

# The balancing act of the liver: tissue regeneration versus fibrosis

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**Epithelial cell loss alters a tissue's optimal function and awakens evolutionarily adapted healing mechanisms to reestablish homeostasis. Although adult mammalian organs have a limited regeneration potential, the liver stands out as one remarkable exception. Following injury, the liver mounts a dynamic multicellular response wherein stromal cells are activated in situ and/or recruited from the bloodstream, the extracellular matrix (ECM) is remodeled, and epithelial cells expand to replenish their lost numbers. Chronic damage makes this response persistent instead of transient, tipping the system into an abnormal steady state known as fibrosis, in which ECM accumulates excessively and tissue function degenerates. Here we explore the cellular and molecular switches that balance hepatic regeneration and fibrosis, with a focus on uncovering avenues of disease modeling and therapeutic intervention.**

## Introduction

Regeneration is the ability to recreate original tissue architecture and function following damage without leaving a scar (ref. 1 and Figure 1). Far from mythological contrivance, this mechanism is very much present in nature yet varies dramatically across metazoan species (2) and with age (3); think of an axolotl or a salamander, which seamlessly regrows its limbs after amputation (Figure 1A). Mammals share a similarly remarkable ability to regenerate tissue during prenatal development but lose most of it in adulthood. Adult injuries are *repaired* as opposed to regenerated, replacing functional tissue parenchyma with a meshwork of extracellular matrix (ECM). The liver is one of the few organs in the mammalian body that defy this paradigm, as it can regenerate efficiently from a wide range of physical and toxic injuries (4). Adult regenerative powers are nonetheless finite, even in the liver. The process of regeneration following an acute insult is characterized by a *transient* cellular and molecular response whose resolution is as important as its emergence for the tissue to reestablish homeostasis (5). It thus follows that switching-off mechanisms must be embedded within the process of wound healing because the same pathways that promote regeneration, when overstimulated, progressively drive scarring and degeneration of the tissue in a process known as fibrosis (6). As a parallel to fibrosis mechanisms, we can think of how cell proliferation, when uncontrolled, may eventually progress into tumorigenesis. In this Review we will explore the delicate balance that exists between regeneration and fibrosis, with a special focus on the liver as an organ that is familiar with both processes.

## Liver regeneration

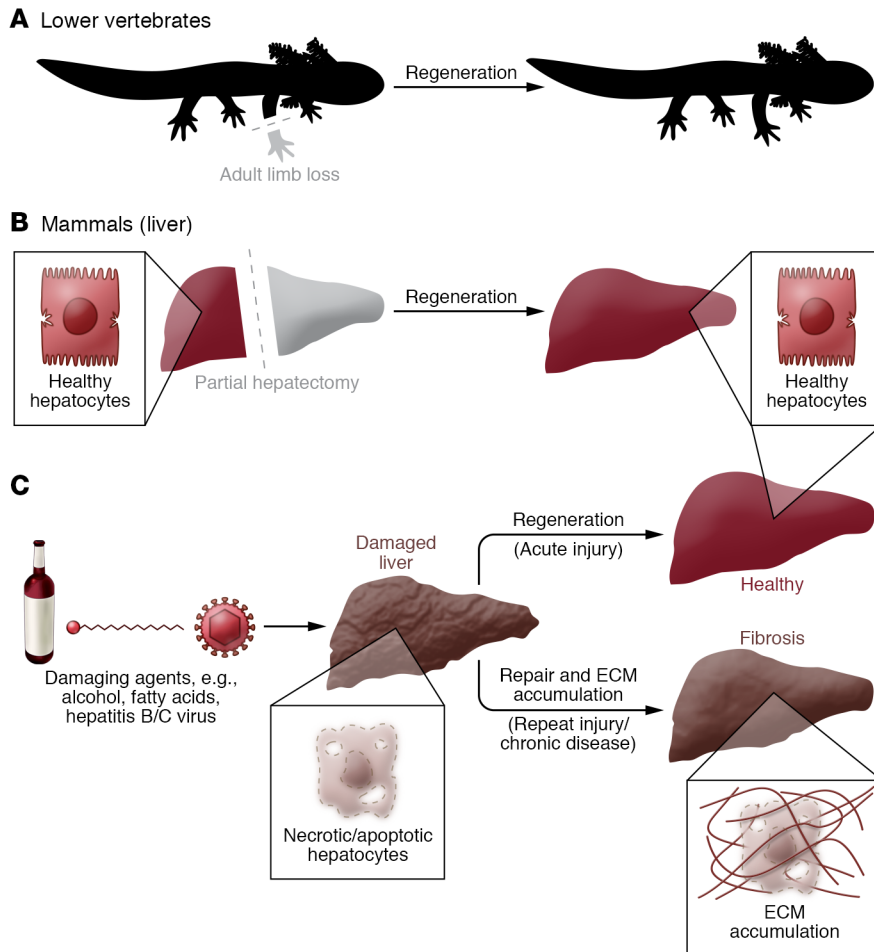
In the absence of injury, the liver epithelium is maintained by the slow turnover of hepatocytes (7) and/or ductal cells (8) within their own compartments. Experiments in rats have shown that between 0.2% and 0.5% of hepatic cells are dividing at any given time point (9). However, this mitotic quiescence is misleading because, if challenged, the hepatic tissue displays a remarkable capacity for regeneration and reinstalls homeostasis within days. Reminiscent of limb regrowth in amphibians, up to 70% of the liver can be surgically resected and the organ will grow back to its original size through compensatory proliferation of both the epithelium (hepatocytes and biliary duct cells) and the stroma, composed of Kupffer cells (macrophages), liver sinusoidal endothelial cells (LSECs), hepatic stellate cells (HSCs), and portal fibroblasts (10). Notwithstanding, the hepatectomized liver is not considered injured nor “damaged”; regeneration occurs from the unscathed lobe(s) as a result of the organ's ability to sense insufficient size (Figure 1B). The hepatectomy-induced healing response thus has clinical relevance for live-donor transplants and tumor resections but is of less consequence to chronic liver pathologies like nonalcoholic fatty liver disease and cirrhosis, which account for high rates of morbidity worldwide (11, 12). Hepatic epithelial cells, hepatocytes in particular, are susceptible to pathologies of this sort because of their daily exposure to exogenous and endogenous toxins (alcohol, viruses, and fatty acids, among others) as part of their metabolic and digestive functions. This has subjected the tissue to a unique evolutionary pressure to develop robust, yet not infallible, mechanisms of regeneration against toxic injury (Figure 1C).

