct populations of hematopoietic-derived "guard cells" (for example, innate lymphoid cells) that are present in most organs and are poised to rapidly secrete cytokines such as interferon-γ, IL-13, and IL-17 (*36*). IL-13 and IL-17 may directly contribute to tissue fibrosis in the liver and lung, respectively.

Table 1. Definitions of terms used in this review.

Protein activation	Refers to the enzymatic conformational change induced in a protein by another protein, resulting in the generation of functionally active form of the protein, for example, by an active protein that can bind to a receptor.
Cell activation	Circumstances in which a cell (typically a mesenchymal cell) undergoes phenotypic changes as part of an injury response. This phenotypic change includes altered transcription leading to synthesis of new factors or augmented production of factors that contribute to disease pathogenesis. Cellular activation may manifest as migration, proliferation, deposition of matrix proteins, cytokine or chemokine production, acquisition of toxic or cytolytic properties, or increased expression or organization of contractile machinery (for example, expression of αSMA by myofibroblasts). Often used synonymously with transdifferentiation, but milder degrees of reprogramming are inferred.
Transdifferentiation	Mature somatic cell transforms into another mature somatic cell. Also known as lineage reprogramming or in the context of mesenchymal cells is sometimes called cell activation. Resident fibroblasts and pericytes transdifferentiate into pathological disease-associated fibroblasts or myofibroblasts.
Myofibroblast	A fibroblast-like cell of mesenchymal origin that has contractile properties and expresses the protein αSMA, with extensive rough ER, indicating a secretory phenotype. The cells generate extensive fibrillar matrix that contributes to the formation of tissue scars.
Fibroblast	A generalized term that may refer to resident mesenchymal cell in normal tissue embedded in normal stromal matrix. Fibroblast may also indicate an activated, disease-associated, fibrillar matrix–forming cell of mesenchymal origin, similar to a myofibroblast except that it does not express αSMA.
Resident fibroblast	Resident mesenchymal cell of healthy tissue embedded in normal stromal matrix.
Pericyte	Resident mesenchymal cell of healthy tissue attached to endothelial cells [EC(s)] by processes embedded in capillary basement membrane.
Hepatic stellate cell	Resident subendothelial mesenchymal cell of the hepatic sinusoids that preserves some features of a resident fibroblast and pericyte and stores vitamin A in cytoplasmic droplets.
Mesangial cell	Specialized pericyte of the renal glomerulus.

Appearance of myofibroblasts

Tissue fibrosis in virtually every organ is accompanied by accumulation of large numbers of mesenchymal cells that have undergone profound changes in cell function, morphology, and transcriptional regulation. These cellular events comprise a transdifferentiation that is typically referred to as "activation" (Table 1). These newly appearing, activated mesenchymal cells secrete both increased amounts and disease-associated forms of collagen (that is, interstitial collagens, types I and III), as well as other ECM proteins that characterize pathologic fibrosis. The origins of these cells are detailed below, but variable fractions of them express α-smooth muscle actin (αSMA) and other contractile proteins, which accounts for their description as myofibroblasts or contractile fibroblasts (Table 1). Myofibroblast accumulation and contraction, combined with loss of epithelial integrity, contribute to the ongoing architectural distortion that adds to the loss of organ function in fibrotic diseases. Combinations of platelet-derived growth factor (PDGF), connective tissue growth factor (CTGF), and TGFβ act on various mesenchymal cell populations to provoke their transdifferentiation into myofibroblasts (*37*). In response to these and other growth factors, the cellular precursors of myofibroblasts (pericytes, resident fibroblasts, hepatic stellate cells, and renal mesangial cells) (Table 1) also up-regulate their expression of PDGF receptor β (PDGFRβ) (*38*), one of many positive feedback loops that can drive myofibroblast transdifferentiation to promote pathologic fibrosis.

As noted above, TGFβ is stored in a tethered, latent form that can be converted or activated to a biologically active form by physical deformation of the latent complex. The crystal structure of latent TGFβ reveals the mechanism through which cells can accomplish this physical deformation—through integrins that attach to the latent complex (*39*). In some organs—for example, lung, kidney, and biliary tract—injured epithelial cells can mechanically activate TGFβ via $\alpha_v\beta_6$ integrin directly, but this integrin is not expressed on epithelial cells in every organ. Instead, myofibroblasts can express their own distinct integrin(s) that activates TGFβ. Indeed, cultured pulmonary myofibroblasts can mechanically activate TGFβ through multiple integrins that all share the α_v subunit (*34*). As highly contractile cells, myofibroblasts are well suited to exert physical force on latent TGFβ (*40*). Therefore, multiple feedback loops act to maintain the myofibroblast state, at least in part through epigenetic events (*41*), although in liver myofibroblasts, they can also revert to a more quiescent phenotype that resembles their original cellular source (*42*–*44*). Nevertheless, even such "deactivated" cells are primed to more easily reactivate upon recurrent tissue injury (*42*).

Several pathways of gene regulation are essential to drive key functions of myofibroblasts. These include molecules downstream of the TGFβ receptor, including Smads (*45*, *46*), JunD (*47*, *48*), and the classical downstream mediators of receptor tyrosine kinase signaling (*49*), which typically control critical features of fibrogenic cells—cell motility, proliferation, and morphogenesis—through well-characterized intracellular signaling pathways (*50*). Autophagic signaling also contributes to myofibroblast function (*51*). This highly conserved pathway is induced during mesenchymal cell activation in tissue injury to maintain energy homeostasis in the face of the increased metabolic demands of proliferation, fibrogenesis, and contractility (*51*).

Properties of the fibrotic ECM

In most healthy organs, ECM proteins are concentrated in the basement membrane between epithelial and endothelial cells (ECs) to provide structural support. Basement membranes are principally composed

of laminins, type IV collagen, and a mixture of proteoglycans. In fibrotic organs, both the composition and location of ECM proteins are markedly altered. The most abundant matrix proteins in fibrotic tissues are types I and III collagen, but there are also marked increases in fibronectin, osteopontin, hyaluronan, and proteoglycans, and different subtypes of collagens and laminins are produced. The secreted collagen is heavily cross-linked and can form extensive, physically integrated structures that replace and distort the normal tissue architecture. Although in thin tissue sections the abnormal ECM can appear to be localized within discrete accumulations (for example, "fibroblast foci" in pulmonary fibrosis), three-dimensional reconstructions indicate that collagen networks actually extend throughout an entire fibrotic organ (*52*).

In addition to displacing and distorting normal tissue structures, the abnormal fibrotic ECM can alter the behavior of both normal resident cells and pathologic myofibroblasts. For example, signaling initiated by locally produced tissue fibronectin contributes to the transdifferentiation of quiescent hepatic stellate cells into classical tissue myofibroblasts (*53*) and facilitates EMT-like reprogramming of lung epithelial cells (*54*).

The abnormal ECM also regulates the availability of cytokines and growth factors, many of which are concentrated by ECM proteins to enable optimal release and conversion to their active forms or signaling or for sequestration and inactivation by specific ECM components.

