Divergent angiocrine signals from vascular niche balance liver regeneration and fibrosis

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Chemical or traumatic damage to the liver is frequently associated with aberrant healing (fibrosis) that overrides liver regeneration1–3. The mechanism by which hepatic niche cells differentially modulate regeneration and fibrosis during liver repair remains to be defined4–6. Hepatic vascular niche predominantly represented by liver sinusoidal endothelial cells deploys paracrine trophogens, known as angiocrine factors, to stimulate regeneration7–9. Nevertheless, it is not known how pro-regenerative angiocrine signals from liver sinusoidal endothelial cells is subverted to promote fibrosis10–13. Here, by combining an inducible endothelial-cell-specific mouse gene deletion strategy and complementary models of acute and chronic liver injury, we show that divergent angiocrine signals from liver sinusoidal endothelial cells stimulate regeneration after immediate injury and provoke fibrosis after chronic insult. The pro-fibrotic transition of vascular niche results from differential expression of stromal-derived factor-1 receptors, CXCR7 and CXCR4 (refs 18–21), in liver sinusoidal endothelial cells. After acute injury, CXCR7 upregulation in liver sinusoidal endothelial cells acts with CXCR4 to induce transcription factor Id1, deploying pro-regenerative angiocrine factors and triggering regeneration. Inducible deletion of Cxcr7 in sinusoidal endothelial cells (Cxcr7i<sup>Cre<sup>Lox/Lox</sup></sup>) from the adult mouse liver impaired liver regeneration by diminishing Id1-mediated production of angiocrine factors9–11. By contrast, after chronic injury inflicted by iterative hepatotoxin (carbon tetrachloride) injection and bile duct ligation, constitutive FGFR1 signalling in liver sinusoidal endothelial cells counterbalanced CXCR7-dependent pro-regenerative response and augmented CXCR4 expression. This predominance of CXCR4 over CXCR7 expression shifted angiocrine response of liver sinusoidal endothelial cells, stimulating proliferation of desmin<sup>+</sup>hepatocellular-like cells22,23 and enforcing a pro-fibrotic vascular niche. Endothelial-cell-specific ablation of either Fgfr1 (Fgfr1<sup>i<sup>Cre<sup>Lox/Lox</sup></sup>) or Cxcr4 (Cxcr4<sup>i<sup>Cre<sup>Lox/Lox</sup></sup>) in mice restored the pro-regenerative pathway and prevented GFGR1-mediated maladaptive subversion of angiocrine factors. Similarly, selective Cxcr7<sup>i<sup>Cre<sup>Lox/Lox</sup></sup> activation in liver sinusoidal endothelial cells abrogated fibrogenesis. Thus, we demonstrate that in response to liver injury, differential recruitment of pro-regenerative CXCR7-Id1 versus pro-fibrotic Fgfr1-CXCR4 angiocrine pathways in vascular niche balances regeneration and fibrosis. These results provide a therapeutic roadmap to achieve hepatic regeneration without provoking fibrosis1,2,4–6.

Despite the liver’s capacity to undergo regeneration, chronic or overwhelming injury often causes liver fibrosis that culminates in cirrhosis and hepatic failure14–17. The integrated process of liver repair includes regeneration and wound healing characterized by synthesis of extracellular matrix proteins. Both processes are modulated by dynamic interplay between parenchymal hepatocytes and non-parenchymal cells18–21, including hepatic stellate cells18,22, inflammatory cells15–19, biliary epithelial cells and liver sinusoidal endothelial cells (LSECs)22,23. As such, defining the multicellular crosstalk that balances regeneration and dysfunctional (maladaptive) healing<sup>16</sup> holds promise for designing treatment for liver diseases.

LSECs that line liver sinusoidal vasculature induce hepatic organogenesis in manners that extend beyond their passive role for metabolite delivery22,24,25. By deploying paracrine growth regulators, which we have defined as angiocrine factors, LSECs trigger regeneration of hepatocytes20–25. However, aberrant activation of LSECs in the context of chronic injury provokes fibrosis26–29. This dichotomy of LSEC niche function in mediating liver repair implies divergent angiocrine signals balance regeneration and fibrosis11. Therefore, we sought to decipher the mechanisms that subvert pro-regenerative capacity of LSECs to a pro-fibrotic state.