Epithelial progenitor cells are thought to compensate for tissue loss in many adult tissues, in what has been hypothesized as a reiteration of developmental mechanisms (13–17). In the liver, this idea resonates loudly, considering that hepatoblasts are bona fide bipotential precursors of bile duct cells and hepatocytes during organogenesis (14). Pioneering work in adult rat livers has

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**Figure 1. Coping with injury: regeneration versus repair.** (A) Lower vertebrates, such as axolotls, salamanders, and fish, are able to regenerate severed limbs through a process that reconstitutes original tissue anatomy and function without leaving a scar (a meshwork of ECM). Mammals may similarly regenerate complex tissues during embryogenesis, but lose most of this capacity in adulthood. (B) The liver is one of the few adult mammalian organs that retains a remarkable ability to regenerate itself. Resection of up to 70% of the liver mass via partial hepatectomy leads to compensatory growth from the intact tissue and fully restores organ size in a matter of days, similarly to axolotl limb regrowth. However, the hepatectomized liver is typically not injured or “damaged,” and regeneration is a result of the organ’s ability to sense insufficient size. (C) The liver may also regenerate following injury by exogenous and/or endogenous agents (e.g., alcohol, hepatitis B/C viruses, fatty acids) that cause hepatocyte death. This process is characterized by an inflammatory reaction and ECM synthesis/remodeling. However, if the damaging insult persists, the tissue will be repaired instead of regenerated, resulting in excessive scarring, known as fibrosis, that alters histoarchitecture and hinders optimal tissue function.

also shown a robust damage-induced expansion of “oval-looking” cells expressing developmental markers and pulse-chasing into both mature hepatocytes and biliary ducts (18–20). Nonetheless, the cell of origin and the regenerative potential of these cells remain contentious to this very day. Evidence supporting biliary ancestry comes from lineage tracing experiments in *Sox9-CreER* (21) and *Opn-CreER* (22) mice, and from the fact that whole ductal tree fragments (23) or ductal marker-enriched (e.g., EpCAM<sup>+</sup>, MIC1-1C3<sup>+</sup>, CD24<sup>+</sup>, CD133<sup>+</sup>) single cells self-renew in vitro as 2D monolayers or 3D organoid cultures (24–27) while maintaining potency toward the hepatocyte lineage. Several recent studies have, however, shown minimal regeneration of the hepatocyte parenchyma by ductal-derived progenitors in vivo, in contrast to the robust contribution from hepatocytes themselves (8, 28–30). Still, the clinical reality is that “ductular responses” are frequently observed in patients with chronic liver diseases (31, 32), where hepatocytes are mostly senescent (33). Conditional deletion of *Mdm2* in up to 98% of hepatocytes, which causes them to senesce, activates a vigorous progenitor response that correlates with the full recovery of liver function in mice (27). Similar results have been observed in zebrafish livers after extensive hepatocyte loss (34). On the other hand, mature hepatocytes have been shown to undergo reversible ductular metaplasia in chronically damaged livers, regenerating up to 60% of their lost cell numbers, which suggests that part of the progenitor pool

could originate from hepatocytes as an injury escape mechanism (35). Recently, using lineage tracing approaches, Raven and colleagues have unequivocally shown that ductal progenitors contribute to the regeneration of the hepatocyte lineage in murine livers with impaired hepatocyte proliferation caused by both p21 overexpression and loss of *Itgb1* (encoding integrin  $\beta_1$ ) (36).

Regardless of the cell-of-origin debate, what we can gather from these studies is an exceptional degree of epithelial plasticity in the regenerating liver (reviewed more exhaustively elsewhere, refs. 37–39). This is intimately linked to the source and extent of tissue damage and suggests an instructive role for the microenvironment. Indeed, the recovery from epithelial-specific injuries relies on auxiliary responses by nondamaged stromal cells that become activated in situ and get recruited from the bloodstream. Several paracrine signaling pathways, whose ligands are of stromal origin — WNT (40–44), hepatocyte growth factor (HGF) (43, 45, 46), fibroblast growth factor (FGF) (47) — can directly stimulate epithelial cells to reenter cell cycle, dedifferentiate, and/or redifferentiate, and have been shown to be essential for regeneration (48). Wound healing is further characterized by the transient remodeling, de novo synthesis, and deposition of ECM, which releases latent cytokines (e.g., pro-HGF) (49) and ensures epithelial cell repositioning within the 3D histoarchitecture (50). A niche of fibrillar collagen and laminin invariably surrounds hepatic progenitor cells