A critical consequence of ECM accumulation is increased tissue stiffness, which markedly affects both normal and pathologic cellular responses. Increased tissue stiffness drives alterations in the actin cytoskeleton in many cell types, enhancing the size and number of focal adhesion signaling complexes and augmenting signaling through integrins and other receptors that concentrate in these structures (*55*). Resident fibroblasts are especially responsive to tissue stiffness, remaining quiescent when they are plated on or within matrices with stiffness resembling that of healthy organs, but transdifferentiating into myofibroblasts when they are plated on stiff matrices similar to fibrotic organs (*56*, *57*). Increased tissue stiffness can also stimulate resident fibroblast proliferation (*58*) and facilitate TGFβ activation by integrins. Fibroblasts or epithelial cells activate TGFβ in proportion to the stiffness of the substrate; when plated on flexible substrates, these cells are unable to generate sufficient force for TGFβ activation. A positive feedback loop is generated through fibrosis-induced tissue stiffness, which further enhances ECM accumulation; targeted reduction of stiffness may break this cycle. One example of such a potential therapy to reduce stiffness is an allosteric antibody inhibitor of lysyl oxidase 2 (LoxL2), a member of a family of enzymes responsible for collagen cross-linking, which can potently inhibit pathologic fibrosis in the lung and liver in animal models (*59*). However, it is not entirely clear that the effects of this inhibitor are a result of its action on stiffness, because in addition to cross-linking collagen, LoxL2 can interact with Snail1 to deaminate a specific lysine residue in histone H3, thereby facilitating Snail-mediated transcriptional regulation (*60*). Regardless of its mechanism, antagonism of LoxL2 has emerged as an appealing antifibrotic strategy (see below).

Immune cell recruitment

Innate immune effector signaling pathways act as important drivers of myofibroblast transdifferentiation by provoking cellular activation (Table 2) and fibrosis. Mediators include tumor necrosis factor–α (TNFα), IL-1β and the NALP3/ASC (Nod-like receptor protein 3/apoptosis-associated speck-like protein) inflammasome, IL-6 and IL-17A, and type 2 cytokines including IL-4 and IL-13 (*61*–*63*). Two T cell cytokines that are activated in response to disease, IL-13 and IL-17, are secreted by innate, as well as adaptive, immune cells. Specifically, innate helper cells, basophils, and eosinophils secrete IL-13, and innate helper cells, γδ T cells, and invariant natural killer T cells secrete IL-17. These cytokines are especially well characterized in the fibrotic reaction to schistosomal parasites, which depends on IL-13 (*64*), and in the bleomycin model of pulmonary fibrosis, which depends on IL-17 (*65*, *66*). IL-13, IL-17, and related cytokines are interesting potential targets for treatment for chronic fibrotic diseases.

Myofibroblasts are also critical innate immune sensors and can themselves generate a wide array of inflammatory effectors including chemokines, cytokines, and oxygen radicals (*67*) (Table 2). In turn, oxidant stress can further amplify inflammation and fibrosis (*68*–*70*) and accelerate transdifferentiation of resident mesenchymal cells into myofibroblasts (*71*–*73*). Although the relative contribution of each of these processes is not known, disruption of proinflammatory or oxidative stress pathways in myofibroblasts or their precursors, or in other cells such as endothelium, epithelium, or leukocytes, are attractive targets for therapeutic intervention.

Monocyte-derived cells

Monocyte-derived cells (macrophages and dendritic cells) play an important role in inflammation and in the subsequent development of fibrosis in a range of organ pathologies (*74*–*81*). Their contributions to fibrosing diseases have been clarified from work in in vivo models that allow for their selective ablation and repopulation. Monocyte-derived cell populations can dynamically control the fibrotic process through both direct effects on matrix remodeling and indirect effects on the regulation of activated myofibroblasts, their precursor populations, and ECs (*74*–*77*, *82*–*84*). For example, macrophages are often found in close association with collagen-producing myofibroblasts in vivo and can produce cytokines and growth factors that either stimulate or suppress myofibroblast activity. The recruitment of distinct functional subsets of macrophages (M1, inflammatory; M2a-like, profibrotic; M_{reg}/M2c-like, regulatory) (*74*, *76*, *77*, *79*, *80*, *82*, *85*) and their relative concentrations during injury can determine whether the injury response leads to productive reepithelialization and healing or to pathologic scarring.

Monocytes promote progression of fibrotic disease when they differentiate into M2a-like macrophages (and possibly fibrocytes), which produce fibroblast stimulatory growth factors and cytokines, including TGFβ1, PDGF, FGF2, insulin-like growth factor–binding protein 5, CCL18, and galectin-3 (*74*, *81*, *86*–*94*). Increased circulating concentrations of these macrophage secretory factors represent potential biomarkers of fibrotic disease progression. Through the stimulation of additional local tissue injury, M1-type macrophages may also provoke myofibroblast activation and fibrosis that are independent of the production of cytokines. Indeed, conditional ablation of macrophages at early stages of fibrosis blocks fibrosis progression in several fibrosis model systems (*74*, *80*, *83*). Many stimuli, including IL-4 and IL-13, macrophage colony-stimulating factor, and CCL17 and CCL2 chemokines, establish a milieu that promotes differentiation of monocytes into fibrocytes (that is, circulating cells that may have the potential to become fibroblasts) and profibrotic macrophages (*74*, *95*–*99*). Once activated, these profibrotic macrophages amplify the amount and number of profibrotic cytokines and growth factors.

Resolution and regression of fibrosis

Tissue fibrosis did not evolve to cause disease, but rather as a protective response to tissue injury. Under normal circumstances, ECM that

Table 2. Pathways that affect fibrosis in myofibroblasts and their mesenchymal cells of origin. Information is derived from published articles on hepatic stellate cells, mesenchymal precursors, resident fibroblasts, and pericytes in one or more organs; contributions by other pathways are likely [modified from (*187*)]. Li, liver; K, kidney; Lu, lung; S, skin; H, heart; P, pancreas; G, gut; FGF, fibroblast growth factor; EGF, epidermal growth factor; VEGF, vascular endothelial growth factor receptor; NGF, nerve growth factor; FGFR, FGR receptor; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; TLR, Toll-like receptor; LDL, low-density lipoprotein; PAF, platelet-activating factor.