In response to tissue injury, cytokines and chemokines, such as stromal-derived factor (SDF)-1 (Cxc12), are upregulated to initiate regeneration by switching on its receptors CXCR4 and CXCR7 (refs 18–21). Although CXCR4 activation both in haematopoietic and in vascular cells modulates angiogenesis and haematopoiesis, expression of another SDF-1 receptor, CXCR7, is mainly restricted to endothelial cells, with its function primarily believed to be pivotal in vascular patterning and tumour neo-angiogenesis. However, elucidating the mechanism by which the SDF-1 pathway orchestrates liver repair is hindered by the lack of cell-type-specific genetic models in defined settings of liver injuries.

To unravel the divergent role of LSECs in modulating liver repair, we used a single injection of carbon tetrachloride (CCL<sub>4</sub>) and acetaminophen, which cause acute liver injury, as well as chronic injury models of repeated CCL<sub>4</sub> injection and bile duct ligation (BDL) (Fig. 1a). At day 2 after single CCL<sub>4</sub> injury, CXCR7 was upregulated specifically in vascular endothelial (VE)-cadherin<sup>+</sup> LSECs (Fig. 1b–e and Supplementary Fig. 1). By contrast, CXCR4 is broadly expressed by other cell types, and its expression remains relatively stable on LSECs after CCL<sub>4</sub> injury. Therefore, we have identified CXCR7 as an inducible LSEC-specific SDF-1 receptor in response to liver injury.

Our group9,10 and others13 have shown that after partial hepatectomy LSECs, by producing angiocrine factors such as Wnt2 and hepatocyte growth factor (HGF), elicit liver regeneration. Activation of transcription factor Id1 in LSECs was essential for this process5. SDF-1 induced Id1 upregulation in cultured human LSECs, which was abrogated by genetic silencing of either Cxcr7 or Cxcr4 (Fig. 1f and Supplementary Figs 2 and 3). Cxcr7-selective agonist TC14012 similarly induced Id1 upregulation. Immunoprecipitation–western blot demonstrated that after SDF-1 stimulation, CXCR7 was associated with CXCR4 and β-arrestin in LSECs (Supplementary Fig. 4). Therefore, SDF-1 stimulates Id1 induction through enabling cooperation between CXCR7 and CXCR4 (refs 27 and 28).

To determine the contribution of CXCR7 in LSEC-mediated liver repair, we used a tamoxifen-inducible endothelial-cell-specific Cre<i>ERT2</i>
Figure 1 | After acute liver injury, upregulation of SDF-1 receptor CXCR7 in LSECs stimulates angiocrine-mediated regeneration. a, Liver injury models for studying the maladaptive transition of pro-regenerative LSEC function to a pro-fibrotic vascular niche. b–e, CXCR7 is specifically upregulated on VE-cadherin+ CD31+ LSECs after acute chemical injury. After injection of CCL_4, CXCR7 and CXCR4 were determined in isolated LSECs (b), liver sections (c, d) and non-parenchymal cells (NPCs) (e). CXCR7 was expressed on LSECs but not large vessels; n = 5. Scale bar, 50 μm in Fig. 1; all data hereafter are presented as mean ± s.e.m. f, SDF-1 stimulation of human LSECs upregulates inhibitor of DNA binding 1 (Id1) protein, a transcription factor inducing production of pro-regenerative angiocrine factors^9. Id1 stimulation by SDF-1 in primary human Factor VIII^− LSECs was abrogated by silencing of CXCR4 and CXCR7 in LSECs; n = 5. g, h, Endothelial cell (EC)-specific inducible deletion of CXCR7 (Ccxr7^ΔEC/ΔEC) in mice. Mice harbouring loxp sites flanking Ccxr7 were crossed with a mouse line with endothelial-cell-specific VE-cadherin promoter-driven Cre^ERT2 (VE-Cad-Cre^ERT2/Cdx5-PAC-Cre^ERT2). 