**Table 1. Experimental animal models of liver fibrosis**

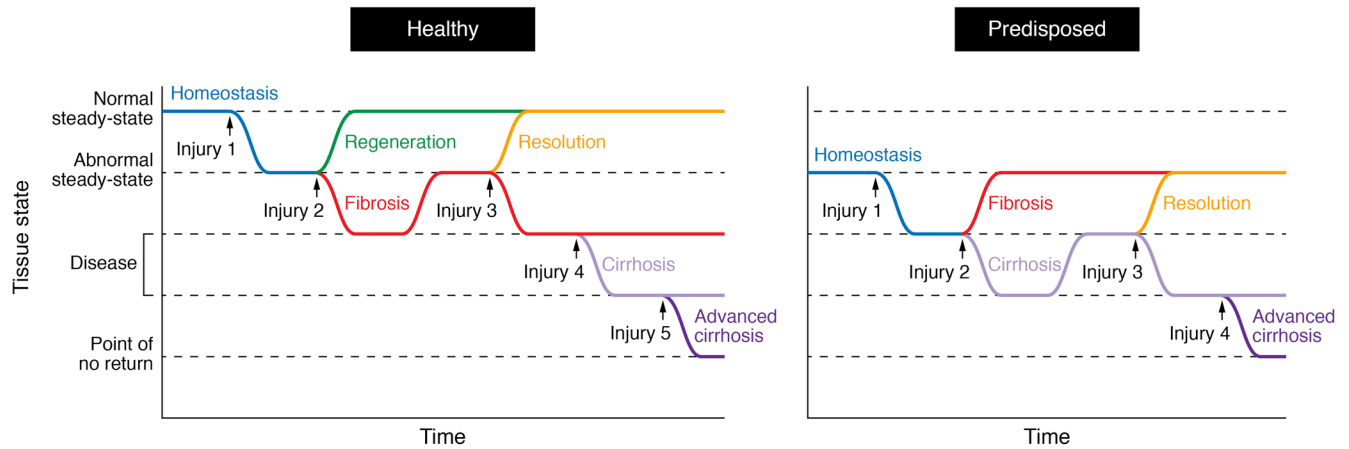
Models of liver fibrosis	Animal	Protocol/method	Onset of fibrosis	References
<b>Toxic/xenobiotics</b>				
Carbon tetrachloride (CCl <sub>4</sub> )	Rats	s.c. or i.p. twice weekly, 0.2 ml/100 mg body weight of CCl <sub>4</sub> in oil (1:1 ratio)	>4–6 weeks	168, 169
	Mice	i.p., every 5 days, 1 μl/g body weight of CCl <sub>4</sub> in oil (1:7 ratio)	4 weeks	170
Dimethylnitrosamine (DMN)	Rats	i.p., 10 mg/kg body weight, twice weekly	>4 weeks	171, 172
	Mice	i.p., 10 mg/kg body weight, thrice weekly	>3 weeks	173
	Dogs	Orally twice weekly or intraperitoneally once weekly	>3–6 weeks	174, 175
Thioacetamide (TAA)	Rats	At 300 mg/l in drinking water	>2–3 months	176
	Mice	At 200 mg/l in drinking water	>3–4 months	177
		i.p., thrice weekly, 150–200 mg/kg body weight	>6 weeks	178
3,5-Diethoxy-carbonyl-1,4-dihydrocollidine (DDC)	Mice	Supplemented (0.1%) in solid diet	>4–8 weeks	179
Ethanol	Rats	Intragastric infusion of ethanol (25%–47% of calories) and high-fat diet	>3 months	180
	Mice	Liquid diet with 5% ethanol for 10 days, then 1 dose 5 g/kg body weight by gavage	Damage, no overt fibrosis	181
	Baboons	Liquid diet with ethanol (50% of calories), twice a day	>6 months	182
<b>Nutritional</b>				
Choline-deficient, ethionine-supplemented (CDE) diet	Mice	Choline-deficient diet supplemented with 0.15% ethionine in drinking water	>2 weeks	183
	Rats		>10 weeks	184
Methionine- and choline-deficient (MCD) diet	Mice	Methionine- and choline-deficient diet	8–10 weeks	185
	Rats		10 weeks	186, 187
Methionine- and choline-deficient, ethionine-supplemented (MCDE) diet	Mice	Methionine- and choline-deficient diet supplemented with 0.15% ethionine in drinking water	1–3 weeks	188
<b>Surgical</b>				
Bile duct ligation	Mice	Common extrahepatic bile duct is ligated	3 weeks	189
	Rats		>4 weeks	190
	Dogs		>4 weeks	191
	Monkeys		>8 weeks	192
<b>Genetic</b>				
TGF-β1	Mice	Dox-repressible expression of TGF-β1 transgene, conditional to hepatocytes ( <i>Cebpb-tTA</i> )	10 inductions	193
	Mice	LPS-inducible expression of fusion transgene CRP-TGF-β1, conditional to hepatocytes	Only mild fibrosis	194
<i>Pdgfb</i>	Mice	Expression of <i>Pdgfb</i> transgene, conditional to hepatocytes (albumin promoter)	>5 months postnatally	195
<i>Mdr2</i> KO	Mice	KO of the phospholipid transporter <i>Mdr2</i>	>3 months postnatally	196
<i>Nemo</i> KO	Mice	KO of the NF-κB essential modulator ( <i>Nemo</i> ) conditional to liver parenchymal cells ( <i>Alfp-Cre</i> )	>6–12 weeks postnatally	197
<i>Tak1</i> KO	Mice	KO of TGF-β-activated kinase 1 ( <i>Tak1</i> ) conditional to liver parenchymal cells ( <i>Alfp-Cre</i> )	>6–12 weeks postnatally	198
<i>Bcl-xl</i> KO	Mice	KO of the antiapoptotic <i>Bcl-xl</i> conditional to hepatocytes (albumin- <i>Cre</i> )	>5 months postnatally	199
<i>Mdm2</i> KO	Mice	β-Naphthoflavone-inducible KO of <i>Mdm2</i> conditional to hepatocytes ( <i>Ah-Cre</i> )	>3 months	27
<b>Immunological</b>				
2-OA-BSA/α-GC	Mice	Immunization with 2-octynoic acid and BSA, then exposure to α-galactosylceramide	>4–12 weeks	200

in the damaged liver, and loss of contact with specific matrix proteins may stimulate differentiation of the progenitor pool, as shown for laminin (51–53). The regeneration of the liver following acute injury thus requires a coordinated process of epithelial and stromal interactions that feed back onto one another until homeostasis is reestablished. The cessation of healing is albeit a process that is poorly understood. Hepatectomy studies point toward the existence of a “hepatostat” system that controls tissue size for optimal performance (4). In other organs, the Hippo pathway has been shown to limit tissue overgrowth by sensing cellular density and inactivating the proliferative, antiapoptotic program driven by Yes-associated protein (YAP) (54) and its paralog, the transcriptional coactivator with PDZ-binding motif

(TAZ), which may also negatively regulate WNT (55) and TGF-β signaling (56), respectively. In the liver, defects in various components of the Hippo pathway lead to hepatomegaly and tumorigenesis due to uncontrolled proliferation of both hepatocytes and hepatic progenitors (57, 58). Moreover, YAP also drives HSC activation and matrix synthesis (59, 60), suggesting that Hippo signaling may simultaneously modulate regeneration and a maladaptive response leading to fibrosis (see below).