Ligand family	Pathway or cytokine	Receptors	Organs	Effects in disease
Developmental				
TGFs, activins	TGFβ, BMP4, BMP7	TGFβRI, II, III	Li, K, Lu, H, P, S, P, G	Fibrogenic/inhibitory
PDGFs	PDGF-A, -B, -C	PDGFRβ, PDGFRα	Li, K, Lu, S, P	Proliferative/fibrogenic
HGF	HGF	c-met	Li, K, Lu	Fibrogenic/regenerative
FGFs	FGF1,2	FGFR2	Li, K, Lu, P	Proliferative/fibrogenic
EGF	EGF, TGFα	EGFR3	Li, K, Lu	Proliferative/antifibrogenic/regenerative
IGFs	IGF-I, -II	IGF-1R	Li, K, Lu	Proliferative/fibrogenic/regenerative
VEGFs	VEGF-A	VEGFR-II	Li, K, Lu, P	Proliferative/fibrogenic
Neurotropins	NGF, BDNF, NT4, NT4/5	P75-NTR, TrkB, TRkC	Li, P	Proliferative/fibrogenic/regenerative
CCN	CTGF, Cyr61	$\alpha_v\beta_3$, LRP6, LRP1 β_1 integrins	Li, K, Lu, P	Proliferative/fibrogenic
RGD-containing ligands		Integrins $\alpha_v\beta_1$, $\alpha_2\beta_1$, $\alpha_6\beta_4$, $\alpha_5\beta_1$, $\alpha_8\beta_1$, $\alpha_v\beta_3$, $\alpha_v\beta_8$	Li, Lu	Proliferative/fibrogenic
Hedgehog	Sonic	Patched, smoothened	Li, K, Lu, P, G	?Fibrogenic
Notch			Li, K, Lu, G	Regenerative/fibrogenic
Wnt	Wnts	LRP5, LRP6, Frizzleds	Li, K, Lu	Regenerative/fibrogenic
Endothelins	ET-1	ETRA/B	Li, K, Lu, P	Proliferative/fibrogenic/inflammatory
Renin/angiotensin	AngII	AT1R	Li, K, Lu, H, P	Proliferative/fibrogenic
Innate immune				
Purine		P2Y	Li, P	Proliferative/fibrogenic
Adenosine		A2(a)R	Li, K, Lu	Proliferative/fibrogenic
TNF	TNFα	TNFR1	Li, K, Lu, P, G	Chemotactic/inflammatory
CD40	CD40L	CD40	Li, Lu	Chemotactic/inflammatory
TLRs	Endogenous and pathogens	TLR2, TLR4	Li, K, G	Chemotactic/inflammatory
AGE		RAGE	Li, K, Lu	Chemotactic/inflammatory
CSFs	M-CSF	CSF1R	Li	Chemotactic/inflammatory
Chemokines	CXCL1, MCP1, RANTES, MIP1, IL-8	CXCR3	Li, K, Lu, P, G	Chemotactic/inflammatory
Thrombin		Thrombin R	Li, K	Proliferative/fibrogenic
PAF	PAF	PAF receptor	Li, K	Chemotactic/inflammatory
Oxidized LDL	oxLDL	CD36	Li	Chemotactic/inflammatory
Homeostatic				
Fibrillar collagens	Coll1, CollIII	DDR1, 2, UPARAP	Li	Fibrogenic
Cannabinoid		CB1R	Li	Proliferative/fibrogenic
Opioids		δ1 and δ2 receptor	Li, K	Chemotactic/inflammatory
Regenerative				
IL-6	IL-6		Li, P, G	Regenerative
IL-8	IL-8		Li, P, G	Inflammatory
IL-10	IL-10	IL-10R	Li, K, G	Antifibrogenic
Adipogenic				
Adiponectin	Adiponectin	CB2 receptor	Li. P	Antifibrogenic
PPARs		PPARγ, α	Li, K, Lu, S, P	Antifibrogenic

accumulates in response to injury is digested and removed. Therefore, under optimal circumstances, normal structure and function are restored after injury, especially if the injury is acute or self-limited. Resolution of fibrosis requires induction of proteases, especially matrix metalloproteinases (MMPs), that cleave the assembled matrix proteins into fragments small enough to be taken up by tissue phagocytes or fibroblasts. Metalloproteinases play multiple roles in regulating fibrotic diseases. Their activity is tightly regulated at several steps, including their rates of production and secretion (usually as inactive precursors), their proteolytic cleavage to yield active enzyme, and their degradation [by the local concentration of protease inhibitors, particularly tissue inhibitors of metalloproteinases (TIMPs)].

Our understanding is fragmentary about which metalloproteinases are important for wound repair at each stage of the injury response. In the early phases of injury, some metalloproteinases can enhance inflammation and injury, for example, MMP-7, which in response to intratracheal bleomycin cleaves the cell surface proteoglycan syndecan-1 from epithelial cells, releasing the neutrophil chemoattractant KC and contributing to lung inflammation (*100*). As a result, mice lacking MMP-7 are protected from bleomycin-induced pulmonary fibrosis (*101*). In contrast, during the resolution phase of fibrosis, metalloproteinases are required to cleave ECM fibrils, in which case reduced metalloproteinase levels or increased TIMPs could worsen fibrosis.

Myofibroblasts, disease-associated fibroblasts, and tissue macrophages use specific cellular machinery, including uPARAP (ENDO180) (*102*), to ingest and remove collagen fragments. Collagen uptake in macrophages depends on the secreted protein Mfge8, which directly binds to collagen and facilitates its uptake by macrophages. Mice lacking Mfge8 develop exaggerated pulmonary fibrosis in response to intratracheal bleomycin, despite normal inflammatory responses to the drug and normal rates of collagen production (*103*).

Studies in liver have been clearest in tracking the fate of myofibroblasts as fibrosis resolves, a key component of fibrosis regression. Apoptosis of myofibroblasts can clear these fibrogenic cells after cessation of experimental liver injury (*104*), or, as noted above, they may instead revert to a more quiescent phenotype (*42*, *44*, *105*). Because quiescent hepatic stellate cells probably support normal liver homeostasis, treatment strategies that preserve their quiescence rather than enhance their depletion may preserve normal liver function.

Because most chronic human fibrotic diseases are progressive, there has been uncertainty whether fibrosis is reversible. However, the success of antiviral therapies for viral hepatitis has conclusively established that, at least in liver, organ function can be markedly restored, with resolution of fibrosis, once the underlying source of injury is controlled (*106*). Specifically, most patients with chronic hepatitis B virus infection and cirrhosis who show suppressed viral replication after antiviral therapy exhibit remarkable regression of cirrhosis over time, often with return of normal liver structure and function (*107*). Similarly, a sustained virologic response (that is, a cure) in hepatitis C virus (HCV) patients to interferon-based treatment not only leads to regression of cirrhosis but also markedly reduces clinical complications and improves portal hypertension (*108*, *109*). These hopeful findings raise two key questions: (i) Is the liver unique in its regenerative capacity and ability to show reversal of advanced fibrosis? (ii) What cellular or matrix component of scar determines when fibrosis is irreversible? Regardless of the answers, these observations in liver indicate that parenchymal organs can harbor the capacity to resorb even extensive scars, increasing optimism that therapeutic agents could lead to elimination of disease, rather than only slowing or stopping the progression of fibrosis.

Monocyte-derived tissue cells, especially M_{reg}/M2c-like macrophages, contribute to the resolution of inflammation and fibrosis. Their depletion at the start of the recovery phase of liver injury suppresses ECM degradation and the clearance of myofibroblasts necessary for resolution (*80*), whereas transferring macrophages from mice without fibrosis into mice with liver injury reduces fibrosis in both kidney and lung injury models (*98*, *110*). In liver, resolution of fibrosis promoted by macrophages and dendritic cells has been a result of their production of MMP-13 and MMP-9 (*79*, *111*). Consistent with this finding, MMP-9 overexpression in macrophages substantially reduces lung fibrosis (*112*). In models of liver fibrosis, macrophage phagocytosis of apoptotic hepatocytes reduces inflammation to prevent the development of fibrosis (*113*), and phagocytosis of apoptotic cholangiocytes reverses preexisting fibrosis (*114*).

The secretion of IL-10 is a defining marker of regulatory macrophage function. Accordingly, administration of IL-10, adoptive transfer of IL-10–stimulated macrophages, and in vivo induction of macrophage IL-10 expression all ameliorate fibrosis and inflammation in kidney, gut, and brain (*77*, *78*, *115*–*117*). Arginase-1–expressing M2 macrophages can also ameliorate liver fibrosis due to chronic *Schistosoma mansoni* infection (*118*). Therefore, monocytes can promote resolution of fibrotic disease by (i) differentiating into regulatory macrophages that produce suppressor cytokines locally, including IL-10; (ii) producing MMPs that can directly degrade interstitial collagen (for example, MMP-1, MMP-2, MMP-8, MMP-9, and MMP-13); (iii) locally depleting essential amino acids required for T cell and myofibroblast proliferation (*1*, *74*); (iv) actively promoting apoptosis of myofibroblasts; and (v) phagocytosing ECM and cellular debris that would otherwise stimulate inflammatory and fibrogenic cell activation. These insights have led to efforts to stimulate regulatory macrophage activity therapeutically.

