REVIEW

Hepatic Progenitor Cells in Action

Liver Regeneration or Fibrosis?

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Accepted for publication
June 29, 2015.

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Liver injury caused by drugs, viruses, and toxins that impede the proliferation of mature hepatocytes results in the activation of hepatic progenitor cells (HPCs), which then participate in the restoration of the damaged liver tissue. HPCs are known to be bipotential cells, capable of forming both hepatocytes and cholangiocytes when regeneration by mature hepatocytes is plagued or impaired. Both clinical studies of liver disease and certain experimental animal models of liver injury conspicuously show the presence of activated HPC response and proliferation. However, in addition to regeneration, the proliferation of HPCs also determines the appearance of a ductular reaction that has been correlated with progressive portal fibrosis, suggesting intricate links between activation of HPCs and fibrogenesis. The current review highlights the role of activated HPCs in both hepatic regeneration and fibrosis during liver injury. (*Am J Pathol* 2015, 185: 2342–2350; <http://dx.doi.org/10.1016/j.ajpath.2015.06.004>)

Hepatic progenitor cells (HPCs) have been described as small oval cells with scant, lightly basophilic cytoplasm and pale blue-staining nuclei. HPCs, as they are called in humans and mice, or oval cells, in rats, represent a heterogeneous population of cells that are activated to proliferate under different conditions of liver pathophysiology.¹ Several studies have shown that these cells represent a clonogenic epithelial population in the adult liver expressing markers of both hepatocyte and cholangiocyte or bile duct cell lineages.^{2,3} It has been suggested that under conditions of massive hepatocyte and cholangiocyte loss and, hence, their impotency to self-restore, there is a facultative response in the liver, in which HPCs contribute to new hepatocytes and/or cholangiocytes.² During chronic liver injury, the normally quiescent resident hepatic HPCs are activated and expanded from the periportal to the pericentral zone of the liver, producing reactive ductules in humans.^{4,5} Reactive ductules or ductular reactions (DRs) refer to an increased number of ductules (the finest ramifications of the biliary tree), represent a regenerative response of the liver, and, thus, are a source of hepatocellular restoration during its damage or senescence. The contribution of HPCs to parenchymal restoration during liver injury is directly proportional to diminishing hepatocyte replicative potential and increasing senescence.⁶ Although

the evolution of DR and HPCs correlates with the regenerative and reparative response of liver during disease, their subsequent perpetuation inevitably leads to hepatic fibrogenesis. In the current review, we discuss the dual character of activated HPCs/DRs and elucidate their participation in both liver regeneration and fibrogenesis.

HPC Niche

HPCs are known to reside in the most distal part of the biliary tree, the canal of Hering (CoH).⁷ The CoH or intrahepatic bile ductules represent smaller branches of the intrahepatic biliary tree in the periductular areas and are lined partly by hepatocytes and partly by cholangiocytes. However, in addition to CoH, bromodeoxyuridine label retention cell studies have suggested three other possible hepatic stem cell compartments/populations in a murine model of acute liver injury (namely, intralobular bile duct cells, periductal null mononuclear cells, and peribiliary hepatocytes).⁸

Supported by the Indian Council of Medical Research, India.
Disclosures: None declared.

Stem/progenitor cells that reside within the CoH and bile ducts seem to generate the peribiliary hepatocytes through a differentiative process. These hepatocytes retain the bromodeoxyuridine label for at least 2 months, thus supporting the concept of streaming liver, which states that new hepatocytes are continuously generated in the portal field and mature on their way to the central vein. It has also been postulated that peribiliary HPC-derived hepatocytes form a specialized compartment that links hepatocytes and cholangiocytes in CoH.⁹

Null cells, whose identity still remains conspicuous, might represent stem/progenitor cells from extrahepatic sites and may originate from circulating bone marrow cells.