### Liver fibrosis and its cellular effectors

Damage-induced matrix deposition is a transient phenomenon of the regenerative response, and successful healing entails its eventual removal (61, 62). Fibrosis occurs when ECM proteins



**Figure 2. Periodicity of damage alters the ability of the tissue to return to homeostasis.** (Left) In healthy individuals, a punctual tissue injury (injury 1) to the liver awakens a regenerative response (green curve) to reestablish homeostasis or steady-state. Repeated injuries (injuries 1 + 2) hinder regeneration and make the system drift into a diseased state known as fibrosis (red curve). The tissue may recover from this as time progresses if no further damage is applied (resolution, injuries 1 + 2 + time, yellow curve). Alternatively, fibrosis will be maintained in the face of new damage (injuries 1+2+3, red curve). Additional injuries deteriorate the tissue until it reaches a cirrhotic (1 + 2 + 3 + 4, light purple curve) or advanced cirrhotic (1 + 2 + 3 + 4 + 5, dark purple curve) state. Recovery from this latter scenario is very unlikely. (Right) The tissue of predisposed individuals (e.g., aged) functions at an abnormal steady-state that makes them prone to develop fibrosis, thus accelerating disease progression and reaching a point of no recovery earlier.

accumulate in excessive amounts, leading to scarring that distorts the normal layout and stiffness of the tissue. Experimental models of hepatic fibrosis in rodents, dogs, and monkeys, whereby tissues are analyzed for collagen deposition (e.g., Sirius red staining), have been fundamental for studying the onset and pathogenesis of this disease (Table 1). As the injury becomes chronic, the once-functional hepatic parenchyma is overtaken by an acellular mesh of connective tissue — mostly collagen and elastin fibers — whose progressive cross-linking restrains access to degrading enzymes and makes scar resolution increasingly difficult (63).

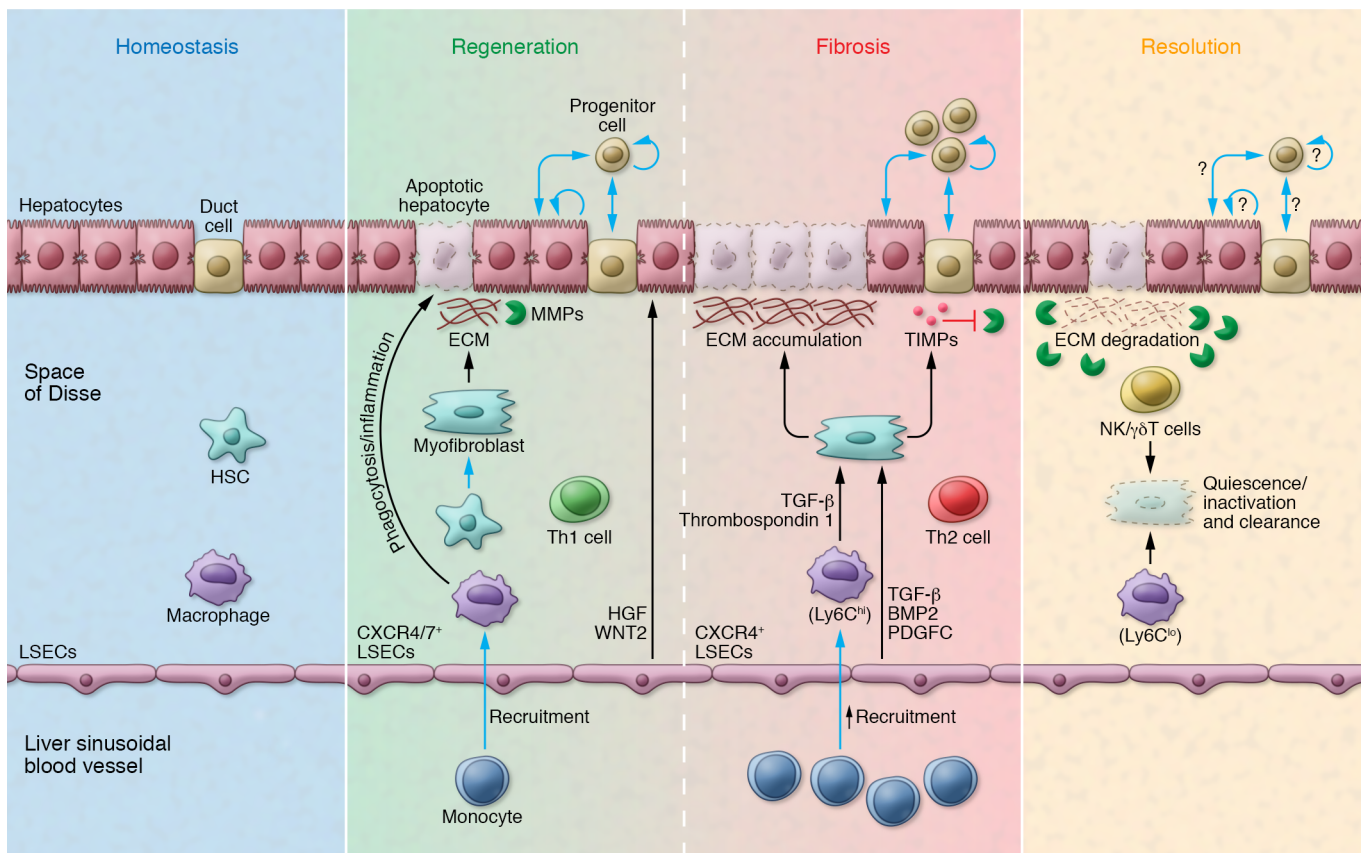
The specialist producers of ECM in many tissues of the body are myofibroblasts: proliferative and migratory cells that express high levels of fibrillar collagens and tissue inhibitors of metalloproteinases (TIMPs) (64, 65). These cells are thought to have diverse origins, but they share a common process of transdifferentiation to acquire profibrotic traits in the context of damage (66). HSCs are microvasculature-associated pericytes and the best-studied precursors of myofibroblasts in the liver. These cells transition from quiescence to an active myofibroblast-like state following injury, and they are the dominant contributors to liver fibrosis, independent of its etiology (67). The ability of HSCs to respond to diverse types of damage may relate to their widespread placement in the liver architecture, which allows them to act swiftly at multiple sites of injury. In contrast, portal fibroblasts are found exclusively around the portal tract and predominantly drive fibrogenesis in biliary disease and cholangiocarcinomas (68). Bone marrow-recruited monocytes may also differentiate into ECM-producing “fibrocytes” as part of the inflammatory response, although transplantation studies suggest that their contribution to the development of hepatic fibrosis is minimal compared with that of tissue-resident mesenchymal cells (69, 70). The activation of “local” ECM producers may not be a universal mechanism in the body, however, considering that the fibrotic lung recruits a large amount of its collagen-producing