SOURCES OF FIBROGENIC CELLS

Resident mesenchymal cells as the source of myofibroblasts

Mesenchymal cells in adult tissues are also known as stromal cells that are prominent during organogenesis because they are in a loose matrix known as stroma that exists between the forming organ units. Stromal cells perform critical functions in organ formation, patterning and maturation, and development of the microvasculature requires these cells to form and stabilize vessels (when they are referred to as pericytes, see below) (*119*). Stromal cells in development regulate these critical functions through signaling pathways downstream of Wnt, PDGF, TGFβ superfamily members, FGF, EGF, VEGF, hedgehog, notch, ephrins, and angiopoietins (*119*, *120*).

Compared with hematopoietic or epithelial cells, however, our knowledge of stromal or mesenchymal precursor cells is in its infancy, but current estimates suggest that these cells represent >5% of all cells in adult organs and are critical for organ development, homeostasis, physiology, and repair. In adult tissues, mesenchymal cells have been variably termed fibroblasts when embedded in stroma between organ units, or mural cells, Rouget cells, perivascular cells, pericytes, or pericyte-like cells when attached to the microvasculature, depending on the context. Pericytes are essential for vasculogenesis, angiogenesis, and vascular

integrity (*67*, *121*) and have specific functions within each organ during development (as above) and in adult tissues.

Although the origin of fibrosis in injured adult tissues has been debated for decades, the bulk of data now implicate the myofibroblast or disease-activated fibroblast as the primary fibrogenic cell (Table 1). Originally identified in wounded skin and contractures and defined as "wound fibroblasts," myofibroblasts express contractile proteins in common with smooth muscle cells and accumulate in virtual spaces between organ structures (*122*). They exhibit dense rough ER, a lack of lysosomal vacuoles, and modest amounts of intermediate filaments (desmin, vimentin, and αSMA). In normal organs, αSMA is expressed by smooth muscle cells of arterioles, veins, and the airways (*123*), but all diseased organs show an increase in αSMA^+ myofibroblasts, which generate interstitial or fibrillar matrix composed of types I and III collagens, among other ECM constituents (*124*, *125*). The use of fluorescent or enzymatic reporters expressed under the control of lineage-restricted promoters has enabled the identification of large populations of mesenchymal cells in all human organs (*126*). Moreover, the use of Cre recombinase to permanently recombine genomic DNA in lineages of murine cells indicates that these mesenchymal precursors are the major—if not the only—source of fibrosis-forming myofibroblasts in many models of organ injury in mice (Table 1) (*127*–*134*).

Each organ has related but distinct resident cell types that give rise to myofibroblasts. In the healthy liver, resident hepatic stellate cells are identifiable by their storage of lipid droplets enriched in vitamin A and long perisinusoidal processes (*135*). Hepatic stellate cells are located in the space of Disse, a virtual space between the fenestrated endothelium of the hepatic sinusoid and the brush border of the hepatocyte. Genetic studies in models of liver disease have confirmed that they are the primary source of myofibroblasts in vivo in a number of liver diseases, although a rarer population of portal fibroblasts contributes to the myofibroblast population in models of biliary disease (*136*, *137*). Pancreatic stellate cells (*138*–*140*) and intestinal fibroblasts (*141*, *142*) are similar to those in liver.

Conversion of resident mesenchymal cells such as stellate cells into myofibroblasts in response to tissue injury, a process termed cellular activation, engages many of the same pathways underlying their embryonic development (*143*). In some ways, tissue damage or injury recapitulates developmental processes to regenerate the damaged tissue (Table 1), although in adult tissues, these pathways frequently promote fibrosis rather than normal structure, especially when the injury is chronic. Chronic recruitment of developmental pathways in adult tissues may cause dysregulated signaling (*68*). In a remarkable study, myofibroblasts in liver were shown to induce heritable epigenetic changes (through a secreted factor that affects sperm) that attenuate fibrogenesis in the offspring, suggesting that transmission of fibrosis propensity can be both epigenetic and genetic (*144*).

Gene ontology analyses with transcriptomics to characterize the conversion of mesenchymal precursor cells into descendent myofibroblasts confirm that cellular activation is a true cellular transdifferentiation. These and other recent functional studies suggest that myofibroblasts have lost the homeostatic properties of pericytes or resident fibroblasts from which they are derived, possibly through epigenetic events such as DNA methylation. Hence, fibrosis may actually represent a state of pericyte deficiency as a result of their conversion to myofibroblasts, which undermines impaired organ homeostasis beyond the simple deposition of fibrotic matrix (*67*). Nonetheless, the reversibility of this phenotype, at least in stellate cells (*42*, *43*), indicates that phenotype plasticity is preserved in these myofibroblasts.

Contribution of other cell types to fibrosis

Leukocytes. Many studies have demonstrated that myeloid lineage cells in the circulation and in tissues can stain positively for collagen I on the cell surface or within their cytoplasm (*83*, *145*), but there is little evidence that they synthesize the protein. Moreover, myeloid lineage cells express collagen receptors and can internalize and degrade ECM, accounting for the occasional detection of collagen within these cell types. In models of kidney fibrosis, <0.1% of collagen I–producing cells in the injured tissue are of myeloid origin. In models of liver fibrosis, a small but significant (5%) fraction of collagen I–producing cells are derived from myeloid leukocytes (for example, fibrocytes), and they may represent a discrete profibrotic myeloid subpopulation similar to M2a macrophages, but their significance is unclear (*78*, *146*).

Epithelial cells. Injured epithelial cells have been considered a progenitor cell for myofibroblasts through EMT (*20*, *54*, *147*), but several fate mapping studies have failed to identify epithelial cells as a source of myofibroblasts in murine models of fibrosis in kidney, liver, and lung (*125*, *130*, *133*, *134*, *137*, *148*). Because interpretation of fate mapping studies is influenced by the specific gene used to mark presumed precursors, it remains possible that a subset of epithelial progenitors not labeled in these studies could give rise to a small fraction of collagen-producing cells in fibrotic organs. Alternatively, epithelial cells may undergo EMT and produce collagen only in culture and not in vivo (*149*).

Endothelial cells (ECs). ECs have been described as myofibroblast progenitors in heart, kidney, and lung (*150*–*152*). ECs cultured on plastic with TGFβ express αSMA, and cells of apparent endothelial origin in vivo are recruited to interstitial spaces and also express αSMA. However, ECs express many receptors that are shared with cells of bone marrow origin, which has confounded the study of endothelial progenitor cells for many years (*153*, *154*). Moreover, as with epithelial cells, sinusoidal ECs in culture may acquire features that are not present in vivo. Thus, there is little rigorous support from in vivo studies for this idea, and careful mapping of mesenchymal lineage cells does not identify ECs as a major myofibroblast progenitor.

PATHWAYS DRIVING MYOFIBROBLAST GENERATION FROM RESIDENT MESENCHYMAL CELLS

Until recently, research has focused on how mesenchymal cells with a resting phenotype undergo a transdifferentiation to an activated cell type, characterized by classic features of myofibroblasts as detailed above. However, a new model has emerged recently. In this view, injury or stress to endoderm- or mesoderm-derived epithelium, or injury to mesoderm-derived endothelium or myocytes, can lead to increased fibrosis, or fibrogenesis, independent of injury to or recruitment of other cells such as leukocytes (*22*). In this view, injured or stressed cells signal via paracellular mechanisms to neighboring mesenchymal cells (Fig. 3). Understanding the nature and regulation of these cell-to-cell signaling pathways is critical to developing therapies that block fibrogenesis. As reviewed above, injured or stressed epithelia of liver, pancreas, lung, and kidney are a major stimulus of fibrogenesis (*155*). Injured epithelial cells generate factors that can transmit signals to nearby mesenchymal cells. These factors include NGF, TGFβ, PDGF-B, VEGF-A, Wnts, and hedgehog ligands (Table 2).

