Published in final edited form as:

Hepatology. 2014 August ; 60(2): 754–756. doi:10.1002/hep.27116.

Sinusoidal Endothelial Cells Direct Traffic at the Intersection of Regeneration and Fibrosis

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Abstract

Chemical or traumatic damage to the liver is frequently associated with aberrant healing (fibrosis) that overrides liver regeneration. The mechanism by which hepatic niche cells differentially modulate regeneration and fibrosis during liver repair remains to be defined. Hepatic vascular niche predominantly represented by liver sinusoidal endothelial cells deploys paracrine trophogens, known as angiocrine factors, to stimulate regeneration. Nevertheless, it is not known how pro-regenerative angiocrine signals from liver sinusoidal endothelial cells is subverted to promote fibrosis. 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 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*^{i EC/i EC} from the adult mouse liver impaired liver regeneration by diminishing Id1-mediated production of angiocrine factors. By contrast, after chronic injury inflicted by iterative hepatotoxin (carbon tetrachloride) injection and bile duct ligation, constitutive FGFR1 signaling 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⁺ hepatic stellate-like cells and enforcing a pro-fibrotic vascular niche. Endothelial-cell-specific ablation of either *Fgfr1* (*Fgfr1*^{i EC/i EC}) or *Cxcr4* (*Cxcr4*^{i EC/i EC}) in mice restored the pro-regenerative pathway and prevented FGFR1-mediated maladaptive subversion of angiocrine factors. Similarly, selective CXCR7 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 fibrosis.

Comment

As a regenerative organ, the liver is unique and remarkable in its ability for self-renewal after significant parenchymal loss. Angiogenic signals, including but not limited to FGFR1 in liver sinusoidal endothelial cells (LSEC), appear to be critically linked to the regenerative process(1-3). Furthermore, in the context of acute hepatocellular injury, successful healing

includes both efficient hepatocyte replication and orderly angiogenesis which are often sufficient to promote tissue regeneration and restoration of hepatic function (4). However, during various states of chronic liver injury, the characteristically precise and efficient processes that regulate liver regeneration are paradoxically misdirected into a chronic wound healing response that is inefficient and fibrogenic. This aberrant form of regeneration occurs in tandem with a pathological form of angiogenesis that promotes portal hypertension and disease progression(5). Tremendous insights have been realized during the parallel evolution of the fields of liver regeneration and fibrosis and it has become increasingly clear that LSEC proactively participate in the inter-cellular crosstalk that mediate these processes, especially those that govern the response to acute and chronic injury.

While multiple complex and inter-dependent mechanisms are likely at play, signaling within and among LSEC appear to be one key regulatory node that helps to orchestrate the delicate balance between the efficient liver regeneration program seen after resection or acute injuries and the inflammatory and fibrotic milieu that defines chronic liver disease. However, the molecular switch in LSEC that mediate these processes remains incompletely defined. In this regard, a recent paper in *Nature* by Ding et al.(6) elucidates mechanistic insights whereby differential endothelial expression patterns of the chemokine receptors, CXCR7 and CXCR4, balance the processes of regeneration and fibrosis in models of acute and chronic injury, respectively. They show that CXCR7 is up-regulated in LSEC after acute injury and that this promotes paracrine regenerative factors via the Id1 transcription factor. In contradistinction, chronic injury augments FGFR1-mediated CXCR4 expression which in turn represses the Id1 pathway and instead stimulates stellate cell activation and subsequent fibrosis. This is especially interesting since the FGFR1 pathway is also a potent mediator of aberrant angiogenesis. These important observations reach across disciplines to inform us about the vascular compartment as a paracrine regulator of both regeneration and fibrosis and raise the possibility of targeted interventions to promote the former process while limiting the latter.

