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Review Series

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Resolution of organ fibrosis

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Introduction

Fibrosis is the excessive accumulation of extracellular matrix (ECM) that can lead to distortion of tissue architecture and loss of organ function. This pathology commonly results from a wound healing response to repeated or chronic injury or tissue damage, irrespective of the underlying etiology, and can occur in virtually any solid organ or tissue. A broad range of prevalent chronic diseases can give rise to fibrosis, including diabetes, hypertension, viral and nonviral hepatitis, heart failure and cardiomyopathy, idiopathic pulmonary disease, scleroderma, and cancer. Fibrosis resulting from these and other diseases can lead to failure of liver, lung, kidney, heart, or other vital organs as excessive ECM replaces and disrupts parenchymal tissue (1). Consequently, severe fibrosis is estimated to account for up to 45% of all deaths in the developed world (2). Current therapies for fibrosis are few and of limited efficacy. Therefore, there is an urgent need to understand how fibrosis may regress and to identify potential therapeutic approaches.

Wound healing in any organ generally proceeds through three broad phases that are temporally overlapping but functionally distinct (3, 4). Immediately following injury, hemostasis is achieved through the formation of a platelet plug and a fibrin matrix, accompanied by the release of cytokines and chemokines that initiate inflammation and recruit immune cells. This leads to the first phase of healing, the inflammatory phase, wherein infiltration of neutrophils and macrophages combats possible infections and removes tissue and cell debris. Cells undergoing apoptosis and the immune cells they recruit promote new tissue formation by producing proinflammatory, vasoactive, and profibrotic effectors, including TGF-β1, PDGF, TNF-α, IL-6, and IL-13, to prompt the proliferative phase of healing (5–7). TGF-β1 plays a particularly prominent role in inducing the differentiation of precursor cells into myofibroblasts, which rapidly produce a prodigious amount of ECM to maintain the integrity of the injured tissue during repair and to enhance cell proliferation for granulation tissue formation

or parenchymal regeneration (Figure 1). In the final maturation phase, the provisional ECM is degraded and remodeled to rebuild the parenchymal tissue architecture. Dysregulation of these processes or repeated or chronic injury allows inadequate opportunity for the ECM to be resolved as myofibroblasts are relentlessly stimulated to produce ECM. Over time, the accumulated ECM begins to form a fibrotic lesion. With some exceptions, fibrosis is associated with chronic inflammation, which drives the production of profibrotic growth factors (3).

The principal cell type that produces ECM to form fibrotic lesions is the myofibroblast, which exhibits features of both smooth muscle cells and fibroblasts, and is characterized by a prominent rough endoplasmic reticulum, stress fibers, enlarged nucleolus, and expression of α-smooth muscle actin (αSMA) and other contractile proteins (8). Myofibroblasts produce interstitial or fibrillar ECM largely composed of collagen I and III, as well as myriad other ECM proteins, including an alternatively spliced form of fibronectin with an extra domain A (EDA-fibronectin) that is important for TGF-β1–induced myofibroblast differentiation (9). In the early granulation tissue, there is a population of fibroblastic cells that express collagen I and III, termed proto-myofibroblasts, that can be fully differentiated into myofibroblasts upon stimulation by TGF-β1 (8, 10). The sources of myofibroblast precursors are heterogeneous and have been controversial (Figure 1), although recent data indicate that resident fibroblasts and pericytes are major contributors (11, 12), with mesenchymal stem cells also playing a role (13, 14). The mechanisms that trigger fibrogenesis have been discussed in several excellent recent reviews (7, 15, 16).

Reversibility of organ fibrosis

Although fibrosis was once thought to be irreversible, there is now a growing body of evidence suggesting that fibrosis is reversible in human fibrotic diseases under some circumstances. Most information on fibrosis regression comes from studies in animal models, where resolution can occur in most cases if the underlying etiology is eliminated. In contrast to animal models where the cause of injury is well defined, human fibrotic diseases are often multifactorial and eradication of the injurious stimuli may not be possible. More-
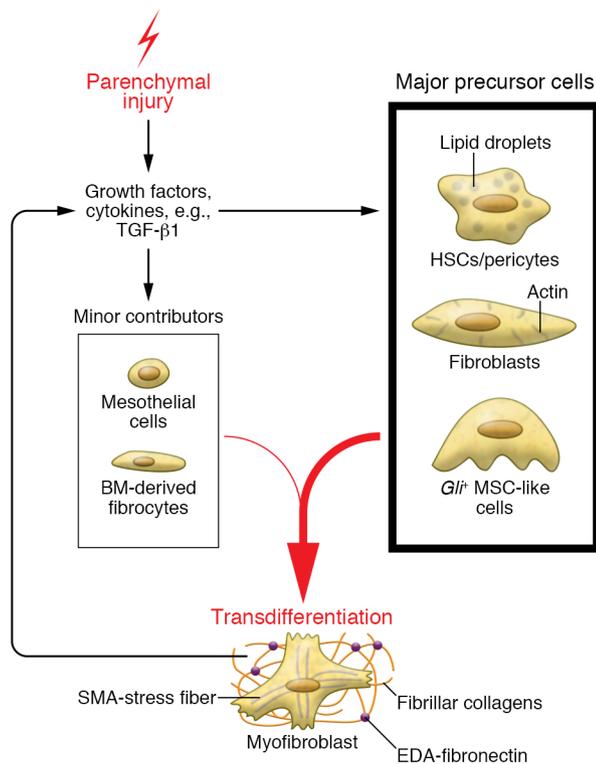
Figure 1. Transdifferentiation of precursor cells into fibrogenic myofibroblasts. Upon parenchymal injury, the wound-healing response leads to the release of fibrogenic growth factors and cytokines, the most prominent of which is TGF-β1. These factors drive the transdifferentiation and proliferation of precursor cells into myofibroblasts. Major precursors of myofibroblasts are cells of mesenchymal origin, including resident fibroblasts, pericytes, and *Gli*+ mesenchymal stem cell–like (MSC-like) cells. In some cases, myofibroblasts may be derived from bone marrow–derived (BM-derived) fibrocytes or through mesothelial-to-mesenchymal transition (MMT). Myofibroblasts produce ECM comprising fibrillar collagens that undergo extensive cross-linking and EDA-fibronectin, which promotes TGF-β1–induced transdifferentiation.