By using a novel technique, on the basis of the biochemical deficiency of cytochrome *c* oxidase in the liver cells, Fellous et al¹⁰ identified defined populations of clonal stem cells in the nonregenerative liver, closely opposed to the portal tracts, plausibly originating from the periportal areas. Cytochrome *c* deficiency that has been attributed to the presence of mutations in the mitochondrial cytochrome *c* oxidase gene, a component of complex IV of the respiratory chain, is proposed to be observed only in the stem cells because these are the only cells that have a sufficient lifespan to accumulate these mitochondrial mutations to a level that results in a detectable biochemical deficiency.¹⁰ Tarlow et al¹¹ unraveled a new origin for hepatic progenitors and stated that human hepatocytes undergo reversible ductular metaplasia, forming biliary-like progenitors that contribute to the parenchymal regeneration during liver injury.

HPC Markers

DRs comprise a mixture of rare hepatic stem cells, transit-amplifying cells, and differentiated cells. In addition to the epithelial cell population, DRs also have a complex of extracellular matrix (ECM), inflammatory cells, vessels, mesenchymal cells, and other diverse components that are fundamental for its maintenance. HPCs represent a heterogeneous population of cells in the transit-amplifying population in DRs. The phenotype and distribution of these cells also vary in different liver disease settings and severities.² In this population, some cells express markers specific for biliary epithelial cells, such as *CK-7*, *CK-8*, *CK-18*, *CK-19*, and *OV-6*, some express markers for immature fetal hepatoblasts, such as *AFP*, and some express one or more stem cell markers, such as *ckit/CD117*, *CD34*, *Sca-1*, *Sox9*, and *Thy1/CD90*. These cells also express neural cell adhesion molecule (*NCAM*), prominin1 (*CD133*), telomerase, and epithelial cell adhesion molecule (*EPCAM*). These markers, however, only highlight cell populations enriching for a subpopulation of HPCs with stem cell-like functions, but do not exclusively and specifically identify HPCs. The

details of various surface markers expressed by HPCs are summarized in a study by Li et al.¹²

Evidence from Cell Fate Tracing

HPCs Contribute to Liver Regeneration

Although the existence of HPCs was proposed almost 65 years ago, their involvement in liver regeneration still remains a subject of controversy. Several studies have reported the clonogenic potential of HPCs and their dual potential to differentiate into hepatocytes and cholangiocytes by *in vitro* cell culture studies and *in vivo* cell transplantation.^{13–15} Although cell transplantation and *in vitro* culture can provide insights into cell potential under specific experimental conditions, cell-lineage tracing allows for a more precise analysis of the endogenous cell fate.

Several groups have used HPC lineage tracing to characterize the origin and fate of HPCs using several HPC markers. Furthermore, several rodent models of chronic liver injuries have been used for studying the activation of HPCs, including hepatocyte injury models prepared by giving a choline-deficient, ethionine-supplemented (CDE) diet, and chronic injection of carbon tetrachloride and biliary or oval cell injury models prepared by 5-diethoxycarbonyl-1,4-dihydrocollidine (DDC)—containing diet, and bile duct ligation. Genetic fate-tracing studies in mice have been based on *Sox9*, osteopontin (*Opn*), or Wnt target gene leucine-rich-repeat-containing *Lgr5* expression within the HPC population.

Because of the use of broader lineage markers of HPCs for genetic marking of HPCs and an absence of specific HPC markers, various studies have yielded highly contrasting results in different injury models. For instance, by using a combination of cell lineage tracing studies and liver injury, it was shown that Sox9-expressing progenitor cells residing in the canals of Hering are the predominant source of new hepatocytes in mouse liver homeostasis, two-thirds partial hepatectomy (2/3 PH), and carbon tetrachloride intoxication or bile duct ligation—induced injury.¹⁶ Lineage tracing using leucine-rich-repeat-containing G-protein-coupled receptor 5 labeling in HPCs has also revealed that damage-induced leucine-rich-repeat-containing G-protein-coupled receptor 5-positive cells generate hepatocytes and bile ducts *in vivo* during liver injury.¹⁷ Another lineage tracing study using Opn-creatinine (Cre)—marked HPCs suggests that HPCs make a negligible contribution to liver regeneration after 2/3 PH, acute liver injury, chronic carbon tetrachloride injury, or even bile duct injury induced by DDC diets.¹⁸ However, HPCs contribute to >2% of functional hepatocytes during recovery in a CDE-induced chronic hepatocyte injury.¹⁸ Shin et al¹⁹ corroborated these results by tracing and ablating Foxl1-Cre—marked HPCs in CDE-induced liver injury models and provided evidence that Foxl1-HPCs and their descendants are requisite for the development of cholangiocytes and hepatocytes in liver after CDE