cells from the bone marrow (71). In severely damaged livers, epithelial cells have also been proposed to feed the myofibroblast pool through an epithelial-to-mesenchymal transition (EMT) (72). The support for EMT has nonetheless been overreliant on the partial upregulation of mesenchymal markers in vitro, and a committed switch toward the myofibroblast lineage in vivo has not yet been formally proven (73, 74).

The transition from quiescent “cell X” (be it HSC, portal fibroblast, or monocyte) to active myofibroblast-like phenotype is a malleable process wherein the chronicity of the damage stimuli may generate a mixed spectrum of profibrotic cell signatures. While HSC-derived myofibroblasts have been reported to revert to quiescence readily (75, 76), myofibroblasts originating from portal fibroblasts are locked in a more mature/committed state and are unlikely to transition back (68). Understanding disease progression over the duration of injury as well as profibrotic cell heterogeneity is thus important when considering antifibrotic treatments.

### Cellular and molecular fluctuations balance regeneration and fibrosis

Regeneration and fibrosis share a common cascade of injury-induced events that bifurcates as a result of the chronicity of the damage (Figure 2). At the core of this cascade lie time-dependent multidirectional interactions between epithelial, mesenchymal, endothelial, and immune cells (Figure 3). Inflammation is one of the earliest processes following injury (1), preceding the actual repair of the lesion, and its mechanism is tightly linked to the type of damaging agent (i.e., underlying etiology). Commonly, dying cells or foreign antigens are recognized by tissue-resident or recruited leukocytes of the innate immune system, causing them to express proinflammatory agents (like TNF- $\alpha$ ) to further relay the “damage” signal (77). In that regard, in alcoholic liver disease and nonalcoholic steatohepatitis, overgrowth of LPS-containing gut bacteria induces liver-resident macrophages to produce reactive oxygen species and



**Figure 3. Distinct cellular landscapes characterize homeostasis, regeneration, fibrosis, and resolution in the liver.** The homeostatic liver is characterized by rare cell proliferation and lack of de novo ECM deposition. During regeneration, epithelial replacement occurs predominantly via hepatocyte proliferation and, to a minor degree, through the activation of ductal progenitors. Resident (Kupffer) and bone marrow–recruited macrophages phagocytose the dead epithelium and launch an inflammatory cascade (e.g., TNF- $\alpha$ , IL-6). CXCR7/CXCR4<sup>+</sup> LSECs provide mitogenic signals (HGF, WNT2) that sustain hepatocyte proliferation. HSCs transdifferentiate into myofibroblasts that deposit ECM on the wound site, although this matrix can be degraded via MMPs. In fibrosis, the hepatocyte compartment is highly senescent and ductal progenitor expansion becomes predominant. Monocyte-derived Ly6C<sup>hi</sup> macrophages (secreting TGF- $\beta$ , thrombospondin 1) and CXCR4<sup>+</sup> LSECs (secreting TGF- $\beta$ , BMP2, and PDGFC) collectively enhance myofibroblast proliferation and survival. Myofibroblasts, in turn, secrete high levels of TIMPs, which inhibit MMPs and cause excessive matrix accumulation. A Th1- versus Th2-skewed immune system favors regeneration versus fibrosis, respectively. The resolution of fibrosis entails the return to quiescence/inactivation of myofibroblasts as well as their clearance by NK cells,  $\gamma\delta$  T cells, and Ly6C<sup>lo</sup> macrophages. High levels of MMPs contribute to matrix degradation. The mechanisms of epithelial replacement at this stage have not been fully elucidated.