over, available animal models often do not fully recapitulate the relevant human disease, and thus treatment strategies that lead to fibrosis regression in animal models may not directly translate into therapy in humans. In the section below, we briefly summarize the etiologies associated with fibrosis in parenchymal tissues, the evidence for fibrosis reversibility, and relevant experimental models.

Liver fibrosis. The primary causes of liver fibrosis are etiologies that drive chronic inflammation, including viral and parasitic infections, excessive alcohol consumption, and nonalcoholic steatohepatitis. The most compelling evidence for the resolution of organ fibrosis in humans is observed in the liver. Patients with liver fibrosis associated with hepatitis B virus (HBV) or HCV infection treated with antiviral therapies have shown fibrosis regression and histological improvements even in cases of cirrhosis (17–19). Liver fibrosis triggered by schistosomiasis, one of the most common causes of liver fibrosis worldwide, is also thought to regress upon treatment of infection (20, 21). Common experimental rodent models for liver fibrosis include administration of a hepatotoxin (e.g., carbon tetrachloride [CCl_4]) to induce acute hepatocellular injury or bile duct ligation (BDL) to induce cholestasis, resulting in pericentral or periportal liver fibrosis, respectively. Genetic lineage tracing studies have found that the vast majority of myofibroblasts in the fibrotic rodent liver are derived from hepatic stellate cells (HSCs), irrespective of etiology (22, 23). HSCs are normally quiescent, pericyte-like vitamin A storage cells located in the space of Disse between hepatocytes and the fenestrated sinusoid. In addition, portal fibroblasts, bone marrow–derived fibrocytes, and *Gli*+ mesenchymal stem cell–like cells also contribute to hepatic myofibroblasts (13, 23), whereas mesothelial cells can give rise to HSCs and myofibroblasts through mesothelial-to-mesenchymal transition (MMT) (24).

Lung fibrosis. Fibrosis of the lung is associated with diverse etiologies, including scleroderma (systemic sclerosis), sarcoidosis, infections, and exposure to toxicants or radiation. Idiopathic pulmonary fibrosis (IPF) is the most common form of idiopathic interstitial pneumonia and is usually fatal, with a median survival of 2 to 3 years. IPF is not associated with substantial inflammation, and antiinflammatory therapy is ineffective (25). In 2014, the FDA granted fast-track approval for the profibrotic signaling inhibitors pirfenidone and nintedanib for treating IPF on the basis of their slowing of lung function decline as measured by forced vital capacity and reduced all-cause mortality (26). However, the efficacy of these drugs to promote fibrosis resolution in IPF has not been demonstrated. The most common experimental mouse model for lung fibrosis is intratracheal administration of the chemotherapeutic drug bleomycin, which induces inflammation followed by fibrosis (27). Spontaneous resolution of fibrosis with restitution of tissue architecture occurs in this model upon cessation of further bleomycin-induced injury. Various studies have found that myofibroblasts in the fibrotic lung are largely derived from pericytes, with contribution from mesothelial cells through MMT (13, 28–31).

Kidney fibrosis. Fibrosis is a major complication in all forms of chronic kidney disease (CKD), of which diabetes and hypertension are principal causes. Even though the initial injury to the kidney may affect the glomerulus, tubules, or interstitium, the final outcome of all progressive CKD is the formation of tubulointerstitial fibrosis (32, 33). Kidney fibrosis in patients with diabetic nephropathy can be ameliorated by pancreas transplantation (34, 35), suggesting that established kidney fibrosis can be reversible to some extent (36). A common rodent model of renal fibrosis is unilateral ureteral obstruction (UUO), in which one ureter is ligated while the other is left as control, causing fibrosis to develop within

Table 1. Targeting regulators of ECM metabolism in fibrosis resolution in animal models