diet-induced injury. However, lineage is deeply dependent on the specificity of the tracing tools used. For example, the *Opn*-, *Sox9*-, or *Foxl1*-based lineage tracing studies all rely on the assumption that Cre-mediated recombination does not occur in hepatocytes. But if this recombination existed in hepatocytes, even to a small extent, the hypothesis that hepatocytes are derived from HPCs may prove wrong. Malato et al,²⁰ instead of marking the HPCs, thus used hepatocyte-specific transthyretin promoter-Cre in an *in situ* hepatocyte fate-tracing model and reported that all newly formed hepatocytes are derived from preexisting hepatocytes during normal liver homeostasis and acute liver injury. HPCs make a small contribution to hepatocyte replacement in response to 2/3 PH and chronic carbon tetrachloride intoxication.²⁰ During biliary injury, these HPCs are converted into bile duct cells.²⁰ The study also rules out the transdifferentiation of hepatocytes to biliary epithelial cells after biliary injury. HPCs likely convert into both hepatocytes and cholangiocytes in the face of substantial hepatocyte deterioration and liver damage.

HPCs Do Not Contribute to Liver Regeneration

There is another school of thought, however, that believes that new hepatocytes and even cholangiocytes are derived only from preexisting hepatocytes and not from HPCs, even during injury conditions. Schaub et al²¹ used multiple cell fate-tracing strategies in CDE diet-induced chronic liver injury mice models and showed that new hepatocytes do not originate from HPCs, cholangiocytes, or mesenchymal liver cells. This study used refined methods for *in vivo* detection of hepatocytes to prove that the non-fate-traced hepatocytes detected after CDE diet feeding in earlier studies are not generated by HPCs or other nonhepatocyte sources but are preexisting hepatocytes that were not detected by the transthyretin-Cre-labeling method.

Yanger et al²² marked three cell populations in the liver (biliary cells, hepatocytes, and rapidly dividing cells) by virtue of genetic and nucleoside analog labeling tools. Their results confirm that both new hepatocytes and cholangiocytes arise from preexisting hepatocytes, not only after partial hepatectomy but also in carbon tetrachloride-, CDE-, and DDC-induced liver injuries. Oval cells or HPCs and other nonhepatocyte populations do not contribute significantly to hepatocyte neogenesis during liver regeneration in all of the injury models tested. A few other recent studies further demonstrate that hepatocytes only transdifferentiate into biliary progenitor cells in DDC-induced biliary injury models, and these progenitors then proliferate and differentiate to functional hepatocytes *in vitro*.¹³

Tarlow et al²³ performed clonal tracing of Sox9(+) mouse liver cells and reported that these cells produce clonal oval cell proliferation and bipotential organoids *in vitro*, but rarely produce hepatocytes *in vivo*. Subsequently, they provided an alternative model for hepatocyte regeneration,

suggesting that in response to chronic liver injury, both murine and human hepatocytes undergo reversible ductular metaplasia, forming biliary-like progenitors that ultimately contribute to the restoration of the damaged hepatocyte mass.¹¹ The genetic lineage tracing study by Sekiya and Suzuki²⁴ has also corroborated these results, stating that hepatocytes, rather than cholangiocytes, are the major source of cells for the primitive ductules formed in response to chronic liver damage. The authors hypothesize that this metaplasia provides a mechanism to shut down the hepatocyte-gene expression program to avoid insults that are specifically toxic to hepatocytes.¹² All these studies, however, do not completely exclude the possibility of hepatocyte differentiation from HPCs. This may be ascribed to two reasons. First, although genetic lineage tracings are initiated in defined populations, it is difficult to exclude the possibility that actual stemness is found in an even smaller subpopulation. Second, the current mouse models of liver injury are insufficient for HPC activation, and because HPCs are thought to serve as a backup system when hepatocytes fail to proliferate, HPC activation requires a true hepatocyte cell cycle arrest. For instance, in lower vertebrates, such as zebrafish, it has been shown that a severe hepatocyte ablation results in a significant HPC or biliary cell contribution to hepatocyte differentiation.²⁵ An inhibition of hepatocyte proliferation by *N*-2-acetylaminofluorene to enhance oval cell proliferation after CDE diet administration or 2/3 PH, along with sophisticated genetic tracing techniques, may yield a further distinct picture.