system to knockdown Ccxr7 in the endothelial cells of adult mice (Fig. 1g). Mice harbouring loxp site-flanking Ccxr7 were crossed with VE-Cad-Cre^ERT2/Cdx5-PAC-Cre^ERT2 mice whereby endothelial-cell-specific VE-cadherin promoter drives Cre^ERT2. Mice carrying tdTomato fluorescent protein following the floxed stop codon were used to exclude off-target effects of VE-Cad-Cre^ERT2 on other liver cell types. Tamoxifen injection specifically activated Cre^ERT2 activity in endothelial cells but not desmin-expressing stellate-like cells (Fig. 1h and Supplementary Fig. 5), demonstrating induced endothelial-cell-specific deletion of Ccxr7 (Ccxr7^ΔEC/ΔEC) in VE-cad-Cre^ERT2/Ccxr7^loxP/loxP mice. Ccxr7^−/− mice were used as control for Cre toxicity.

Compared with control mice, hepatocyte proliferation in Ccxr7^ΔEC/ΔEC mice was significantly decreased after CCL_4 injury (Fig. 1i, j). Id1-dependent deletion of angiocrine factors HGF and Wnt2 from LSECs of Ccxr7^ΔEC/ΔEC mice was reduced both after CCL_4- and after acetaminophen-induced liver injuries (Fig. 1k). The extent of liver injury, as determined by the concentration of serum alanine aminotransferase (ALT), was exacerbated (Fig. 1l and Supplementary Fig. 6). Thus, after liver injury, SDF-1 through activation of CXCR7^+ LSECs triggers an angiocrine response to initiate liver regeneration (Fig. 1m).

Although hepatocytes regenerate after acute liver injury, chronic liver damage more frequently leads to activation of myofibroblasts and causes fibrosis^2,3. To address how the pro-regenerative angiocrine signals of LSECs are diverted to provoke this maladaptive healing, we used a mouse model of chronic liver injury by repeated CCL_4 injection^8, (Fig. 2a, b). The CXCR7-Id1 pathway in LSECs was counterbalanced by CXCR4 upregulation after chronic injury (Fig. 2c–e and Supplementary Figs 7 and 8). After repeated CCL_4 injection, protein concentrations of α-smooth muscle actin (SMA) and extracellular matrix protein collagen were augmented in Ccxr7^ΔEC/ΔEC mice, compared with control mice (Fig. 2f–h and Supplementary Figs 7 and 10). Injection of CXCR7-specific agonist TC14012 reduced the upregulation of SMA and collagen I in control but not Ccxr7^ΔEC/ΔEC mice (Fig. 2g, h and i).
Supplementary Fig. 11). Therefore, chronic liver injury interferes with pro-regenerative CXCR4-Id1 angiocrine pathway in LSECs and promotes fibrosis.

The requirement of CXCR7 in LSECs in resolving liver fibrosis was tested5,23. After three CCl4 injections, SMA and collagen protein concentrations were enhanced in control mice; they peaked at day 8 after last injection and approached basal (vehicle-injected group) amount at day 20 (Fig. 2i–j and Supplementary Figs 12 and 13). By contrast, time-dependent resolution of liver injury was impaired in Ccr7-/- mice. SMA and collagen protein concentration were enhanced in control mice; they peaked at day 8 after last injection and approached basal (vehicle-injected group) amount at day 20 (Fig. 2i–j and Supplementary Figs 12 and 13). By contrast, time-dependent resolution of liver injury was impaired in Ccr7-/- mice.

We then used BDL, a clinically relevant liver cholestasis model, to define how CXCR4 and CXCR7 modulate pro-fibrotic transition of LSECs, we examined the activation of the FGF-2 receptor FGFR1 on LSECs after BDL. There was a time-dependent upregulation and activation of FGFR1 (refs 16, 29, 30) concomitant with phosphorylation of MAPK (Erk1/2) in the injured VE-cadherin+ LSECs (Fig. 3g, h and Supplementary Figs 16 and 17), suggesting that FGF-2 induced CXCR4 upregulation and CXCR7 suppression in LSECs negate the Id1 pro-regenerative pathway.