ECM target	Liver	Lung	Kidney	Heart	References
MMP1 overexpression	Attenuated thioacetamide fibrosis			Attenuated cardiac fibrosis	52, 53
MMP2 KO	Exacerbated fibrosis in CCl_4 and BDL model				159
MMP3 KO		Protection from bleomycin fibrosis			160, 161
MMP7 KO		Protection from bleomycin fibrosis			160, 161
MMP8 overexpression (liver)/KO (lung)	Protection from CCl_4 and BDL fibrosis	Reduced bleomycin fibrosis			162, 163
MMP9 overexpression (lung)/KO (kidney)		No effect in bleomycin fibrosis	Protection from UUO fibrosis		164–166
MMP12 KO		No effect on fibrosis	No effect on UUO fibrosis		167–169
MMP13	Reduced fibrosis in KO mice; overexpression accelerated resolution	Less fibrosis in radiation injury; no effect in hyperoxia injury in KO mice			170–173
MMP19 KO	Protected from CCl_4 fibrosis				174
TIMP1 Tg (liver)/KO (liver, lung, kidney)	Enhanced fibrosis in KO and Tg mice	No effect in bleomycin fibrosis	No effect in UUO fibrosis		57, 58, 175, 176
TIMP2 siRNA	Protection from CCl_4 fibrosis				177
TIMP3 KO		Reduced resolution of fibrosis	No effect on UUO fibrosis		59, 178, 179
LOXL2 mAb	Reduction in fibrosis	Reduction in fibrosis		Reduction in fibrosis	62–64
TG2 KO	No effect in CCl_4 fibrosis	Reduction in bleomycin fibrosis			66, 67

7 days (37). Myofibroblasts in kidney fibrosis are derived primarily from resident fibroblasts, bone marrow–derived fibrocytes, and *Gli*+ progenitors (13, 38). Interestingly, recent studies have indicated that tubular epithelial cells can undergo partial epithelial-to-mesenchymal transition, leading to recruitment of inflammatory cells and release of fibrogenic cytokines, which exacerbate myofibroblast activation and fibrogenesis (39, 40).

Cardiac fibrosis. Cardiac fibrosis results from pathological myocardial remodeling triggered by heart diseases of nearly all etiologies (41). In contrast to organs discussed above, the parenchymal cells in the heart are muscle cells (cardiomyocytes) rather than epithelial cells and display very limited regenerative capacity. Consequently, extensive scarring is necessary to prevent rupture following myocardial infarction and other injuries. Nevertheless, in patients with hypertension and left ventricular hypertrophy or stiffness, regression of biopsy-proven cardiac fibrosis was observed after treatment with the hypotensive drugs lisinopril or losartan (42, 43). Experimental models of cardiac fibrosis include permanent occlusion of the left anterior descending (LAD) coronary artery to induce myocardial infarction, and transverse aortic constriction (TAC) to trigger pressure overload–induced cardiac hypertrophy. Regression of collagen deposition occurs after constriction is removed in TAC-treated mice (44) and in hypertensive rats following treatment with angiotensin-converting enzyme inhibitors (45, 46). Genetic lineage tracing found that myofibroblasts in the injured heart are primarily derived from resident fibroblasts (13, 47), which constitute a significant percentage of cardiac cells (48).

Degradation of the fibrotic ECM

Since fibrosis develops as a healing response to injury that leads to excessive production of ECM by myofibroblasts, effective resolution of fibrosis requires three critical components: (a) eradication of the cause of injury; (b) degradation and removal of the fibrotic ECM; and (c) elimination of fibrogenic myofibroblasts. Treating the cause of injury is perhaps the most efficacious approach to fibrosis resolution, as exemplified by antiviral treatment of HBV-induced liver fibrosis (17, 18). In the absence of further injury, matrix remodeling may proceed as part of wound healing and eventually lead to fibrosis resolution. However, when etiology is unclear or effective treatments for the underlying cause of injury are unavailable, tackling the fibrotic ECM directly may help to ameliorate the pathological effects of the lesion (Table 1).

There are two major types of ECM: basement membrane, which forms a lattice of support for epithelial and endothelial cells, and interstitial ECM secreted mainly by fibroblasts in the connective tissue. The basement membrane is composed of collagen IV, laminins, heparan sulfate proteoglycans, and proteins such as nidogen and entactin, whereas fibrotic ECM adopts more interstitial-like characteristics, with a preponderance of fibrillar collagen I and III, as well as fibronectin, hyaluronan, elastin, and various proteoglycans (49). As fibrosis progresses, secreted collagen becomes more heavily cross-linked by lysyl oxidase (LOX) and tissue transglutaminase (TG2), thereby forming a rigid structure that is more resistant to proteolytic degradation and can alter cellular function and tissue architecture. Tg mice expressing a nondegradable form of collagen I displayed persistent HSC activation and CCl_4-induced liver fibrosis, underscoring the importance of ECM degradation in fibrosis resolution (50).