Bidirectional signaling between the endothelium and pericytes may be critical to amplify injury or stress responses that drive the fibrogenic process (Fig. 3). Injury to the endothelium of peritubular capillaries transmits signals directly to underlying pericytes, with microvascular injury as a major stimulus for fibrogenesis (*156*). Microvascular toxins, irritants, changes in blood pressure and flow, or interactions with activated platelets or leukocytes are all sufficient to stimulate ECs, which generate TGFβ, PDGF, sphingosines, CTGF, and other profibrotic factors that can signal directly to neighboring pericytes (*121*). Once stimulated, ECs also respond differently to ligation of the VEGF receptor 2, which may be a central coordinator of EC responses to injury. Activation of the pericyte also leads to increased expression of factors that stimulate ECs, including VEGF-A isoforms, matrix factors including ADAMTS1 (*67*), and CTGF, as well as to up-regulation of receptor signaling pathways that render pericytes more susceptible to activation, including the PDGFRβ signaling pathway.

TISSUE-SPECIFIC FEATURES OF FIBROSIS FOR THERAPEUTIC TARGETING

Researchers are wary of targeting the conserved, or core, pathways of fibrosis for therapy because of potential collateral effects of antagonizing widespread mediators in nonfibrotic or unaffected tissues. For example, systemic TGFβ inhibition would be expected to inhibit fibrosis but could also impair tumor suppression or cause chronic inflammation. Although antagonism of these mediators in a tissue-specific manner could prove useful, an alternative approach is to pinpoint targets that are unique to diseased tissue or are only expressed in a specific organ. One example is the PDGFRβ, which is relatively restricted to arteriolar smooth muscle cells in normal liver but is markedly induced in hepatic stellate cells upon transdifferentiation (that is, activation) to myofibroblasts during liver injury (*157*). Other targets in liver fibrosis that have an altered profile of expression during injury include neuropilin-1 (*158*, *159*), endothelin signaling (*160*), and TGFβ receptors (*161*), among many others. A particularly interesting target on myofibroblasts in liver is a receptor heterodimer composed of the angiotensin II type I receptor and the cannabinoid CB1 receptor (*162*). Because this molecule is restricted to injured liver, antagonists designed to inactivate it should not have systemic effects. Additional fibrosis-specific targets may be uncovered by unbiased proteomic identification of molecular species that are confined to fibrogenic cells in injured tissues.

Although cell surface molecules are appealing targets because of their accessibility to therapeutic antagonists, unique intracellular targets also merit consideration—yet few have emerged. The high degree of specificity conferred by protein-protein interactions and by post-translational modifications that regulate gene expression suggests that these features are also fertile targets for drug discovery. One example is a small molecule that promotes nuclear translocation of YB-1, an intracellular TGFβ signaling antagonist (*163*). Uncovering targets like this one becomes especially pertinent as methods evolve for antagonizing molecules previously considered undruggable (*164*). To date, there have been no systematic efforts to identify signaling pathways or patterns of gene expression unique to fibrosis in a particular organ, yet many public data sets are available that could be interrogated for this purpose.

OBSTACLES TO TRANSLATION OF BASIC SCIENCE INTO CLINICAL PRACTICE

To translate basic molecular mechanisms of fibrotic diseases into clinical development of new therapeutics, there is a pressing need to overcome several challenges (Fig. 4). The paucity of adequate treatments

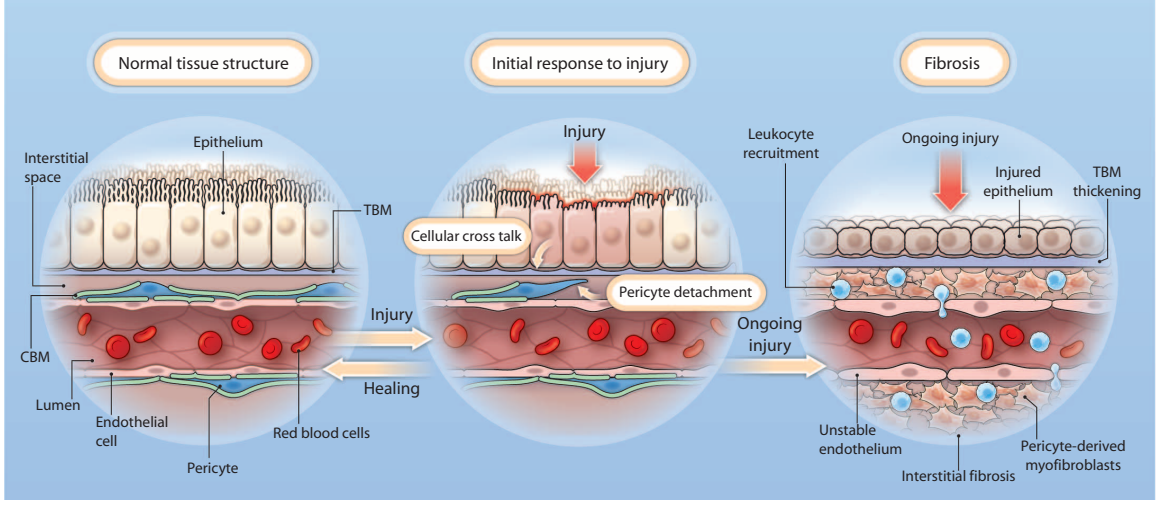

Fig. 3. Fibrosis in the kidney in response to injured epithelium or endothelium. In health, pericytes are attached to peritubular capillaries, partially embedded in the capillary basement membrane (CBM), where they perform critical homeostatic functions. In response to injury, epithelial cells or ECs send signals such as PDGF to pericytes, with reciprocal signaling from, for example, VEGF-A coming from pericytes. The pericytes are activated, detach, and migrate into the virtual interstitial space. If injury is sustained or iterative, then pericytes proliferate and exhibit marked changes in gene transcription that cause their transdifferentiation into myofibroblasts. These myofibroblasts have lost pericyte functions, deposit ECM, and secrete pro-inflammatory factors. The consequence is an unstable endothelium, leukocyte recruitment, thickening of the tubular basement membrane (TBM), and further injury to epithelial cells, which respond by losing epithelial function and developing further phenotypic changes as described in Fig. 2.