To test how FGF-2 signalling modules angiocrine response of LSECs, we examined the activation of the FGF-2 receptor FGFR1 on LSECs after BDL. There was a time-dependent upregulation and activation of FGFR1 (refs 16, 29, 30) concomitant with phosphorylation of MAPK (Erk1/2) in the injured VE-cadherin+ LSECs (Fig. 3g, h and Supplementary Figs 16 and 17), suggesting that FGF-2 induced CXCR4 upregulation and CXCR7 suppression in LSECs negate the Id1 pro-regenerative pathway.

Human LSECs were then stimulated with angigenic factors VEGF-A and FGF-2 to investigate the mechanism whereby CXCR4 expression is enhanced to dominate over the CXCR7 pathway. FGF-2, but not VEGF-A, increased CXCR4 messenger RNA (mRNA) and protein concentrations, and attenuated CXCR7 expression (Fig. 3g and Supplementary Fig. 14). Specific inhibition of mitogen-activated protein kinase (MAPK) blocked FGF-2-driven CXCR4 induction and CXCR7 inhibition in LSECs15 (Fig. 3h). As a result, treatment of human LSECs by FGF-2 suppressed Id1 induction by SDF-1 (Fig. 3i, j and Supplementary Fig. 15), suggesting that FGF-2 induced CXCR4 upregulation and CXCR7 suppression in LSECs negate the Id1 pro-regenerative pathway.

Figure 2 | Iterative hepatotoxic injury perturbs CXCR7 pro-regenerative pathway in LSECs and forces the generation of a pro-fibrotic vascular niche. a, b, Mouse liver fibrosis is induced by repeated injection of CCl4. Sirius red staining was used to detect collagen in the injured liver. Scale bar, 50 μm in Fig. 2c–e. Chronic liver injury suppresses CXCR7 pathway and upregulates CXCR4 expression in LSECs. Quantitative PCR (c), immunostaining (d) and flow cytometry (e) showed the abrogation of CXCR7-Id1 pathway in VE-cadherin (VE-Cad)+LSECs after chronic CCl4 injury. CXCR4 is expressed in both endothelial cells and non-endothelial cells (white arrow). *P < 0.05 versus vehicle-treated mice; n = 5. f–h, CXCR7 activation in LSECs negates liver fibrosis. The extent of fibrosis was augmented in Ccr7-/-EC mice, as evidenced by elevated hepatic amounts of α-SMA and collagen 1 (g, sirius red staining). CXCR7-selective agonist TC14012 reduced fibrosis in control but not Ccr7-/-EC mice. i, j, Impaired resolution of liver injury in Ccr7-/-EC mice. The amount of SMA in the injured liver was tested to assess the resolution of injury (i). Compared with control mice, the amount of SMA in Ccr7-/-EC mice was enhanced after last CCl4 injection and remained stable afterwards. The amount of collagen 1 was similarly assessed (Supplementary Fig. 13); *P < 0.05, **P < 0.01, versus control mice; n = 4. k, CXCR7 activation restores Id1 induction in chronically injured LSECs. TC14012 prevented Id1 suppression in LSECs during repeated CCl4 injury. *P < 0.05, **P < 0.01, compared with vehicle group; n = 5. l, Interference with pro-regenerative CXCR7-Id1 pathway in LSECs causes pro-fibrotic transition of vascular niche. After injury, upregulation of the CXCR7-Id1 pathway in LSECs induces generation of hepatic-active angiocrine factors and stimulates regeneration. Chronic injury perturbs CXCR7-Id1 signalling, counteracting regeneration and provoking fibrogenesis.
FGFR1-mediated MAPK activation in LSECs, resulting in CXCR4-dominated pro-fibrotic transition of angiocrine response during liver repair (Fig. 3m).