The most important enzymes contributing to the extracellular pathway of collagen degradation are matrix metalloproteinases (MMPs), a family of Zn^{++}- and Ca^{++}-dependent endopeptidases produced by a broad spectrum of cell types. Intact fibrillar col-
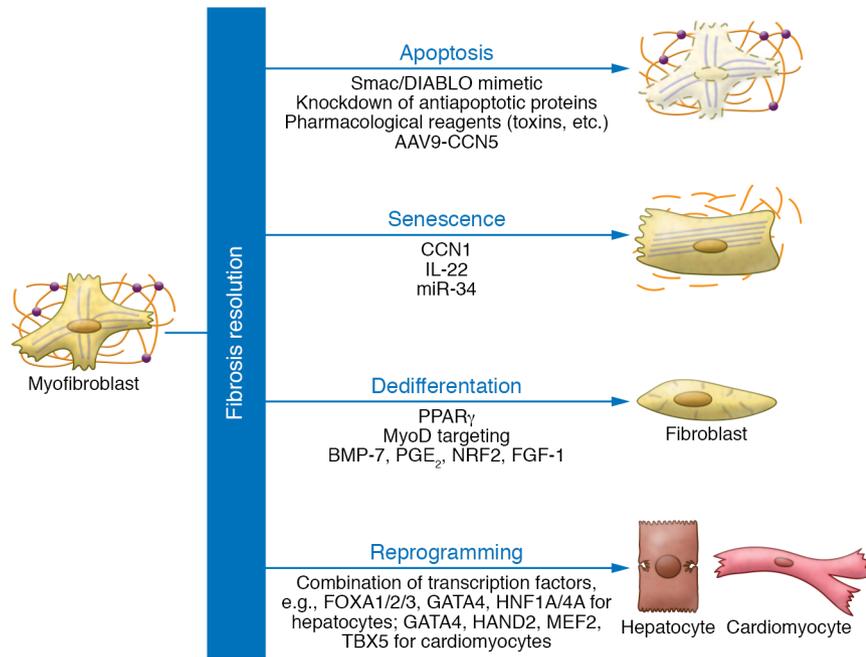
Figure 2. Fate of myofibroblasts during fibrosis resolution. Fibrogenic myofibroblasts can be eliminated during fibrosis resolution through one of several alternative cell fates: apoptosis, senescence, and dedifferentiation. Cellular lineage reprogramming also serves as an alternative route to eliminate myofibroblasts. These cell fates are not mutually exclusive and can be driven through genetic or pharmacological manipulations to promote fibrosis resolution.

lagen can be degraded by collagenases (MMP1, MMP8, MMP13, MMP18) and two membrane-anchored MMPs (MMP14 and MMP16) (51), with MMP1, MMP13, and MMP14 being the most critical in fibrotic matrix degradation (52–54). However, some MMPs can promote fibrosis through activities unrelated to direct degradation of ECM (55). MMP activities are inhibited by tissue inhibitors of metalloproteinases (TIMPs) through their binding in a 1:1 stoichiometric ratio (56). In contrast to the 25 MMPs in mammals, there are 4 TIMPs (TIMP1–TIMP4) that can preferentially interact with distinct subsets of MMPs. The resultant MMP-TIMP complexes are recognized by scavenger receptors and engulfed by macrophages. Paradoxically, both overexpression and KO of *Timp1* in mice resulted in more severe liver fibrosis (57, 58), and *Timp3*-KO mice experienced aggravated fibrosis despite an overall increase in MMP activity (59). Since TIMPs can regulate cell growth, migration, and survival (60), they may regulate cellular processes that directly or indirectly influence myofibroblast activation and ECM turnover.

LOX and its homologs, LOX-like enzymes 1–4 (LOXL1–LOXL4), are extracellular copper-dependent enzymes that catalyze aldehyde formation from lysine residues in collagen and elastin precursors (61). LOXL2 inhibition was shown to attenuate and reverse fibrosis of the heart, lung, and liver in experimental models, fueling considerable enthusiasm for LOXL2 blockade as a therapeutic approach (62–64). However, a recent clinical trial using the humanized mAb simtuzumab to inhibit LOXL2 activity did not improve progression-free survival in IPF patients (65). It is likely that the mouse bleomycin model does not adequately recapitulate human IPF, the etiology of which is unknown. TG2 can also cross-link proteins to form inter- and intramolecular bonds between lysine and glutamine residues. Although *TG2*-null mice developed reduced lung fibrosis, they did not show any difference in the extent or reversibility of CCl_4-induced liver fibrosis, suggesting that the effects of TG2 on matrix stability are tissue-specific (66, 67).

In addition to the extracellular pathways for collagen degradation, macrophages and myofibroblasts can internalize intact or fragmented collagen for degradation. For example, integrins $\alpha_2\beta_1$ and $\alpha_3\beta_1$, as well as the fibroblast-derived transmembrane urokinase plasminogen activator receptor–associated protein (uPARAP or Endo180), can bind and internalize fibrillar and nonfibrillar collagens to the lysosome for degradation (51). uPARAP-deficient mice exhibited increased total lung collagen after bleomycin injury (68). Uptake of fragmented collagen in macrophages depends on the extracellular glycoprotein milk fat globule–epidermal growth factor 8 (MFGE8), and *Mfge8*-null mice develop aggravated pulmonary fibrosis induced by bleomycin (69). MFGE8 also promotes inflammation resolution by inhibiting inflammasome-induced IL-1β production (70) and stimulating the clearance of apoptotic cells by acting as a bridging molecule that binds apoptotic cells and the phagocytic receptor integrin $\alpha_v\beta_3$ on macrophages (71).