HPCs in Liver Injury

DRs and HPC activation have been independently correlated with progressive fibrosis in different etiologies of cirrhosis.^{25–27} It has not yet been established that DR causes fibrosis, but profibrogenic factors from the cells of the DR may exacerbate fibrosis. Few studies have shown a significant correlation between liver disease severity and the magnitude of HPC activation or DR response.^{28,29} It has been reported that the degree of progenitor cell activation increases with the severity of liver disease. In moderate and severe degrees of inflammation, intermediate hepatocytes occur, having a phenotype intermediate between progenitor and ductular cells and mature hepatocytes. The number of these intermediate hepatocytes gradually increases with higher degrees of inflammation, higher degrees of necrosis in necrotizing hepatitis, and more advanced stages of nonalcoholic steatohepatitis.^{28–31} This correlation strongly proposes a higher degree of differentiation of progenitor cells into hepatocytes when more hepatocyte damage is present.

In chronic hepatitis, progenitor cell activation correlates with the degree of inflammation, degree of fibrosis, and stage of disease.^{29,32} This indicates that HPC expansion occurs concomitantly with fibrogenic wound healing in the

Figure 1 The activation of hepatic progenitor cells (HPCs) by other resident components in the injured liver. Senescent or dead hepatocytes directly or indirectly activate the HPCs. The necrotic hepatocytes are phagocytosed by the liver macrophages, which then secrete Tweak. Tweak interacts with its receptor, Fn14, on HPCs, leading to their activation. Macrophages also activate the HPCs by secreting Wnt proteins. The activated neighboring hepatic stellate cells (HSCs) secrete factors, including hepatocyte growth factor (HGF) and fibroblast growth factor (FGF)-7, to stimulate the HPCs. HPCs also interact with the HSCs via the Notch-Jagged and LT-β–LT-βR pathways. Abnormal deposition of extracellular matrix (ECM) components, such as laminin and osteopontin, also activates the HPCs. Liver sinusoidal endothelial cells stimulate HPCs by secreting angiogenic factors, such as vascular endothelial growth factor (VEGF). HPCs express various cell surface receptors, including Notch, LT-βR, and Fn14, to interact with these signal proteins.

liver. It is well known that the key players of the liver fibrogenic response are hepatic stellate cells (HSCs). HSCs, occupying the space of Disse between hepatocytes and sinusoids, have a unique and critical role in liver physiology and pathology, because they regulate several functions, including storage and release of retinoids and presentation of lipid antigens to natural killer cells in a normal liver. During chronic liver disease, quiescent HSCs activated by inflammatory cytokines begin to express α -smooth muscle actin (α -SMA) and switch to a myofibroblastic phenotype.^{33,34} After this conversion, activated HSCs coerce fibrosis by depositing ECM proteins, which assist to control HPC proliferation and differentiation. HSCs are found within the progenitor cell niche in normal and regenerating liver near the canals of Hering.³⁵ An outgrowth of both HPCs and myofibroblasts (MFs) is a prominent feature during liver scarring. Henceforth, mutual interactions of HPCs/reactive ductular cells and HSCs/MFs during progressive chronic liver injury are discernible.

Interactions between HPCs and HSCs

Several injury signals, such as lypopolysaccharide, tumor necrosis factor (TNF)- α , IL-1 β , reactive oxygen species, and leptin, can activate fibrogenic gene expression in HSCs via distinct signaling pathways, including transforming growth factor (TGF)- β , Hedgehog (Hh), phosphatidylinositol 3-kinase/AKT, and Janus-activating kinase/STAT/extracellular signal-regulated kinase pathways. In the initial stages after liver injury, HSC activation is critical, because it leads to the release of growth factors, such as

hepatocyte growth factor (HGF), that promote hepatocyte proliferation. HSCs are a major source of HGF.³⁵ Activated HSCs have also been implicated in assisting liver regeneration by producing angiogenic factors that modulate endothelial cell and hepatocyte proliferation.³⁶ As the injury progresses, HSCs closely associate with HPCs and secrete a multitude of growth factors, including TGF- α , HGF, fibroblast growth factor, and TNFs, that are necessary for oval cell growth and proliferation^{36,37} (Figure 1).