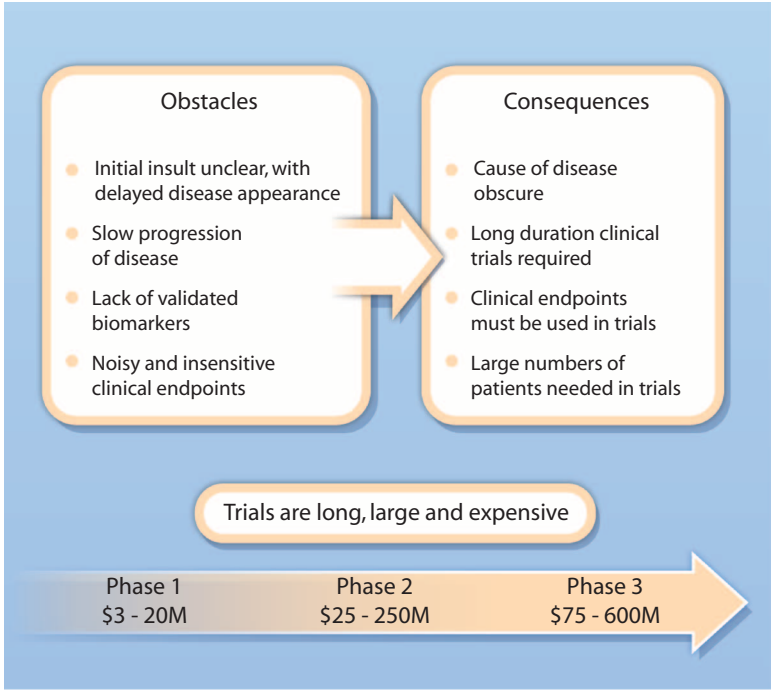

Fig. 4. Obstacles to translation in developing antifibrotics. In the lower arrow are shown the estimated clinical trial costs for progressive stages of clinical development in fibrotic diseases. The cost of each trial depends on the number of patients enrolled, the duration of the study, and the endpoints selected.

stems from several causes. We do not completely understand the etiology and pathogenesis of many fibrotic human diseases, limiting our ability to model these processes effectively in animals (*165*). This is especially true in IPF, where the initial insult is obscure; this is in contrast to liver fibrosis, for example, where patients typically have known precipitants, including viral infection, alcohol abuse, obesity, or immunologic attack. In addition, differences between the pathology in proof-of-concept animal studies and the human disease limit progress. Indeed, most animal models use toxic injuries that are rarely, if ever, seen in human disease (for example, CCl_4 in liver and bleomycin in lung).

Several approaches are under way to narrow this gap (Fig. 5). Studies carried out with human samples such as tissue, blood, serum, and urine can uncover molecular pathways and gene alterations that correlate with fibrosis, disease progression, and clinical outcomes (*166*–*171*). Once a molecular pathway has been linked to the onset or progression of human disease, these findings can be replicated in preclinical animal models to provide an experimental platform for interrogating the pathogenic process. Examples include rodent models in which a gene is mutated or silenced, or a molecular pathway is activated, in a way that mimics the human disease (*172*–*175*). Such models allow testing of therapeutics for their ability to interrupt a disease-relevant pathway to block the onset or progression of disease.

One recent example emerged from the identification of surfactant C mutations in familial forms of IPF, which leads to increased expression of markers of ER stress in type II pneumocytes from patients with either familial or sporadic forms of IPF (*176*). These findings in humans were then replicated in rodents to investigate how ER stress can promote lung fibrosis. To do so, a mutation in the surfactant C gene present in IPF patients was introduced into type II pneumocytes of mice, which sensitized the animals to lung fibrosis induced by bleomycin. These and similar models can be used to evaluate the role of ER stress and apoptosis in a specific cell type on tissue injury and fibrosis.

Similarly, Alport syndrome is a hereditary disease caused by mutations in the genes encoding Col4A3, Col4A4, and Col4A5 (*172*). These patients develop progressive glomerulonephritis that culminates in fibrosis within the glomerular and interstitial regions of the kidney. An animal model of autosomal recessive Alport syndrome was created by genetically deleting the Col4A3 gene (*172*, *177*). At 6 to 12 weeks of age, these mice spontaneously develop progressive fibrotic kidney disease similar to that of humans. Key molecular pathways from the murine mutant kidneys have been linked to histopathological and functional changes in human disease (*178*). Therefore, $Col4A3^{-/-}$ mice provide an in vivo system for testing pharmacological agents that are antifibrotic and/or restore kidney function.

In other animal models, the TGFβ pathway is stimulated or inhibited, which creates opportunities to clarify the role of the TGFβ pathway in fibrotic disease (*175*).

Despite this progress, each of these animal models only captures one aspect of a complex pathogenesis; new models must better reproduce the complete spectrum of pathogenic events. For instance, in renal transplant patients with nephropathy, transcriptional profiling of the kidney allografts identified gene activation patterns associated with histopathological changes in the kidney that are correlated with renal function and graft survival (*168*, *169*, *179*). This approach points to candidate pathways and genes that merit evaluation in vivo, such as molecules regulating epithelial injury, immune cell activation, and tissue repair. Many of these targets are also affected in other fibrotic diseases of kidney, as well as in other tissues, revealing stereotypical transcriptional changes for the inciting injury across several organs (*3*, *180*).

Currently, there is a high degree of uncertainty associated with the clinical development of antifibrotic therapies, and a more streamlined, accurate, and targeted approach is critical to accelerate the development of new therapies for fibrotic diseases. The uncertainty arises from patient heterogeneity and imprecise clinical endpoints that necessitate lengthy clinical trials. Disease heterogeneity—either in the form of differing subtypes of disease or different rates of fibrosis progression—also contributes to wide variability among patients enrolled in a clinical trial, which in turn can lead to large differences in patients' responses to a particular therapy. Successful efforts to stratify the risk of fibrosis progression with single nucleotide polymorphisms in patients with hepatic fibrosis due to hepatitis C (*181*, *182*) illustrate how genetic information might be exploited to enrich study populations by restricting enrollment to high-risk patients. Similar stratification based on genetic determinants of disease progression is needed for all chronic fibrotic disorders.

Endpoints used to monitor changes in fibrosis in clinical trials tend to be variable and insensitive, requiring enrollment of a large number of patients to yield sufficient statistical power to detect a significant benefit of a drug. Moreover, most fibrotic diseases progress over years, so that clinical trials require lengthy study periods to reveal a clinically meaningful benefit. Consequently, large, long, and costly clinical trials

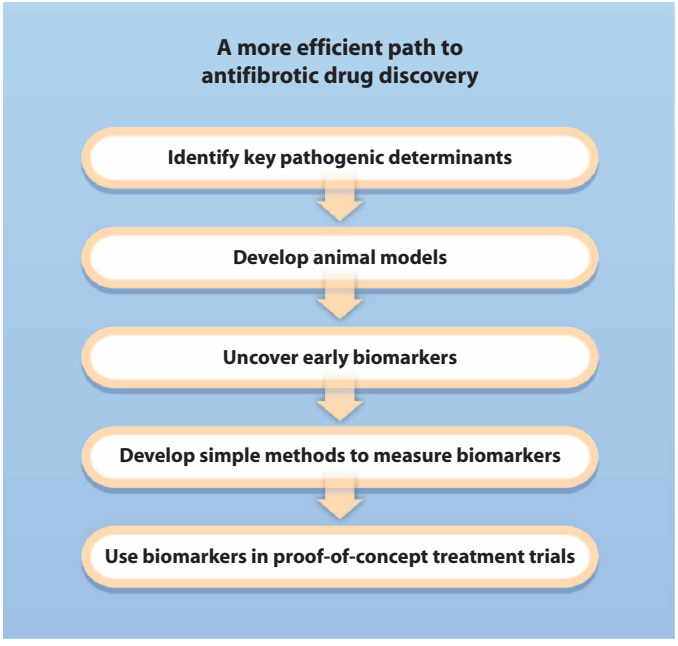

Fig. 5. Narrowing the translational gap for antifibrotic therapy development. Several sequential steps will accelerate translation of preclinical data into clinical trials and new antifibrotic therapies. These include (i) identification of key pathogenic determinants and features of human disease by tissue and body fluid analysis; (ii) development of animal models that recapitulate features of human disease; (iii) the use of human studies and animal models to uncover biomarkers that are early signals of altered disease activity; (iv) the development of techniques to detect biomarkers noninvasively and frequently; and the (v) employment of biomarkers in proof-of-concept treatment trials to establish either efficacy or futility after a shorter interval than is presently possible.

have been required for drug approval in fibrotic disease. This obstacle illustrates the critical need to validate the effectiveness of new therapies earlier in the clinical development process to de-risk the later, more lengthy, and expensive trials.