Another critical component in ECM metabolism is the protean function of macrophages, which are recruited during the inflammatory phase and produce profibrotic growth factors, including TGF-β1. Selective depletion of macrophages during CCl_4-induced injury resulted in reduced fibrosis, whereas deletion of macrophages in the recovery phase impaired ECM degradation and fibrosis resolution, indicating that macrophages have both pro- and antifibrotic effects at different phases of injury and repair (72). During liver injury, inflammatory cytokines such as CCL2 (also known as MCP-1) recruit Ly6C^{hi} monocytes and macrophages, but these macrophages switch to a Ly6C^{lo} phenotype and produce MMPs during fibrosis resolution (73). Selective depletion of Ly6C^{lo} macrophages during fibrosis regression results in impaired scar remodeling, consistent with the matrix-degrading functions of these macrophages. Macrophage therapy using mature macrophages, but not their monocyte precursors, reduced liver fibrosis in mice by expressing MMP13 and MMP9 (74). In addition, the antifibrotic functions of macrophages may be mediated in part

Table 2. Effects of manipulating myofibroblast cell fate in animal models of fibrosis resolution

Target	Liver	Lung	Kidney	Heart	Skin	References
Apoptosis						
Hsp1a/b KO IKKβ inhibition	Accelerated regression					83, 180
Inhibition of XIAP, ROCK, HDAC, SRF/MRTF, *Mkl1* KO		Attenuated bleomycin-induced fibrosis				84, 85, 89, 90
Gliotoxin/proteasome inhibitor	Reduced fibrosis					86, 88
Ccn5 expression				Reversion of TAC fibrosis		91
Senescence						
p53/p16 KO	Exacerbated CCl$_4$ fibrosis		Enhanced UUO fibrosis	Aggravated TAC fibrosis		95, 104–106
CCN1 (protein delivery or gene expression)	Reduced CCl$_4$ and BDL fibrosis, accelerated resolution			Reduction of TAC fibrosis	Accelerated resolution in wound healing	96, 101, 102, 105
IL-22 (protein or gene expression)	Reduced fibrosis and accelerated resolution of CCl$_4$ fibrosis					103
miR-34		Reduced bleomycin-induced fibrosis				113
Dedifferentiation						
PPARγ activation (nitrated fatty acids)		Reduced severity and promoted fibrosis reversal				121
BMP7	Suppression of fibrosis		Reversal of chronic renal injury			122, 123
FGF-1		Reduced TGF-β–induced fibrosis				126
Reprogramming						
Combination of transcription factors	Attenuation of CCl$_4$-induced fibrosis			Reduced LAD-fibrosis		134, 136, 137

through their expression of the angiogenic factor VEGF. Ablation of VEGF specifically in myeloid cells, including macrophages, prevented resolution of CCl$_4$-induced liver fibrosis concomitant with reduced expression of *Mmp2* and *Mmp14* in endothelial cells and increased expression of *Timp1* and *Timp2* (75). These results point to multiple roles of macrophages in fibrosis resolution through degradation of the ECM.

Elimination of the fibrogenic myofibroblasts

Since activated myofibroblasts are the primary sources of the fibrotic ECM, elimination of these cells is a prerequisite for sustained fibrosis resolution. Fibrogenic myofibroblasts can be eliminated through several alternative cell fates, which may be exploited for potential therapeutic intervention (Figure 2 and Table 2).

Myofibroblast apoptosis. It has been well established that myofibroblasts can undergo apoptosis during fibrosis resolution, and the apoptotic cell debris is removed by macrophages and dendritic cells (76, 77). Apoptosis is coordinated by two major pathways: the extrinsic pathway, involving death receptors (e.g., TNFR-1 and Fas); and the intrinsic pathway, mediated through mitochondria, resulting in activation of caspases (78). Fas ligand–expressing immune cells induce Fas-dependent myofibroblast apoptosis during the resolution of lung fibrosis, underscoring the importance of the extrinsic apoptotic pathway (79). However, myofibroblasts

of fibrotic tissues often exhibit increased resistance to apoptosis, most commonly by overexpressing antiapoptotic and prosurvival proteins. Some of the cytokines and growth factors expressed in the inflammatory milieu can activate NF-κB, a master transcription factor that promotes cell survival through activation of antiapoptotic genes (80). Moreover, both TGF-β1 and endothelin-1 (ET-1), potent activators of myofibroblastic differentiation, can promote resistance to apoptosis by activating FAK and PI3K/AKT (81).

In the liver, activated HSCs are resistant to many apoptotic stimuli, in part because of upregulation of the antiapoptotic proteins BCL-2 and HSP1A/B (82, 83). Ablation of HSP1A/B renders HSCs more susceptible to TNF-α–induced apoptosis, and *Hsp1a/b*-deficient mice show accelerated regression of CCl$_4$-induced liver fibrosis. In addition, ECM stiffening in fibrotic conditions can modulate fibroblast resistance to apoptosis through a mechanotransduction pathway involving Rho/ROCK and the mechanosensitive transcription factor megakaryoblastic leukemia 1 (MKL1), resulting in upregulation of profibrotic and prosurvival genes (84, 85). Inhibition of ROCK by fasudil or genetic ablation of *Mkl1* protected mice from experimental lung fibrosis (84).