In order to disentangle the pathways responsible for effective repair in the acute setting and fibrosis progression after chronic injury, the authors begin with a variety of murine models of acute and chronic liver injury. Acute injury was modelled via bolus injection of carbon tetrachloride (CCl₄) in conjunction with acetaminophen administration, a situation in which regenerative pathways are activated leading to effective adaptation and repair. In contrast, serial administration of CCL₄ or bile duct ligation (BDL) were used to model chronic hepatotoxic and biliary injury, respectively. In these settings, regeneration is inhibited and instead a pro-fibrotic milieu emerges that drives maladaptive repair and disease progression. All three models were studied in parallel in conjunction with a variety of elegant endothelial cell (EC)-specific genetic knockouts and pharmacologic manipulations.

First, they used the CCL₄/acetaminophen acute injury model to demonstrate that Cxcr7 is significantly over-expressed in LSEC in the period following acute injury. Following up on a previous *Nature* paper (7), they show that this pathway mediates release paracrine factors such as HGF and Wnt2, promotes regenerative processes, and involves the Id1 transcription factor. The key role of CXCR7 is supported by the use of EC-specific Cxcr7 knockout mice and the selective CXCR7 agonist TC14012. They go on to use the chronic CCL₄ and BDL

models to show that during evolution of chronic injury, early and transient Cxcr7/Id1 expression is temporally overtaken by Cxcr4 expression which then counteracts the regenerative pathway and instead nurtures a pro-fibrogenic niche that drives disease progression. Lastly, they demonstrate a further mechanistic link with persistent FGFR1/MAP kinase activation as a means for CXCR4-mediated suppression of the regenerative Cxcr7/Id1 pathway. This work is supported by the use of the specific MAPK inhibitor U0126 and EC-specific knockout of either Cxcr7 or FGFR1. The net result is a novel and mechanistic picture of how differential signaling within the vascular compartment can promote regeneration in the setting of acute liver injury but also drive pathological angiogenesis and fibrosis during chronic liver disease (Figure 1).

While this study sheds light on the role of the endothelial niche as a regulatory node, the overall picture is clearly even more complex and involves other cellular compartments as well. For example, activation of liver progenitor cell compartments differs markedly in response to acute (8) or chronic injury (9). Liver regeneration after acute injury typically involves reactivation of cell division in terminally differentiated and relatively mitotically quiescent hepatocytes. However, in the setting of massive parenchymal loss or in circumstances where underlying regenerative processes are impeded, such as chronic liver disease, hepatocyte replicative ability is overwhelmed(10). In this setting, the peri-biliary progenitor cell niche is activated to proliferate and differentiate in an often inefficient attempt to compensate for the lack of hepatocyte replication. It also appears that this mechanism may in fact promote further fibrogenesis (11). The molecules and cell sources that subvert these stem cell responses are unclear but it would come as no surprise if altered LSEC signaling plays a role, especially since CXCR4 is essential to initiate and maintain stem cell niches(12).

One of the most common queries from patients with cirrhosis is, “If the liver can regenerate, then why won’t my liver get better?” Clearly, the fibrogenic program impedes the regeneration program in liver. Indeed, it appears that extracellular matrix proteins can actually suppress hepatocyte proliferation through integrin dependent pathways (13). The work here provides a molecular model that at least partially accounts for the divergent fibrogenic and regenerative programs in the liver with the LSEC and its differential angiogenic responses at the nexus. Clinically relevant questions for the future that are raised by the work include: 1) How hardwired are these distinct programs?; 2) Are they reversible?; and 3) How will emerging anti-fibrotic, anti-angiogenic, and regenerative medicine therapies affect these complex mechanisms? As with any great study, this one raises as many questions as it answers.

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Figure 1.

Differential LSEC Signalling in Models of Acute or Chronic Injury. A. The acute injury model with bolus administration of CCl_4 in conjunction with acetaminophen allows liver regeneration to proceed efficiently as predominant CXCR7 signalling promotes Id1-mediated release of paracrine factors such as HGF and Wnt2. B. The chronic injury models of iterative CCl_4 or bile duct ligation promote fibrosis as persistent FGFR1 signalling promotes MAPK-mediated CXCR4 activation along with attenuation of the CXCR7/Id1 pathway.