To achieve this goal, early trials must rely on biomarkers rather than only clinical endpoints. Ideally, biomarkers can provide rapid and accessible readouts of efficacy, drug exposure, or clinical response. Biomarker readouts that are based on a strong scientific rationale and that reflect effects on a target molecular pathway highly correlated with disease outcomes can provide critical early evidence of potential efficacy. In many forms of fibrotic disease, this strategy could include monitoring biomarkers of TGFβ pathway activation such as SMAD2/3 protein phosphorylation, or altered expression of plasminogen activator inhibitor-1, TIMP-1, thrombospondin, and collagen. Alternatively, a biomarker strategy could include monitoring markers of epithelial injury such lipocalin 2, Kim-1, and SLC22A8, or markers of matrix remodeling such as TIMP-1, LoxL2, and transglutaminase 2. Early evidence of efficacy based on validated biomarkers could be used to justify continued development of new therapeutics in larger and longer pivotal trials. Conversely, an early, inadequate response to therapy based on biomarkers could spare the enrollment of patients in whom there is unlikely to be a meaningful clinical benefit and thereby release these patients to other trials.

Because there are many core features common to all organs affected by fibrosis (*3*, *180*), biomarkers validated for one fibrotic tissue are likely to prove valuable in other tissues. In addition to molecular readouts in tissue or serum [for example, serum microRNAs (miRNAs)], biomarkers can include noninvasive technologies that quantify fibrosis to monitor response and to assess fibrosis progression rates. To this end, more precise technologies for quantifying fibrosis are needed, such as computed tomography– (*183*) and positron emission tomography–based approaches (*184*), or other methods that monitor matrix production or accumulation.

Ultimately, we must improve basic research tools and preclinical animal model systems to evaluate the significance of target pathways and their relevance to human disease. The integration of clinically annotated human disease data sets with preclinical animal models can identify shared target pathways so that responses to therapy in animals can be more predictive of drug efficacy in humans.

THERAPIES FOR FIBROSIS

Sustained progress in uncovering mechanisms of fibrosis has accelerated the clinical development of several therapies. Early preclinical efficacy and toxicity studies in animals have provided justification for pursuing clinical trials. There are dozens of drugs in development as antifibrotics, as described in recent reviews (*63*, *185*–*189*), so, here, we illustrate examples for which observations from animal studies have led to unique approaches in human trials.

The TGFβ pathway has been known for more than 20 years to be a central mediator of the initiation and maintenance of fibrosis in many fibrotic diseases (*190*–*192*), as discussed above. In vitro mechanistic studies, preclinical animal studies, and solid evidence that this pathway is up-regulated in human disease all support antagonism of the TGFβ pathway for treatment of fibrosis (*191*, *193*).

Nevertheless, TGFβ has important normal homeostatic activities, including immune regulation and tumor suppression (*194*, *195*). Consequently, design of clinical studies must account for and minimize the potential adverse effects of systemic inhibition of TGFβ activity.

GC1008 (Genzyme) is a humanized antibody that binds and blocks the function of TGFβ1, -2, and -3. It has been evaluated in multiple early clinical trials in both fibrotic disease and cancer, where it attenuates signaling pathways between tumor and stroma that enhance neoplastic growth. In a recent phase 1 clinical trial, GC1008 was well tolerated as a single dose up through 4 mg/kg in patients with advanced focal segmental glomerular sclerosis (*196*). An ongoing phase 1 trial of GC1008 in scleroderma patients is evaluating its effects on biomarkers of fibrosis in skin biopsies (ClinicalTrials.gov NCT01284322). LY2382770 (Lilly) is a humanized antibody that selectively binds and blocks the TGFβ1 cytokine. This antibody is currently being evaluated in a phase 2 trial with multiple dosing in patients with chronic kidney disease (ClinicalTrials.gov NCT0113801).

A more selective approach to block the TGFβ pathway only at sites of fibrogenesis exploits the $\alpha_v\beta_6$ integrin. This target molecule is expressed at low levels in healthy adult tissues but is strongly up-regulated on epithelial cells in response to tissue injury and fibrosis (*26*, *27*). Blocking $\alpha_v\beta_6$ binding to the latent precursor form of TGFβ locally inhibits the TGFβ pathway. This approach attenuates fibrosis in multiple animal models. STX-100 (Biogen Idec) is a humanized anti-$\alpha_v\beta_6$ antibody that is being evaluated in a phase 2 trial with multiple

dosing in IPF patients (ClinicalTrials.gov NCT01371305). A biomarker strategy is integrated into this trial to evaluate whether STX-100 effectively inhibits $\alpha_v\beta_6$-mediated TGFβ activation in the lung by monitoring pSMAD2 levels and TGFβ-regulated genes in bronchoalveolar lavage (BAL) cells. Reduced TGFβ signaling in BAL cells should indicate whether the drug is reaching its target and inhibiting its function.

The LPA receptor LPA1 is expressed on multiple cell types and plays a pathogenic role in tissue injury and fibrosis in mouse models (*197*, *198*). LPA1 promotes fibroblast migration, vascular leak, epithelial apoptosis, and resistance of fibroblasts to apoptosis, all of which have been implicated in the pathogenesis of IPF. The pleiotropic disease-promoting activities of LPA1 in a variety of human fibrotic diseases suggest that the receptor is an attractive molecule for therapeutic targeting. BMS-986202 (Bristol-Myers Squibb) is a small-molecule antagonist of the LPA1 receptor with protective effects in a mouse model of skin fibrosis (*198*). Developed as AM152 by Amira Pharmaceuticals (*198*, *199*), this drug has been tested in a phase 1 trial in healthy volunteers and is to be tested in additional trials.

The IL-4 and IL-13 cytokines are important mediators of innate immune activation and T helper cell 2 responses, as reviewed above. Likewise, they regulate fibrosis in several preclinical animal models, and there is increased expression of these cytokines or their cognate receptors in human fibrotic diseases, prompting several efforts to target them for the treatment of asthma and fibrotic disease (*180*, *200*). Some groups have developed antibodies that selectively target the individual cytokines, whereas others have used antibodies that bind to the shared IL-4 receptor α, thereby inhibiting both IL-4 and IL-13. Recent positive clinical data with lebrikizumab (Genentech), an anti–IL-13 antibody, in asthma patients with elevated serum periostin suggest that biomarkers could be used to identify patients who respond to anti–IL-13 treatment in fibrotic diseases as well (*201*). Several biotech and pharmaceutical companies are developing IL-4 and IL-13 antagonists for the treatment of asthma; those currently in clinical development for fibrotic disease include QAX576 (Novartis), an anti–IL-13 antibody; tralokinumab (MedImmune), an anti–IL-13 antibody; and SAR156597 (Sanofi), a bispecific-antibody that targets both IL-4 and IL-13 (ClinicalTrials.gov NCT01266135, NCT01629667, and NCT01529853).