Several pharmacological strategies targeting antiapoptotic mechanisms of myofibroblasts can reduce the formation of fibrosis and accelerate resolution of pre-established fibrosis. Treatment with an IκB kinase (IKK) inhibitor (sulfasalazine), a

proteasome inhibitor (bortezomib), or the fungal toxin gliotoxin enhanced myofibroblast apoptosis and reduced liver fibrosis (86–88). Administration of suberoylanilide hydroxamic acid, a histone deacetylase (HDAC) inhibitor, drove apoptosis in IPF patient myofibroblasts in vitro by upregulating proapoptotic BAK and downregulating antiapoptotic BCL-XL, and ameliorated bleomycin-induced pulmonary fibrosis in mice (89). Likewise, inhibiting the caspase inhibitor XIAP, which is highly expressed in fibrotic lung fibroblasts, protected mice from bleomycin-induced lung fibrosis (90). Expression of the matricellular protein CCN5 in the heart reversed pre-established cardiac fibrosis in part by inducing myofibroblast apoptosis through activation of the intrinsic pathway (91). Together, these results suggest that targeting myofibroblast apoptosis can alleviate fibrosis burden as a potential therapeutic approach for the treatment of organ fibrosis.

Myofibroblast senescence. Cellular senescence is an essentially irreversible form of cell cycle arrest. A variety of cellular injuries or stresses, including DNA damage, oncogene activation, oxidative stress, and telomere erosion, can trigger the p53/p21 and/or the p16^{INK4a}/pRb tumor suppressor pathways to establish and maintain senescence (92, 93). Senescent cells cease to proliferate and exhibit the senescence-associated secretory phenotype (SASP), a characteristic gene expression profile that includes upregulation of ECM-degrading enzymes such as MMPs (93, 94). In myofibroblasts of the liver and skin, SASP also includes downregulation of collagen and TGF-β1 (95, 96). Thus, by undergoing senescence, myofibroblasts switch from being proliferating, ECM-producing profibrotic cells to being growth-arrested, ECM-degrading antifibrotic cells. These cells are ultimately eliminated by NK cells, CD4$^+$ T cells, and macrophages, which selectively target senescent cells for immunological clearance (95, 97, 98).

Myofibroblast senescence has been shown to play important roles in fibrosis resolution in multiple organ systems, including the liver, skin, heart, kidney, and lung (Table 2), underscoring its broad significance. Senescent myofibroblasts can be recognized as αSMA-positive cells that express senescence markers, including p21, p16^{INK4a}, and senescence-associated β-galactosidase (SA-β-gal) (95). Mice with genetic ablation of p53 and p16^{INK4a} suffered exacerbated liver fibrosis when subjected to CCl$_4$-induced injury concomitant with reduced myofibroblast senescence, elevated *Tgfb1* expression, and reduced MMP expression (95).

The secreted matricellular protein CCN1 (also named Cyr61) is upregulated during injury repair in many tissues and has been shown to regulate myofibroblast senescence in diverse contexts of injury repair (99, 100). CCN1 induces myofibroblast senescence through direct binding to integrin α$_6$β$_1$ on the cell surface, thus activating Rac1 and NADPH oxidase 1 to generate sustained accumulation of ROS, leading to activation of p53 and p16^{INK4a} and senescence (96). In excisional skin wounds, CCN1 is required for myofibroblast senescence at the maturation phase of healing to restrict fibrosis (96). Skin wounds of mice that expressed a mutant CCN1 unable to bind integrin α$_6$β$_1$ did not exhibit senescent cells; lacked expression of SASP, including MMPs; and suffered exacerbated fibrosis. Moreover, topical application of recombinant CCN1 protein to cutaneous wounds of CCN1 mutant mice restored induction of myofibroblast senescence and reduced collagen deposition to dampen fibrosis (96).

Similarly, deletion of *Ccn1* specifically from hepatocytes, the major *Ccn1*-expressing cell type in the liver, resulted in reduced myofibroblast senescence, diminished expression of MMPs, and exacerbated fibrosis upon hepatic injury due to either hepatotoxin (CCl$_4$) or cholestasis (BDL) (101). Conversely, mice overexpressing *Ccn1* in hepatocytes showed increased myofibroblast senescence and reduced fibrosis with elevated expression of SASP. CCN1 also induces cellular senescence and expression of SASP in activated HSCs and portal fibroblasts. Injection of CCN1 protein accelerated resolution of pre-established liver fibrosis (101), and adenoviral *Ccn1* expression attenuated BDL-induced fibrosis (102). In addition to CCN1, IL-22 can also induce senescence in activated HSCs via p53 activation through a STAT3-dependent pathway, leading to increased production of MMPs and reduced fibrosis (103). Consistently, ectopic expression of IL-22 reduced CCl$_4$-induced liver fibrosis and accelerated resolution of fibrosis during recovery (103).

In the heart, senescent cardiac fibroblasts accumulate after LAD ligation–induced myocardial infarction or TAC-induced cardiac hypertrophy and fibrosis (104, 105). Genetic ablation of p53 and/or p16^{INK4a} in mice abrogated the accumulation of senescent myofibroblasts, decreased expression of MMPs, and aggravated fibrosis (104, 105). Cardiac-specific expression of *Ccn1* engendered increased cardiofibroblast senescence and reduced perivascular fibrosis with improved cardiac function after TAC (105). In the kidney, both tubular epithelial cells and interstitial fibroblasts undergo senescence upon UUO-induced injury (106). Mice with ablation of p16^{INK4a} do not exhibit SA-β-gal–positive senescent cells and fibrosis is enhanced, consistent with senescence playing an antifibrotic role (106). Interestingly, inducing senescence in renal epithelial cells leads to enhanced fibrosis in aristolochic acid–induced renal fibrosis (107). Since epithelial cells are not major producers of the fibrotic ECM, senescence in these cells is unlikely to terminate fibrogenesis. Instead, senescence in epithelial cells may potentially aggravate fibrosis through the expression of proinflammatory cytokines (92, 93).