TNF- $\alpha$  (78, 79). On the other hand, viral hepatitis is different in that the hepatitis C virus (HCV) escapes immune surveillance and infects hepatocytes directly, causing oxidative stress as well as apoptosis (80, 81). Mesenchymal cells reinforce the inflammatory cascade by upregulating leukocyte-recruiting chemokines (82–85) and adhesion molecules (86), although they may also engage directly in classical innate immune roles like phagocytosis, antigen presentation, and T cell activation, as shown in isolated human HSCs (87). Acetaldehyde, the major metabolic product of alcohol, and HCV proteins directly stimulate the proinflammatory and fibrogenic profile of HSCs (88, 89). Studies in damaged skin (and after LPS or TNF- $\alpha$  stimulation *in vitro*) have shown that pericytes upregulate ICAM-1 and secrete macrophage migration inhibitory factor (MIF) to attract macrophages and neutrophils, which they later instruct with pattern recognition and motility programs (90). Macrophages can in turn activate quiescent HSCs into scar-forming myofibroblasts by secreting factors such as TGF- $\beta$  (91), PDGF (92), galectin 3 (93, 94), and TNF- $\alpha$  (95). Indeed, activated myofibroblasts and macrophages

spatially colocalize in areas of scar tissue. Depletion of macrophages with chemicals like gadolinium chloride and liposomal clodronate (96–98) or in *ITGAM-DTR* (also known as *CD11B-DTR*) transgenic (99) mice consistently dampens myofibroblast activation and tissue fibrosis in response to chronic injury. Yet macrophage populations are heterogeneous, and not all of them contribute equally to fibrosis (100). Liver-resident macrophages, known as Kupffer cells, ensure immunosurveillance of the tissue in homeostasis and contribute to the immediate response following injury partly through TNF and IL-6 (101, 102); however, these cells drop in numbers as inflammation progresses, while monocyte-derived macrophages increasingly colonize the tissue from the bloodstream (103–105). This latter population, described as CD11B<sup>hi</sup>F4/80<sup>int</sup>Ly6C<sup>hi</sup>, secretes high levels of TGF- $\beta$  and the TGF- $\beta$ -activating protein thrombospondin 1, thus supporting fibrinogenesis (104, 106).

Although seemingly profibrotic, macrophages have a far more complex role in the process of wound healing (99, 107). Mice exhibiting impaired infiltration of monocyte-derived

macrophages (e.g., *Ccr2*<sup>-/-</sup> mice) develop an attenuated form of hepatic fibrosis when persistently damaged, yet present defects to regress to homeostasis (108). Indeed, as part of a late-stage regenerative mechanism, recruited macrophages undergo a phenotypic switch from profibrotic (CD11B<sup>hi</sup>F4/80<sup>int</sup>Ly6C<sup>hi</sup>) to scar-resolving (CD11B<sup>hi</sup>F4/80<sup>int</sup>Ly6C<sup>lo</sup>), characterized by the increased expression of matrix metalloproteinases MMP13, MMP9, and MMP12 to clear away excess ECM, and upregulation of the TNF ligand superfamily member 10 (TNFSF10 or TRAIL), which can specifically trigger apoptosis in activated myofibroblasts due to their high expression of TRAIL receptors 1 and 2 (109). This phenotypic switch of macrophages, which is governed partially by phagocytosis (106), occurs in many other tissues, like muscle (110), skin (111), and lung (112), and hampering it may reinforce the pathogenic cycle of fibrosis. In wound healing, acute inflammation is also accompanied by a long-lasting adaptive immunity that is mounted by lymphocytes like T, B, and natural killer (NK) cells and is customized to the type of damage. This concept is interesting because it signifies that lifestyle, age, and disease history may pre-establish an important bias toward either regeneration or fibrosis (Figure 2). Th2 cells exacerbate fibrosis by secreting IL-4 and IL-13, which instigate macrophages to produce TGF- $\beta$  (113) and myofibroblasts to deposit ECM (114–116); on the other hand, Th1 cells prevent scar formation by producing IFN- $\gamma$  and IL-12, which counteract TGF- $\beta$  production (117, 118). As a result, mice with a Th2-dominant immune system (e.g., BALB/c mice) develop fibrosis more readily than Th1-skewed mice (e.g., C57BL/6 mice) (119).

The liver's resident endothelium acts as a conduit for blood-borne proinflammatory agents that regulate early wound healing and actively secretes trophic factors for epithelial regrowth (43); yet somewhat paradoxically, angiogenesis is observed in progressive liver fibrosis (120). Recent work by Ding et al. puts in evidence how divergent signaling from LSECs can indeed balance regeneration and fibrosis. In acute injury, LSECs upregulate the chemokine receptor CXCR7, which cooperatively with CXCR4 signals through the DNA-binding protein inhibitor ID1 to produce pro-regenerative signals like WNT2 and HGF that drive hepatocyte expansion (43, 121). In chronic liver damage, constitutive FGF receptor 1 (FGFR1) signaling in LSECs decreases their ratio of CXCR7 to CXCR4 expression, overriding ID1 activation and instead stimulating the proliferation of HSCs via secretion of profibrotic cytokines like TGF- $\beta$ , BMP2, and PDGFC. Endothelial cell-specific ablation of either *Cxcr4* or *Fgfr1* or, conversely, upregulation of CXCR7 prevents fibrosis and restores the regenerative program (121).

The hepatic wound healing response is characterized by temporal fluctuations in gene expression, as if dictated by a molecular clock, and tampering with these dynamics may hinder the reacquisition of homeostasis. For instance, LSECs express angiopoietin 2 (ANG2) in a biphasic pattern following acute parenchymal damage: by sharply reducing their levels of ANG2 soon after injury, LSECs downregulate TGF- $\beta$ , which in turn allows hepatocyte proliferation; recovered expression of ANG2 later boosts VEGFR2 levels in the LSECs in order to promote their own proliferation (122). BMP9, a TGF- $\beta$  family member secreted by HSCs, exhibits remarkably similar dynamics in vivo, whereby low levels initially

promote hepatocyte expansion and higher ones are thought to stimulate HSC migration afterward (123). In fibrotic/cirrhotic livers, TGF- $\beta$  levels are notoriously elevated as a result of continuous activation of the profibrotic program (124, 125). We could then hypothesize that raising the baseline of TGF- $\beta$  may hinder the required drop in its concentration to induce hepatocyte proliferation, so that epithelial restitution (at least through the hepatocyte lineage; ref. 126) is overtaken by fibrosis.