Mouse fetal liver-derived Thy1⁺ cells, which express classic features of HSCs (α -SMA, desmin, and vimentin), have been demonstrated to promote maturation of regenerating hepatocytes and hepatic progenitors through cell-cell contact in culture.³⁸ In DDC-fed rats, Thy1⁺ periportal cells in the niche of HPCs are known to produce a key regulatory factor, fibroblast growth factor 7, that causes considerable expansion of the HPC compartment.³⁹ Also, after chronic biliary injury, activated HSCs or MFs express Jagged-1, a Notch ligand, which activates Notch signaling in and around the hepatic ductular/progenitor niche to promote biliary regeneration (Figure 2).⁴⁰ The study also suggests that there is a requirement for an activated Notch signaling pathway to specify biliary epithelium from HPCs.²⁹ Furthermore, depletion of activated HSCs by gliotoxin and L-cysteine treatment has been shown to inhibit oval cell response around the portal tracts and lower gene expression level of *CK-19*, *AFP*, and *Ov6* in the periportal zones.^{41,42} However, these studies should be accepted with caution because gliotoxin is not an HSC-specific treatment. It not only affects HSCs but also Kupffer cells and liver endothelial cells.⁴³

In a dialogue with nearby HSCs, the reactive ductules or proliferating HPCs also produce an array of growth factors,

Figure 2 Different fates of activated hepatic progenitor cells (HPCs) in an injured liver are shown. After substantial hepatocellular damage, there is an initiation of ductular reaction in the liver, marked by the activation of HPCs in the periportal area. From here, HPCs may be directed toward biliary regeneration via up-regulation of the Notch pathway [mediated by hepatic stellate cells (HSCs)]. Hepatic differentiation of HPCs is mediated via macrophage-secreted Wnt- β -catenin pathway (via interaction with macrophages). HPCs may result in fibrosis and inflammation by diverse mechanisms. HPCs may cause activation of HSCs via transforming growth factor (TGF)- β and Sonic Hedgehog (SHh) signals to produce abnormal extracellular matrix (ECM); they may recruit activated macrophages via chemokines, such as chemokine ligand (CCL) 2, which then invite inflammatory cells by secreting Tweak, chemokine ligand 5 (CCL5)/RANTES, and intercellular adhesion molecules (ICAMs). HPCs may also enhance fibrosis by stimulating abnormal angiogenesis by liver endothelial cells.

including platelet-derived growth factor, TGF- β , and Sonic Hh, which further activate the portal HSCs leading to periportal fibrogenesis. It is known that, although pericentral fibrogenesis is because of the activation of HSCs by damaged hepatocytes, the activation of portal MFs is responsible for periportal fibrogenesis.⁴⁴ In an experimental model of carbon tetrachloride-induced liver injury, newly formed bile ductules have been shown to express monocyte chemoattractant protein-1 and platelet-derived growth factor- β ,⁴⁵ capable of recruiting and activating HSCs to produce collagen.

HSCs Contribute to HPCs and Vice Versa

HSCs that surround DRs may not only influence the proliferation and/or differentiation of progenitor cells, but may also themselves contribute to their population. Fate-mapping experiments using glial fibrillary acidic protein-Cre- and α -SMA-Cre-based promoters have shown that HSCs may, through a process of mesenchymal-to-epithelial transition, produce hepatic progenitors and hepatocytes.⁴⁶ Because HSCs represent liver-resident mesenchymal stem cells, they have the potential to differentiate into adipocytes, osteocytes, and other cell types. These studies have also hypothesized that HSC and ductular cells share a common lineage on the basis of the observation that freshly isolated primary HSCs express several other biliary epithelial markers, such as *CK-19*, *aquaporin-1*, and *CK-7*.³⁰ However, fate-tracing studies using HSC-specific markers, lecithin retinol acyltransferase and mesoderm

posterior 1 homolog, have implicated that HSCs are not progenitor cells, and do not contribute to the generation of hepatocytes in the injured liver.^{47,48} The studies have demonstrated that glial fibrillary acidic protein Cre label efficiently labels cholangiocytes but not a significant amount of HSCs, suggesting that previous studies using glial fibrillary acidic protein Cre are likely not HSC specific.⁴⁸