To elucidate the mechanism underlying the pro-fibrotic drift of LSEC niche, we conditionally ablated Fgfr1 and Cxcr4 in endothelial cells of adult mice (Fgfr1<sup>lacZ/+</sup> and Cxcr4<sup>lacZ/+</sup>) (Fig. 4a). After BDL, periportal expansion of desmin<sup>+</sup> cells, deposition of collagen and SMA, MAPK activation and CXCR7 suppression in LSECs of Fgfr1<sup>lacZ/+</sup> mice were all prevented compared with control mice (Fig. 4b–g and Supplementary Fig. 18). CXCR7-dependent angiocrine expression of Wnt2 and HGF was restored in Fgfr1<sup>lacZ/+</sup> mice (Fig. 4e). Therefore, endothelial-cell-specific deletion of Fgfr1 and SMA, MAPK activation and CXCR7 suppression in LSECs of Fgfr1<sup>lacZ/+</sup> mice prevented the aberrant transition of LSECs into a pro-fibrotic state by BDL.

To unravel the altered angiocrine response in chronically injured LSECs, we isolated and analysed LSECs from BDL and sham-operated mice (Supplementary Fig. 19). In injured LSECs, there was significant upregulation of pro-fibrotic factors, including transforming growth factor (TGF)-β, bone morphogenetic protein (BMP)2 and platelet-derived growth factor (PDGF)-C, concomitant with suppression of anti-fibrotic genes, such as follistatin and apelin. This divergent drift of angiocrine factor production in LSECs after BDL was diminished in Fgfr1<sup>lacZ/+</sup> mice, as evidenced by restoration of anti-fibrotic genes and reduced expression of fibrotic factors (Fig. 4h).

The extent of fibrosis after BDL was further tested in Cxcr4<sup>ΔEC/ΔEC</sup> mice. Compared with control Cxcr4<sup>ΔEC/+</sup> mice, hepatic deposition of SMA and collagen, and perisinusoidal accumulation of desmin<sup>+</sup> stellate-like cells, were reduced in Cxcr4<sup>ΔEC/ΔEC</sup> mice after BDL (Fig. 4i–m and Supplementary Fig. 20). The reduction of liver fibrosis in Cxcr4<sup>ΔEC/ΔEC</sup> mice implies that constitutive CXCR4 activation in LSECs by chronic injury establishes a pro-fibrotic vascular niche, activating adjacent myofibroblast cells and provoking fibrogenesis (Fig. 4n).

Although liver regeneration after partial hepatectomy proceeds impeccably without fibrosis, liver repair after chronic injury is associated with fibrosis that compromises restoration of liver function. Therefore, identifying the molecular pathways modulating liver regeneration and aberrant healing will open up therapeutic avenues for treatment of liver cirrhosis and failure. We have shown that after 70% partial hepatectomy, activation of the VEGFR2-Id1 pathway in LSECs leads to liver regeneration<sup>9</sup>. Here we demonstrate that FGFR1-mediated CXCR4 upregulation and CXCR7 suppression in LSECs counterbalance the pro-regenerative function of LSECs and lead to fibrosis.

We used complementary acute and chronic injury models to decipher the contribution of LSECs in liver repair (Fig. 1a). In the mouse liver, we have identified a preferential induction of pro-regenerative CXCR7-dependent signalling in LSECs that responds to acute injury (Fig. 1).

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**Figure 3** | Cholestatic liver injury by FGFR1 overactivation in LSECs shifts CXCR7-dependent pro-regenerative response to a CXCR4-dominated pro-fibrotic vascular niche.