Re-epithelization is the ultimate goal of the regenerative response. Be it through cell cycle reentry of mature cells or activation of facultative progenitors, this process relies on stromal signals (from LSECs, myofibroblasts, and/or macrophages) to fine-tune epithelial cell-fate choices according to local demand (48, 127). It is enticing to suggest that the expanding epithelium may concomitantly modulate stromal cell behavior in a positive-feedback loop to ensure appropriate regeneration, or, conversely, fibrosis when dysregulated. In line with that, transplantation of hepatocytes in healthy livers leads to the expansion of activated smooth muscle actin-positive HSCs, while HSC depletion diminishes hepatocyte cell engraftment (128). Similarly, exposure to free fatty acids induces HSC activation and collagen synthesis only in the presence of hepatocytes, at least in an in vitro model of nonalcoholic fatty liver disease (129). Although live hepatocytes do signal to their surrounding stroma, hepatocyte death prevails in chronic liver injuries and is typically recognized by professional phagocytes like Kupffer cells. Phagocytosis activates the proinflammatory program of macrophages (130) but also induces them to secrete WNT to specify hepatocyte differentiation of ductal progenitors (40). Myofibroblasts have similarly been observed to engulf hepatocyte-derived apoptotic bodies, which enhances their survival and production of matrix (131). In addition, the severity of liver fibrosis in many human pathologies — including chronic hepatitis C and alcoholic and nonalcoholic steatohepatitis — correlates closely with the amount of ductular expansion (132–134). Although ECM deposition chronologically precedes ductular proliferation, as shown in a choline-deficient, ethionine-supplemented (CDE) model of liver damage (135), activated ductal progenitors may reinforce pathogenesis by virtue of their expression of profibrotic mitogens/cytokines like PDGF (136), TGF- $\beta$  (137), insulin-like growth factor (IGF) (138), and monocyte chemoattractant protein-1 (MCP-1) (139). Prosurvival factors like IGF act on the biliary epithelium itself through autocrine signaling, leading some to hypothesize that “selfish” biliary maintenance unintentionally feeds the fibrotic response (140). Ductal cells may also sustain fibrosis indirectly by regulating the inflammatory cell milieu. Ductal cell-derived lymphotoxin- $\beta$  is profibrotic, not through direct HSC activation but instead through NF- $\kappa$ B-mediated induction of the leukocyte-recruiting molecules ICAM-1 and CCL5 in these cells (141). Other chemokines reported within the ductular repertoire include IL-6 (142) and IL-8 (143), albeit their expression requires priming by proinflammatory factors like IFN- $\gamma$ .

## Reversal of fibrosis: potential for therapy

For many years tissue fibrosis was considered to be a degenerative disease with no possibility of regression. A seminal study by Okazaki and Maruyama in 1974 was the first to show collagenase activity in fibrotic livers, hinting at the feasibility of

disease resolution under certain contexts (144). Since then, the liver has provided exceptional evidence of the plasticity of this process, where even advanced fibrotic tissues are capable of reacquiring homeostatic traits (refs. 6, 145, and Figures 2 and 3). The most effective therapy for treating liver fibrosis to date is still to remove the damaging agent. Case in point are clinical cases of cirrhotic livers arising from chronic HCV infection, which achieve remarkable histological regression following antiviral treatment (146, 147). From this we can infer that the liver does contain built-in mechanisms for scar resolution, but these become smothered or inactivated in the face of relentless damage. The removal of profibrotic inputs, or, conversely, the strengthening of antifibrotic ones, should then stimulate scar resolution to at least some extent.

At the ground level, the battle is enzymatic: matrix-degrading enzymes must overcome the inhibitory action of TIMPs for a scar to be broken down. Overexpression of enzymes like MMP1 and MMP8 through adenoviral delivery has proven to ameliorate established fibrosis in rat livers (148, 149). Compounding the problem is the chronic persistence of myofibroblasts, which continuously pump TIMPs into the microenvironment, owing to prosurvival signaling via TGF- $\beta$  and the TNF- $\alpha$ /NF- $\kappa$ B axis (150). A single injection of the fungal toxin gliotoxin following chronic carbon tetrachloride (CCl<sub>4</sub>) damage promotes myofibroblast apoptosis through NF- $\kappa$ B inhibition in these cells and significantly resolves hepatic fibrosis in a matter of days (151). Whether this is solely due to myofibroblast death is still to be determined, given that NF- $\kappa$ B is also critical for the transcription of multiple immune cell-derived cytokines like IL-1 (152), whose direct blockade has shown potential for reducing liver fibrosis (153).

Clearance of myofibroblasts by phenotypically apt immune cells can prove of great benefit to resolve scarring in chronically damaged tissues. Indeed, fibrosis regression in the liver is accompanied by increased numbers of dendritic cells (DCs) (154), NK cells (155), and macrophages (106) in the tissue parenchyma. While DCs directly target ECM degradation through MMP9 secretion (154), NK cells target activated and senescent myofibroblasts for apoptosis through IFN- $\gamma$ -induced NKG2-D type II integral membrane protein (NKG2D), TRAIL, and FasL (156, 157). T cells expressing the  $\gamma\delta$  T cell receptor can also induce myofibroblast apoptosis via the Fas/FasL axis and thereby limit hepatic fibrosis (158). Tissue-restorative CD11B<sup>hi</sup>F4/80<sup>int</sup>Ly6C<sup>lo</sup> macrophages are of particular therapeutic interest because of their double-hit strategy: high secretion of MMPs and induction of myofibroblast apoptosis. A promising antifibrotic therapy would then be to increase the effective number of CD11B<sup>hi</sup>F4/80<sup>int</sup>Ly6C<sup>lo</sup> macrophages within the tissue through autologous transplants. In conjunction, blocking the influx of profibrotic CD11B<sup>hi</sup>F4/80<sup>int</sup>Ly6C<sup>hi</sup> macrophages (accomplished by targeting of the CCL2/CCR2 axis, which attracts monocytes to the liver; refs. 108, 159) could further tilt the balance toward regeneration.