Both lecithin retinol acyltransferase and mesoderm posterior 1 homolog, which are suggested to be specifically expressed by HSCs or their precursors, are also expressed by embryonic and adult stem cells from mice and humans.^{49,50} Transplanted HSCs contribute to liver regeneration in host animals by forming mesenchymal tissue, progenitor cells, hepatocytes, and cholangiocytes.⁵¹ HSCs contribute to the formation of K19⁺ and α -fetoprotein-positive cells during DRs *in vivo*, thus sustaining the notion that mesenchymal cells that surround DRs may not only influence the differentiation of progenitor cells, but also contribute to their population. A few studies have also indicated the possibility that in response to TGF- β , the ductular cells may undergo epithelial-to-mesenchymal cell transition via the Sonic Hh pathway, suggesting that a portion of the MF pool may be derived from the phenotypic transformation of proliferating cholangiocytes and HPCs.⁵²

Signaling Pathways of HPCs and HSCs

The mesenchymal-to-epithelial conversion and vice versa in HSCs and HPCs is regulated by Sonic Hh and Notch

signaling pathways, and both HSCs and HPCs are responsive to Hh signals. During bile duct ligation injury, increased Notch and Hh pathway activity in HSCs and HPCs stimulates epithelial-to-mesenchymal-like transition, which promotes fibrosis, whereas disrupting Hh signaling in MF inhibits Notch signaling and promotes mesenchymal-to-epithelial transformations.⁵³ *Smoothed*, an obligate intermediate in the canonical Hh signaling pathway, controls injury-related accumulation of MFs and liver progenitors in adult liver. A conditional deletion of *Smoothed* in α -SMA-positive cells after PH has been shown to enhance accumulation of quiescent HSCs but reduce accumulation of MFs, fibrosis, and progenitors, as well as inhibition of hepatocyte and cholangiocyte proliferation.⁵⁴ Hence, fibrosis is an essential component of successful liver regeneration, and blocking of canonical Hh signaling in MFs not only inhibits liver fibrosis but also prevents accumulation of liver progenitors, thus further validating a close relationship between these two cell types during liver injury.⁵⁵

Another pathway by which the HSCs and HPCs are known to interact and contribute to enhanced liver fibrogenesis during liver injury is via the lymphotoxin (LT)- β pathway. During liver injury, LT- β -positive HPCs initiate an NF- κ B-dependent signaling pathway in LT- β receptor-positive HSCs that results in the expression of chemotaxis mediators, such as intercellular adhesion molecule-1 and regulated on activation normal T-cell expressed and secreted, which, in turn, recruit regulated on activation normal T-cell expressed and secreted receptor-positive HPCs and other leukocytes. Thus, the pathway induces the migration of HPCs and immune cells to the sites of injury. The LT- β signaling pathway plays a role in fibrogenesis, because chronically injured *LT- β R* knockout mice show fewer α -SMA-positive HSCs and decreased collagen deposition, as evidenced by reduced Sirius Red staining.⁵⁶

Interactions between HPCs and ECM

Activated HSCs are also the main source of ECM, matrix metalloproteinases, and their inhibitors that participate in ECM remodeling. ECM remodeling is tightly coupled with the production of cytokines/growth factors because the ECM is capable of sequestering biologically active molecules.⁵⁷ An aberrant ECM production by hepatic MFs and a subsequent distortion of the growth signals play a critical role in the generation of a specific HPC microenvironment (Figure 1).