**a, b,** Pro-fibrotic transition of LSECs caused by BDL-induced cholestatic injury. After BDL injury (a), most VE-cadherin<sup>+</sup> LSECs were covered by perisinusoidal desmin<sup>+</sup> fibroblasts (b, inset). By contrast, desmin<sup>+</sup> stellate-like cells were sparsely distributed in sham-operated liver. Scale bar, 50 μm in Fig. 3e, b. BDL suppresses CXCR7-Id1 pathway and upregulates CXCR4 expression in LSECs. Loss of CXCR7 protein in LSECs after BDL is shown at bottom panel; *<i>P</i> < 0.05, compared with the amount at day 0; n = 5. f–h, Liver fibrosis caused by BDL is exacerbated in Cxcr4<sup>ΔEC/ΔEC</sup> mice. After BDL, Cxcr4<sup>ΔEC/ΔEC</sup> mice showed higher hepatic concentrations of SMA protein than that of control mice; n = 4. g, j, FGF-2 through MAPK activation favours CXCR4 signalling in LSECs, counteracting CXCR7-Id1 pathway. FGF-2, but not VEGF-A, upregulated CXCR4, suppressed CXCR7 and inhibited SDF-1-dependent Id1 protein induction in LSECs. This FGF-2-mediated predominance of CXCR4 over CXCR7 was attenuated by MAPK inhibitor U0126; dashed line indicates the base level of protein expression in vehicle-treated LSECs, relative to FGF-2 stimulated cells. Note that DMSO served as control for MAPK inhibitor U0126. n = 5.

**k, l,** Activation of FGFR1 and MAPK pathway in LSECs after BDL. There was time-dependent enhancement in phosphorylation/activation of FGFR1 downstream effector FRS2 (p-FRS2) and Erk1/2 (p-Erk1/2) in VE-cadherin<sup>+</sup> LSECs after BDL; n = 6. m, Constitutive FGFR1 signalling in LSECs by MAPK activation forces a CXCR4-dominated pro-fibrotic vascular niche. During chronic liver injury, FGFR1-mediated aberrant MAPK activation in LSECs upregulates CXCR4 and perturbs CXCR7-Id1 pathway. This predominance of FGFR1/CXCR4 activation in LSECs determines the pathological progression from adaptive (pro-regenerative) to maladaptive (pro-fibrotic) liver repair.

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During chronic liver injury, loss of CXCR7 and upregulation of CXCR4 in LSECs causes progression to fibrosis. Indeed, the critical function of CXCR7 activation in promoting regeneration and countering fibrosis is evidenced by the attenuated fibrosis by selective activation of CXCR7 in LSECs, as well as by impaired regeneration (Fig. 1) and enhanced fibrogenesis (Figs 2 and 3) in CXCR7 Δ/−/− mice.

The shift in SDF-1 signalling from the CXCR7-dependent pro-regenerative response to a CXCR4-dominated pro-fibrotic function in LSECs is due to persistent FGFR1 that drives chronic MAPK activation14,16,29,30 (Fig. 3). We used an inducible endothelial-cell-specific mouse genetic deletion system to demonstrate the contribution of FGFR1–CXCR4 pathway in the pro-fibrotic drift of LSECs. After chronic liver injury, the enhanced CXCR4 expression relative to CXCR7 in LSECs was prevented in Fgr1 Δ/−/− mice, and both Fgr1 Δ/−/− and Cxcr4 Δ/−/− mice were resistant to fibrosis (Fig. 4). Therefore, suppression of anti-fibrotic genes such as follistatin and apelin (Supplementary Fig. 19). This pro-fibrotic drift of angiocrine factor in LSECs after BDL was mitigated in Fgr1 Δ/−/− mice, n = 4. Reduction of liver fibrosis in Cxcr4 Δ/−/− mice after BDL. The extent of liver fibrosis after BDL was significantly lower in Cxcr4 Δ/−/− mice than that of control mice, as evidenced by a decreased deposition of collagen (i, j, sirius red staining), SMA protein (k, l), and perisinusoidal enrichment of desmin+ stellate-like cells (m, white arrow); n = 5. Divergent angiocrine signals from LSECs balance liver regeneration and fibrosis. After acute liver injury, activation of CXCR7-Id1 pathway in LSECs stimulates production of hepatic-active angiocrine factors. By contrast, chronic injury causes persistent FGFR1 activation in LSECs that perturbs CXCR7-Id1 pathway and favours a CXCR4-driven pro-fibrotic angiocrine response, thereby provoking liver fibrosis. Therefore, in response to injury, differentially primed LSECs deploy divergent angiocrine signals to balance liver regeneration and fibrosis.
activation diverts SDF-1 signalling in LSECs to a CXCR4-dominated maladaptive (pro-fibrotic) angiocrine response. Taken together, we demonstrate that endothelial cells are not just inert cellophane conduits delivering metabolites but also establish a vascular niche that instructively dictates regeneration and healing. Identifying molecular pathways orchestrating divergent angiocrine responses in the hepatic vascular niche will lay the foundation for therapeutic strategy that ensures liver repair without causing fibrosis.