Pentraxin-2 (serum amyloid P) is an endogenous human protein that functions as a pattern recognition receptor regulating the innate immune response and inhibiting the differentiation of monocytes into fibrocytes or profibrotic, alternatively activated (M2a) macrophages (*76*). Pentraxin-2 localizes to sites of injury and both inhibits fibrosis and promotes repair, a dual therapeutic effect. PRM-151 (Promedior) is a human recombinant form of pentraxin-2 with protective effects in a variety of preclinical models of fibrosis in multiple tissue types (*76*). PRM-151 has been tested in a phase 1 trial with multiple dosing in patients with IPF and is to be tested in a phase 2 trial in patients undergoing a trabeculectomy for glaucoma.

The recognition that tissue stiffness and mechanical stress drive fibrosis has led to efforts to antagonize processes that cross-link and stabilize ECM as a means to attenuate fibrogenesis, focusing on LoxL2, which promotes cross-linking and may have independent effects on gene expression, as described above. GS-6624 (Gilead) is a noncompetitive allosteric antibody inhibitor of LoxL2 in clinical development for the treatment of fibrotic disease and cancer. Blocking LoxL2 activity in animal models leads to fewer myofibroblasts, decreased production of ECM, and reduced expression of TGFβ (*59*). These findings suggest that inhibiting LoxL2 decreases the pathogenic response of myofibroblasts, blocking a feedback loop that amplifies the fibrotic process. GS-6624 is currently being tested with multiple dosing schedules in several clinical trials in fibrotic disease and cancer.

Another approach exploits our rapidly growing knowledge about miRNAs. miRagen and Regulus Therapeutics have developed chemically synthesized oligonucleotides that behave as miRNA inhibitors or mimetics and regulate the stability and translation of specific genes implicated in fibrogenesis (*202*, *203*). Although early studies with miRNAs exploited their roles in cardiac and vascular tissue remodeling, miRNAs also modulate tissue remodeling and fibrogenesis in other tissues (*202*, *203*). Effective tissue delivery of miRNA mimetics and inhibitors remains a challenge but is under active investigation, and continued progress is anticipated.

IPF is a particularly important fibrotic target disease because of its catastrophic clinical course and the huge unmet need that it represents. Drug development efforts for this illness provide a valuable illustration of how the field of antifibrotic therapies is maturing. First, the diagnostic criteria for IPF have been refined (*204*), which will help improve and standardize patient identification for enrollment in clinical trials. Second, data from earlier IPF trials have allowed refinement of patient enrollment criteria to better identify patients with more rapidly progressive disease. This, in turn, will facilitate the detection of a statistically significant change in pulmonary function after drug treatment for 6 to 12 months. Finally, increased clarity regarding the potential path to drug approval for IPF in the United States and Europe has helped decrease the financial risk associated with drug development for this disease. Much of this insight emerged from trials of pirfenidone by InterMune for IPF. Pirfenidone is a small molecule with anti-inflammatory activity that inhibits TGFβ and p38 signaling. The drug is currently being tested in the phase 3 ASCEND trial in IPF patients in the United States (ClinicalTrials.gov NCT01366209 and NCT01504334) and is approved in Europe and Japan for this disease. The results of this study will provide additional data to evaluate the efficacy of pirfenidone in IPF patients. Pirfenidone had previously been evaluated in the CAPACITY phase 3 trials (*205*), but data from that trial were not sufficient for Food and Drug Administration approval in the United States.

Other agents under study for IPF include BIBF 1120 (Boehringer Ingelheim), a small-molecule, broadly active tyrosine kinase inhibitor that inhibits PDGFR, FGFR, and VEGFR. BIBF 1120 showed promise in a phase 2 trial in IPF patients (*206*) and is being evaluated in phase 3 trials in IPF and multiple phase 2 trials in cancer. In addition, GSK2126458 (GlaxoSmithKline) is a small-molecule inhibitor of phosphatidylinositol 3-kinase and mammalian target of rapamycin that is currently planned for a phase 1 trial in patients with IPF (ClinicalTrials.gov NCT01725139). FG-3019 (FibroGen), a humanized antibody to CTGF, has been tested in many early clinical trials in fibrosis and has advanced to phase 2 trials in IPF (ClinicalTrials.gov NCT01262001) and liver fibrosis (ClinicalTrials.gov NCT01217632), and carbon monoxide is being tested in patients with IPF (ClinicalTrials.gov NCT01214187).

A handful of trials for liver fibrosis have not yielded any successes yet, but valuable lessons have emerged. For example, antifibrotic trials for HCV cirrhosis with drugs that were not successful indicated that fibrosis continues to progress even in advanced disease when HCV is not cured, albeit at nonlinear rates (*207*, *208*). Moreover, treatment cannot inadvertently cause the primary disease to worsen, a conclusion that emerged from a study of IL-10 for HCV cirrhosis, which markedly

increased viral loads (*209*). Finally, at least one potential biomarker, interferon-inducible T cell α chemoattractant, was uncovered in a trial of interferon-γ (*207*).

In several fibrosis clinical trials in the last ~15 years, primary endpoints failed to show statistically significant changes, leading to the conclusion that these trials had failed (*187*, *210*). However, many of these trials have provided information that can facilitate fibrosis trials in the future. In some, a trend toward a positive outcome was reported (*206*, *210*). Such findings underscore the difficulty of conducting trials where the clinical manifestations progress slowly, which necessitates longer trial durations than previously realized to see a clinical benefit. These studies have also highlighted the variability in progression across a patient population, which informs power calculations to estimate the number of patients required to detect a statistically significant effect in future clinical trials. From this experiences, we conclude that it is essential to stratify patients to minimize interpatient variability, either on the basis of genetic polymorphisms that define risk of progression such as those used to stratify progression risk in hepatitis C infection (*181*, *182*, *211*). Last, it is better appreciated now that many previously tested targets do not contribute significantly to disease pathogenesis. The lengthy and costly nature of clinical trials in fibrosis underscores the need to pursue targets for which there is strong scientific rationale, with evidence that effects on these targets correlate well with disease progression in humans.

Although success in treating fibrosis has been limited, future clinical trials in fibrotic disease are likely to provide more promising results. We now have substantially more insight into both shared and unique molecular pathways that drive fibrotic disease in various organs. We also have more clarity on guidelines for proper diagnosis of fibrotic diseases and a clearer understanding of rates of disease progression and how to monitor these changes. As research in fibrosis continues to improve our understanding of basic biology and human disease pathogenesis, we should be better positioned to test therapeutic targets for which there is strong supporting scientific rationale in well-designed clinical trials with meaningful biomarker and functional endpoints.

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Funding: S.L.F. is supported by DK56621 from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and AA020709 from the National Institute on Alcohol Abuse and Alcoholism. D.S. is supported by HL53949, HL102292, and HL108794 from the National Heart, Lung, and Blood Institute and AI0774349 from the National Institute of Allergy and Infectious Diseases. J.S.D. is supported by DK84077, DK94768, and DK93493 from NIDDK, the University of Washington, the Institute for Stem Cell and Regenerative Medicine, the Genzyme Research-in-Progress Grant, the Nephcure Foundation, and the American Heart Association 12040023.

Submitted 31 July 2012
Accepted 6 December 2012
Published 9 January 2013
10.1126/scitranslmed.3004700

Citation: S. L. Friedman, D. Sheppard, J. S. Duffield, S. Violette, Therapy for fibrotic diseases: Nearing the starting line. *Sci. Transl. Med.* **5**, 167sr1 (2013).