Cellular senescence in epithelial cells versus myofibroblasts is also likely to cause divergent effects in the lung. SA-β-gal–positive senescent cells are observed predominantly in alveolar and bronchial epithelial cells, but also in lung fibroblasts (108–112). Recent studies showed that the microRNA miR-34a is upregulated in lung myofibroblasts of IPF patients and in bleomycin-injured mice, and induces senescence of lung fibroblasts (113). Young mice with miR-34a ablation accumulated fewer senescent cells and suffered exacerbated pulmonary fibrosis upon bleomycin exposure, suggesting that miR-34a inhibits lung fibrosis by inducing lung myofibroblast senescence (113). In contrast to these antifibrotic effects, miR-34a is profibrotic when it is highly expressed in alveolar epithelial cells in aged mice, but not when it is expressed in fibroblasts (114). These results suggest that senescence in myofibroblasts has an antifibrotic effect, whereas senescence in lung epithelial cells may trigger further damage and exacerbate fibrosis (114, 115). Recent studies also show that selective elimination of senescent cells by senolytic agents or suicide gene expression improved lung functions, although lung fibrosis was apparently unaltered (111). In this model, both senolytic agents and suicide gene expression targeted senescent cells generally and did not distinguish between senescent epithelial cells and myofibroblasts.

Myofibroblast dedifferentiation. Fibrogenic myofibroblasts can revert to an inactive phenotype characteristic of myofibroblast precursor cells during fibrosis resolution. In CCl$_4$-induced or alcohol intoxication–induced liver fibrosis, regression is accompanied by the reversion of activated HSCs to an inactive, quiescent-like phenotype (83, 116). HSCs are normally quiescent cells with vitamin A and lipid stores that express the transcription factor PPARγ, which regulates the expression of genes relevant to adipogenesis. HSC differentiation into myofibroblasts is accompanied by PPARγ depletion, whereas activation or overexpression of PPARγ results in the phenotypic reversal of activated HSCs to quiescent cells in vitro (117, 118). When activated HSCs revert to an inactivated phenotype during fibrosis resolution, they re-express PPARγ and downregulate the expression of fibrogenic genes, including *Acta2* (encoding αSMA), *Col1a1*, *Col1a2*, *Timp1*, and *Tgfbr1* (83). Interestingly, inactivated HSCs are more responsive to further fibrogenic stimuli and express fibrogenic genes more strongly when stimulated by TGF-β than naive HSCs that have not been previously activated (83, 116). Therefore, recovering livers are likely more susceptible to recurring fibrogenic insults.

In the lung, lipofibroblasts (lipid interstitial cells) transdifferentiate into myofibroblasts in response to hyperoxia or nicotine exposure (119, 120). As in HSCs, depletion of PPARγ in lung fibroblasts induced a profibrotic phenotype, and activation of PPARγ promoted dedifferentiation in lung myofibroblasts (121). Lineage tracing studies show that lipofibroblasts are contributing precursors of myofibroblasts after bleomycin-induced injury, and some of the myofibroblasts revert back to lipofibroblast-like cells following fibrosis resolution, with downregulation of αSMA and upregulation of lipofibroblast markers (31). Myofibroblast dedifferentiation is also observed in the heart, where lineage tracing showed that cardiac myofibroblasts are derived from periostin-expressing *Tcf21*⁺ resident fibroblasts (47). Periostin-traced myofibroblasts revert to a less active state upon injury resolution, losing expression of myofibroblast-associated genes including *Acta2*, collagen, and fibronectin, but regaining expression of genes associated with the fibroblast phenotype including *Tcf21* and *Pdgfa* (47).

The observation that myofibroblasts can revert to a phenotype similar to their precursors suggests that their dedifferentiation may be subject to manipulation to promote fibrosis resolution. BMP7 can inhibit liver and lung fibrosis and reverse kidney fibrosis in experimental models, in part by antagonizing TGF-β1 functions and causing reversal of the myofibroblast phenotype (122–124). Overexpression of FGF-1 attenuates TGF-β1–induced lung fibrosis and can reverse the myofibroblast phenotype by downregulating αSMA expression (125, 126). In lung fibroblasts, prostaglandin E$_2$ (PGE$_2$) was shown to block TGF-β1– and ET-1–induced myofibroblast differentiation and induce dedifferentiation of myofibroblasts through inhibition of FAK activation (127, 128). However, the efficacy of inducing myofibroblast reversion pharmacologically in vivo for fibrosis resolution is unclear. For example, administration of PGE$_2$ in mice subjected to bleomycin exposure failed to engender therapeutic effects (129). Likewise, although activation of nuclear factor erythroid 2–related factor 2 (NRF2) can increase antioxidant defenses and induce myofibroblast dedifferentiation in vitro, administration of the NRF2 activator sulforaphane did not protect mice from bleomycin-induced pulmonary fibrosis (130).