METHODS SUMMARY

Generation of endothelial-cell-specific inducible gene deletion was performed by treating VE-Cad-CreERT2/+/Cdhs5-PAC-CreERT2/+ harbouring mice with tamoxifen15. Mice carrying loxP sites flanking Cxcr7 were provided by ChemoCentryx (generated by L. Gan (University of Rochester)). Floxed Cxcr4 mice were offered by Y.-R. Zou (the Feinstein Institute for Medical Research, Manhasset, New York). Sex-, age- and weight-matched littermate animals with indicated genotypes were used and compared in all experimental groups. All animal experiments were performed under the guidelines set by the Institutional Animal Care and Use Committee at Weill Cornell Medical College.

Single and repeated injections of CCI4 were used to induce acute and chronic liver injuries, respectively. To perform BDL, the common bile duct was ligated and severed by incision between ligation. Livers were collected for the analysis of fibrogenesis, including collagen deposition by Sirius red staining, and desmin protein by immunoblot (Abcam). TC14012 (R&D) was injected intraperitoneally at least three times. Statistical analysis used two-way analysis of variance. All data were expressed as mean ± s.e.m.

Online Content Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

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1. Friedman, S. L., Sheppard, D., Duffield, J. S. & Violette, S. Therapy for fibrotic diseases: nearing the starting line. Sci. Transl. Med. 5, 167rl1 (2013).
2. Bataller, R. & Brenner, D. A. Liver fibrosis. J. Clin. Invest. 115, 209–218 (2005).
3. Iredale, J. P. Models of liver fibrosis: exploring the dynamic nature of inflammation and repair in a solid organ. J. Clin. Invest. 117, 539–548 (2007).
4. Gurtner, G. C., Werner, S., Barrandon, Y. & Longaker, M. T. Wound repair and regeneration. Nature 453, 314–321 (2008).
5. Wynn, T. A. Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. J. Clin. Invest. 117, 524–529 (2007).
6. Duffield, J. S. et al. Selective depletion of macrophages reveals distinct, opposing roles during liver injury and repair. J. Clin. Invest. 115, 55–65 (2005).
7. Diehl, A. M. Neighborhood watch orchestres liver regeneration. Nature Med. 18, 497–499 (2012).
8. Boulter, L. et al. Macrophage-derived Wnt opposes Notch signaling to specify hepatic progenitor cell fate in chronic liver disease. Nature Med. 18, 572–579 (2012).
9. Ding, B. S. et al. Inductive angiocrine signals from sinusoidal endothelium are required for liver regeneration. Nature 468, 310–315 (2010).
10. Ding, B. S. et al. Endothelial-derived angiocrine signals induce and sustain regenerative lung alveolization. Cell 147, 539–553 (2011).
11. Kobayashi, H. et al. Angiocrine factors from Akt-activated endothelial cells balance self-renewal and differentiation of haematopoietic stem cells. Nature Cell Biol. 12, 1046–1056 (2010).
12. Sakaguchi, T. F., Sadler, K. C., Crosnier, C. & Stainier, D. Y. Endothelial signals modulate hepatocyte apicobasal polarization in zebrafish. Curr. Biol. 18, 1565–1571 (2008).
13. Wang, L. et al. Liver sinusoidal endothelial cell progenitor cells promote liver regeneration in rats. J. Clin. Invest. 122, 1567–1573 (2012).
14. Matsumoto, K., Yoshihomi, H., Rossant, J. & Zaret, K. S. Liver organogenesis promoted by endothelial cells prior to vascular function. Science 294, 559–563 (2001).
15. LaCouter, J. et al. Angiogenesis-independent endothelial protection of liver: role of VEGFR-1. Science 299, 890–893 (2003).
16. Huerford, R. C. et al. Aquaporin-1 facilitates angiogenic invasion in the pathological neovasculature that accompanies cirrhosis. Hepatology 52, 238–248 (2010).
17. Zeiseberg, E. M. et al. Endothelial-to-mesenchymal transition contributes to cardiac fibrosis. Nature Med. 13, 952–961 (2007).
18. Miao, Z. et al. Cxcr7 (RDC1) promotes breast and lung tumor growth in vivo and is expressed on tumor-associated vasculature. Proc. Natl Acad. Sci. USA 104, 15735–15740 (2007).
19. Yu, S. et al. Deactivation of hepatic stellate cells during liver fibrosis resolution in mice. Gastroenterology 143, 1073–1083 (2012).
20. Zaret, K. S. & Grompe, M. Generation and regeneration of cells of the liver and pancreas. Science 322, 1490–1494 (2008).
21. Woo, D. H. et al. Direct and indirect contribution of human embryonic stem cell-derived hepatocyte-like cells to liver repair in mice. Gastroenterology 142, 602–611 (2012).
22. Huehme, S. et al. Prediction and validation of cell alignment along microvessels as order principle to restore tissue architecture in liver regeneration. Proc. Natl Acad. Sci. USA 107, 10371–10376 (2010).
23. Decaillot, F. M. et al. Cxcr7/Cxcr4 heterodimer constitutively recruits ‘decoy’ receptor CXCR7. Proc. Natl Acad. Sci. USA 107, 628–632 (2010).
24. Yue, C. et al. Role of fibroblast growth factor type 1 and 2 in carbon tetrachloride-induced hepatic injury and fibrogenesis. Am. J. Pathol. 163, 1653–1662 (2003).
25. Bohm, F. et al. FGF receptors 1 and 2 control chemically induced injury and compound detoxification in regenerating livers of mice. Gastroenterology 139, 1385–1396 (2010).