ECM deposition and activation of MFs, matrix-producing cells, are known to occur before HPC expansion, most probably to provide a support matrix for migration and anchorage of HPCs.^{57,58} In a diet-induced liver injury model, ECM deposition is seen as early as day 3, whereas a significantly increased DR by image analysis is only seen after a week, indicating that ECM changes are independent of HPC proliferation. However, an association between

ECM changes and HPC activation is well established. An important role for the ECM component, laminin, in the maintenance and expansion of HPCs in an undifferentiated biliary state has been reported.⁵⁹ Experiments using *r/r* mice, which produce mutated collagen I resistant to matrix metalloproteinase degradation, have shown that recovery after carbon tetrachloride-induced chronic liver injury is associated with an attenuated HPC response in these mice compared with that observed in the wild types, together with persistence of collagen I and failure to deposit ECM laminin.⁵⁸ Thus, the study suggests a paramount role of ECM remodeling in HPC activation.

Opn, another ECM protein, is also a significant driver of HPC response that contributes to scarring and liver fibrosis.⁶⁰ Opn leads to a significant activation of biliary epithelial cells via TGF- β , which further correlates with the expression of collagen-1 in the co-cultured HSCs.⁶⁰ It has been suggested that a dense ECM around pseudoductular structures shields HPCs from macrophage-secreted signals for hepatic differentiation.⁶¹ A decreased density of ECM, laminin, and/or other connective tissue growth factor-dependent profibrotic signals, on the other hand, is known to decrease the oval cell response, but at the same time, stimulate hepatocyte differentiation in HPCs.^{12,62} Thus, the signals that decrease HPC self-renewal and instead enhance their controlled differentiation to hepatocytes may lead to an attenuation of the liver fibrotic response.

HPCs, HSCs, and Kupffer Cells

Kupffer cells or liver macrophages play a central role in the normal physiology and homeostasis of the liver as well as in initiating acute and chronic responses of the liver to toxic compounds. Kupffer cells function as both a promoter and a protector of liver injury in different conditions. Activation of Kupffer cells, either directly or indirectly, results in the release of a multitude of inflammatory and growth control mediators. These molecular mediators may participate in liver fibrosis progression (by generating reactive oxygen species, proinflammatory factors, and profibrotic factors) and/or fibrosis regression (by degrading ECM, exerting anti-inflammatory action, and eliminating activated matrix-producing cells).⁶³ The factors released by Kupffer cells could also influence the liver inflammatory responses by modulating the activation of HPCs. For instance, in CDE diet-induced liver injury, the HPC response has been associated with elevated numbers of Kupffer cells and invading CX(3)CR1(high)CCR2(high) macrophages secreting persistently high levels of TNF- α , which is involved in HPC response. HPCs themselves act as a source of chemokines, such as chemokine ligand 2 and CX(3)CL1, to invite infiltrating macrophages (Figure 2).⁶⁴ In a macrophage-depletion, diet-induced liver injury model, it has been reported that, although the proliferation of HPCs is not affected by the absence of Kupffer cells, the portocentral parenchymal invasion of HPCs is

significantly disrupted. In these models, aggregates of HPCs have been observed in the portal areas only. This effect has been ascribed to a defect in the expression of macrophage-associated growth factors and cytokines, such as HGF, TNF- α , intercellular adhesion molecule (ICAM)-1, and chemokine ligand 5 (CCL5)/RANTES, a decrease in the associated myofibroblastic reaction, and a reduction of ECM framework, which is essential for cell migration.⁶⁵

HPCs and Kupffer Cells: Signaling Pathways

Liver macrophages are a major source of Wnt proteins, a diverse family of signaling glycoproteins (Figure 1). During hepatocellular injury, where MFs enhance Notch signaling for biliary regeneration, macrophages engulf the hepatocyte debris and induce the expression of Wnt3a. This leads to an activation of the Wnt/ β -actin signaling pathway in nearby HPCs, thus maintaining expression of Numb (a cell fate determinant) within these cells and the promotion of their differentiation to hepatocytes. Hence, the Notch signaling pathway is a pathway for biliary regeneration, whereas activation of the Wnt signaling pathway by the Kupffer cells leads to hepatic specification, once again emphasizing the role of a microenvironment in the differential differentiation of HPCs.⁴⁰ Increased phagocytosis of hepatocyte debris encourages intense Wnt up-regulation in the macrophages and HPC differentiation into hepatocytes, providing a critical link between hepatocyte death, macrophages, and HPC fate (Figure 2).