Myofibroblast reprogramming: potential route of fibrosis resolution. Recent studies have demonstrated remarkable cellular plasticity in myofibroblasts, and differentiated cells can dedifferentiate or transdifferentiate into other cell types, either physiologically or under experimental manipulation (131, 132). Although lineage reprogramming has long been observed in amphibians during injury repair, it was demonstrated only recently that mammalian skin myofibroblasts can undergo reprogramming for tissue regeneration (133). This process requires BMP ligands supplied by hair follicles present in close proximity. Human keloid fibroblasts cultured in the presence of BMP4 or human scalp hair follicles can be induced to become lipid-laden adipocytes, suggesting the potential of manipulating myofibroblast reprogramming to accelerate fibrosis resolution (133). The feasibility of reprogramming fibroblasts in vivo to promote tissue regeneration and reduce fibrosis has been shown in the heart, where fibroblasts can be directly reprogrammed to cardiomyocytes by expressing certain transcription factors (GATA4, HAND2, MEF2, and TBX5) (134, 135). Moreover, cardiac fibroblast reprogramming reduced LAD ligation–induced fibrosis (134). More recent studies have shown that hepatic myofibroblasts can be reprogrammed into hepatocyte-like cells to promote fibrosis resolution (136, 137). Using either an adeno-associated viral vector (AAV6) that preferentially targets liver myofibroblasts to express six murine transcription factors (HNF1A, HNF4A, FOXA1, FOXA2, FOXA3, FOXA4) (136), or targeted expression of four human transcription factors (FOXA3, HNF1A, HNF4A, GATA4) in hepatic myofibroblasts (137), some of the transduced hepatic myofibroblasts underwent conversion to hepatocytes, albeit at low efficiency (~4%). In vivo reprogramming of myofibroblasts was able to attenuate hepatic fibrosis induced by up to 8 weeks of repeated CCl$_4$ injections, but was unable to resolve severe fibrosis developed after 12 weeks of CCl$_4$ injections in a mouse model of cirrhosis (136, 137). Thus, even a rather low efficiency of myofibroblast reprogramming can produce beneficial antifibrotic effects. Notably, a fate-tracing study did not observe the transdifferentiation of hepatic myofibroblasts into hepatocytes during spontaneous resolution of CCl$_4$-induced liver fibrosis (116), further suggesting that spontaneous lineage reprogramming rarely occurs, if at all. Nevertheless, in vivo lineage reprogramming may represent a promising therapeutic approach (138, 139).

Conclusions and perspectives

Production of ECM and subsequent remodeling of the provisional matrix are components of normal wound healing. Therefore, it may be possible to harness and amplify the endogenous mechanisms of matrix resolution to treat organ fibrosis, which develops largely due to repeated or chronic injuries. Eradication of the underlying deleterious stimuli is perhaps the most effective strategy toward fibrosis resolution. Where treating the root cause of fibrosis is not possible, it appears that elimination of fibrogenic myofibroblasts is a promising approach. Current information indicates that myofibroblasts can be removed through several alternative cell fates: apoptosis, senescence, dedifferentiation, and reprogramming. It is presently unclear how the specific cell fate of myofibroblasts in a given context is determined, and understanding this aspect will be a significant advance. Accumulating evidence indicates that autophagy, a cellular pathway of organelle and protein degradation, can

influence the determination of cell fate, including apoptosis, senescence, differentiation, and reprogramming (140–143). Autophagy is known to play either an enhancing or an inhibitory role in fibrosis depending on the target cell type through selective degradation of cellular components (144–146). For example, autophagy in HSCs promotes their fibrogenic activation (147), whereas autophagy in macrophages and hepatocytes is antifibrotic by reducing injury-induced inflammation (148, 149). Further studies into genetic or pharmacological manipulations that can dictate specific myofibroblast cell fate may help achieve therapeutic objectives (Table 2).

In animal models of fibrosis and in human liver fibrosis where resolution is clearly documented, restoration of the parenchymal tissue is also observed. However, it is unclear whether fibrosis resolution and parenchymal restitution are mechanistically linked. It is plausible that fibrosis resolution promotes parenchymal repair, which may in turn signal to accelerate or dampen fibrosis resolution. Ideally, the rate of fibrosis resolution should be coordinated with parenchymal regeneration, since removal of the fibrotic scar without concomitant replacement with parenchyma may result in weakened tissue structure and potentially cause rupture. This coordination is particularly important in organs in which the regenerative capacity of the parenchyma is limited. Little is known about the possible crosstalk between fibrosis resolution and parenchymal regeneration, and elucidation of this interaction will be important in future studies.

Since mammalian aging is associated with reduced tissue regeneration (150) and increased risk and severity of fibrotic diseases (151, 152), targeting molecular alterations associated with aging may lead to reversal of persistent fibrosis in some instances (109).

Current treatments for fibrosis are extremely limited. A major challenge is that animal models do not fully recapitulate human pathology in fibrotic diseases, and preclinical models are often not predictive of the efficacies of novel therapies in humans. In this regard, substantial progress is being made in the development of mouse models with humanized immune system or parenchymal tissue (153, 154), some of which have been used for studying specific aspects of fibrosis (155–158). Although significant challenges still remain in the application of these experimental systems, the development of improved animal models will likely lead to new insights into the resolution of fibrosis.

Acknowledgments

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