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Author Contributions B.-S.D. and Z. C. conceived the project, performed experiments and wrote the paper. R. L., K. S. and P. G. performed the experiments and analysed the data. M. E. P., M. S., K. S. and S. Y. R. interpreted the data. S. R. designed the project, analysed the data and wrote the paper. All authors commented on the manuscript.

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METHODS
Endothelial cell (EC)-specific gene deletion strategy. Inducible endothelial-cell-specific gene deletion was achieved by treating VE-Cadherin-CreER\(^{T2}\), harbouring mice with tamoxifen\(^{31}\). Cre\(^{\text{ER}^{\text{T2}}}\) mice were previously described\(^{31}\). Cre\(^{\text{ER}^{\text{T2}}}\) mice were treated with tamoxifen at a dose of 250 mg kg\(^{-1}\) intraperitoneally for 6 days interrupted for 3 days after the third dose. After 3 weeks of tamoxifen treatment, deletion of target genes in LSECs was corroborated by quantitative PCR and immunoblot analysis. All animal experiments were performed under the guidelines set by the Institutional Animal Care and Use Committee at Weill Cornell Medical College, using sex-, age- and weight-matched littermate animals.

Liver injury and fibrosis models. Single and repeated injections of CCL\(_4\) were used to induce acute and chronic liver injuries, respectively, as previously described\(^{29}\). CCL\(_4\) was diluted in oil to yield a final concentration of 40% (0.64 mg ml\(^{-1}\)) and injected into mice at 1.6 mg kg\(^{-1}\) body mass. Eight- to ten-week-old mice were subjected to BDL. Acute liver injury was also induced in mice by intraperitoneal injection of 400 mg kg\(^{-1}\) acetaminophen. To perform BDL, mice were subjected to a mid-abdominal incision 3 cm long, under general anaesthesia. The common bile duct was ligated at two adjacent positions approximately 1 cm from the porta hepatitis. The duct was then severed by incision between the two sites of ligation.

To selectively activate CXCR7, the agonist TC14012 (R&D Systems) was intraperitoneally injected into the mice after CCL\(_4\) injury or BDL injury every other day for 1 week. To perform BDL, mice were injected with 1.6 mg kg\(^{-1}\) of M U0126 was used to inhibit the activity of MAPK.

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