It has also been reported that during excessive hepatocyte damage, Kupffer cells release a cytokine, TNF-like weak inducer of apoptosis (Tweak), the receptors (fn receptors) of which are highly expressed on HPCs (Figure 1). TWEAK-fn14 signaling results in the induction of HPC proliferation in a NF- κ B-dependent manner.⁶⁶ In chronic liver injury, HPC proliferation is induced via macrophage/natural killer cell-produced TWEAK activation of the NF- κ B pathway. TWEAK-fn14 signaling is also known to affect HPC-mediated recruitment of inflammatory cells and leukocytes (Figure 2). The leukocytes further support the establishment of fibrosis in chronic liver injury by affecting HSC activation, liver fibrosis initiation, and injury progression.⁶⁷ It has been recently reported that the inhibition of progenitor cell proliferation using anti-TWEAK antibody prevents fibrogenic response and augments liver regeneration in carbon tetrachloride-induced liver fibrosis.⁶⁸ Modulation of HPC response by Tweak signaling presents a novel strategy to target HSC-mediated liver fibrosis.

HPCs and Angiogenesis

An activated HPC response has also been correlated with the appearance of neovessels or angiogenesis in the HPC niche. In normal liver, HPCs are almost negative for vascular endothelial growth factors (VEGFs; A and C) and VEGF receptors (1, 2, and 3). In cirrhotic livers, however, there is an increased expression of VEGF-A, VEGF-C, VEGF receptor 1, and

VEGF receptor 3 in cirrhotic nodules and in reactive ductules.⁶⁹ VEGF not only acts as a mitogen for liver sinusoidal endothelial cells proliferation but also activates cholangiocyte and HPC proliferation by both autocrine and paracrine mechanisms.⁷⁰ The number of new vessels and the degree of angiogenesis also show a strong correlation with reactive ductules and HPC activation in primary biliary cirrhosis.⁶⁹ The process of angiogenesis is intricately twined with both regeneration and fibrogenesis during liver injury.⁷¹ Hence, it may be proposed that prolonged hepatocyte injuries leading to the activation of HSCs elicit the proliferation and activation of HPCs, which then drive fundamental repair mechanisms, such as neo-angiogenesis, resulting further in HSC-mediated fibrogenesis in the portal regions. The contribution of the angiogenic mediators toward HPC activation, the cross talk between HPC and liver sinusoidal endothelial cells, and the role of HPC-mediated angiogenic mechanisms in liver fibrosis need further exploration.

Conclusions

Although liver regeneration after PH proceeds impeccably without fibrosis, liver repair after chronic injury is associated with fibrosis. The resident HPCs do make a small, but important, contribution to liver repair during chronic hepatocellular damage. The hepatobiliary DR mediated by HPCs during chronic human liver injury represents a unique reaction different from all other organs in the body. The cells and the ECM surrounding HPCs act as an instructive niche, which, through elaboration of several molecular mediators, stimulates these cells and affects liver regeneration during disease. However, in an injured liver, amid substantial hepatocyte damage, HPCs are faced by the challenge of restoring the liver mass, when signals and cellular controls underlying homeostatic replacement of healthy tissue are awry. These signals from the niche not only commence HPC-mediated regeneration but also lead to an aberrant activation of HPCs and subsequent commencement of DRs that result in fibrogenesis and also possibly tumorigenesis. A closer scrutiny of the origin and differentiation of the HPC population and the DRs, their niche components, and signals in different animal models of liver injury and their relative relevance in specific patients is indispensable for the designing of HPC-based antifibrotic or proregenerative therapies for liver diseases.

Many questions need to be answered in this direction, such as whether profibrotic signals dictate the efficacy of liver repair or whether increased liver regenerative response leads to fibrosis per se during disease? Whether phenotypically different populations of HPCs respond to various signals differently? Whether hepatocytes and cholangiocytes are reprogrammed into HPCs during injury or whether some resident HPC populations assume new cell fates under injury conditions, such as stellate cells and MFs? HPCs have the potential to differentiate into hepatocytes and cholangiocytes and, thus, a deeper analysis of the mechanisms and pathways that modulate the activation of HPCs into the right direction will facilitate the development of

therapeutic avenues to accelerate liver restructuring and functioning after a chronic injury.

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