Profibrotic cells can also be inactivated or induced to senesce, as opposed to targeted for cell death. There is now growing evidence that myofibroblasts can revert to their original quiescent-like state, albeit the cell of origin may affect the plasticity of a given myofibroblast population (see above). Moreover, reversal to quiescence

is never 100% successful; deactivated cells do not fully suppress their profibrotic gene signature and instead remain in a “primed” state that is capable of aggravating fibrosis upon further stimuli (75, 76). Senescence of a myofibroblast cell dampens its ability to synthesize matrix and profibrotic cytokines, yet in contrast to deactivation, this phenotype is associated with cell cycle exit and confers susceptibility to immune cell-mediated killing, particularly by NK cells (160). Replicative exhaustion, overstimulation, and oxidative stress are some of the mechanisms suggested to induce HSC senescence. In particular, one study has shown that signaling through the IL-22/STAT3 axis promotes HSC senescence, and accordingly, IL-22 treatment ameliorates liver fibrosis in vivo (161).

The disentangling of fibrosis requires multitargeted efforts because of the redundancy of the pathways that sustain it, mirroring the complexity of anticancer therapies. What this means in clinical terms is that “precision medicine” based on targeted single therapeutics (e.g., a blocking antibody against PDGFB; ref. 162) is unlikely to have robust and durable effects. Cellular therapies are attractive because they signify a continuous/responsive supply of diverse antifibrotic effectors. Interestingly, Lu and colleagues have shown that transplanting in vitro-expanded EpCAM<sup>+</sup>CD24<sup>+</sup>CD133<sup>+</sup> ductal progenitors in damaged livers not only repopulates hepatocellular parenchyma but also reduces liver scarring, the latter through mechanisms currently unknown (27). Still, incorporating the right cell in the “wrong” niche can be futile; thus, there is an increasing need to accurately assess and model disease progression beyond the current invasive method of tissue biopsy. The clinical efforts to modulate regeneration and fibrosis are, after all, a game of timing: an antifibrotic therapy aimed at quenching ECM deposition or inflammation in the very early stages of fibrosis could paradoxically impair hepatic regeneration. Similarly, engrafting ductal progenitors in advanced fibrotic livers could potentiate myofibroblast activation.

## Future perspectives and conclusion

Much of what we understand about fibrosis — its pathology and potential treatments — has been gathered from in vivo animal models of chronic tissue damage. Advanced liver fibrosis is, however, much less reversible in humans because of the decades, instead of weeks, of tissue damage and collagen cross-linking (163). Even after accounting for the variable of time, we cannot rule out species-specific differences in regeneration that may hinder our ability to treat patients. Thus, it may be time for the field to develop innovative human models of liver fibrosis.

Historically, in vitro studies of fibrosis have relied on “stripped-down” strategies involving short-lived primary cells and/or immortalized hepatic lines from healthy and diseased livers. Although simplistic, this has led to critical discoveries in the field, such as the molecular mechanism behind myofibroblast transdifferentiation (a process that occurs spontaneously when HSCs are cultured on plastic) (164). Human adult-derived hepatic progenitors can now be expanded in vitro as highly proliferative, yet genomically stable, 3D organoid structures that incorporate epithelial heterogeneity from the bile duct and hepatocyte lineages (26), proving of great biomedical potential for hepatic disease modeling (165). Still, the lack of stromal cells in these organoids restricts their ability to model complex diseases like fibrosis,

whose pathogenesis involves matrix-depositing, inflammatory, and endothelial cells. Takebe and colleagues have pioneered the idea of a truly organotypic human liver culture, albeit embryonic, by coculturing hepatic progenitors derived from human induced pluripotent stem cells with mesenchyme and endothelium (using human mesenchymal cells and HUVECs) in order to recapitulate key cell-cell interactions that lead to liver-bud formation in the embryo (166). Adult hepatic cultures will likely be challenged by the higher diversification of stromal cells in the tissue — HSCs, portal fibroblasts, LSECs, Kupffer cells, and recruited inflammatory cells — as well as the time-dependent changes that occur following acute versus chronic damage. Multiscale mathematical models, as have been constructed with mouse liver data (167), may be further required to integrate and predict the evolution of cell-cell interactions in space and time. In vitro and in silico approaches like these will never recapitulate the entirety of the fibrotic response at the whole-organism level, but may provide crucial mechanistic information about the complex cellular and molecular crosstalk that underlies human pathology, especially at the early stages of the fibrotic response.

In conclusion, injury of the hepatic tissue activates a spatiotemporally controlled reaction involving inflammatory cell recruitment, matrix deposition, and epithelial cell replacement. Although the liver's plasticity accommodates for a multitude of damaging insults, tissue degeneration and scarring often develop over time. Understanding the mechanisms that balance tissue regeneration and fibrosis is thus essential to identify new avenues of therapeutic intervention as well as to predict disease progres-

sion. The studies reviewed here have highlighted the time-dependent duality (pro-regenerative versus profibrotic) of the cells and molecules that drive wound healing. Be it by deactivation or clearance, regenerative pathways must be terminated; otherwise they become appropriated for fibrosis. In chronically damaged livers, the vicious cycle of cell death, inflammation, and excessive ECM deposition overrides epithelial restoration. Yet even cirrhotic livers may partially regress to homeostasis if antifibrotic inputs outbalance profibrotic ones, proving once again the malleability of the liver tissue. Focus should, then, be placed on developing multi-targeted therapies that cripple the self-maintenance of fibrosis. Lastly, the field would benefit from complementary organotypic cultures and in silico models to shed light on the dynamics that govern regeneration and fibrosis in humans.

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