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BRIEF REVIEW

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Links Between Hepatic Fibrosis, Ductular Reaction, and Progenitor Cell Expansion

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Interactions between cells and their extracellular matrix have been shown to be crucial in a wide range of biological processes, including the proliferation and differentiation of stem cells. Ductular reactions containing both hepatic progenitor cells and extracellular matrix are seen in response to acute severe and chronic liver injury. Understanding the molecular mechanisms whereby cell-matrix interactions regulate liver regeneration may allow novel strategies to enhance this process. Both the ductular reaction in humans and hepatic progenitor cells in rodent models are closely associated with collagen and laminin, although there is still debate about cause and effect. Recent studies have shown a requirement for matrix remodeling by matrix metalloproteinases for the proliferation of hepatic progenitor cells and suggested defined roles for specific matrix components. Understanding the interactions between progenitor cells and matrix is critical for the development of novel regenerative and antifibrotic therapies.

Keywords: Liver Regeneration; Extracellular Matrix; Collagen; Laminin.

The liver has a remarkable regenerative capacity and is unusual in its ability to regenerate from both mature cells and facultative stem cells.[1,2] In normal liver undergoing partial hepatectomy or acute injury, hepatocyte-mediated regeneration predominates. In chronic and severe injury, however, ductular reactions (DRs) of activated biliary epithelial cells that contain hepatic progenitor cells (HPCs) appear in the periportal regions.[3] DRs refer to both the epithelial component and their associated inflammatory niche.[4] HPCs are bipotential adult stem-like cells that are defined through their capacity to differentiate under clonogenic conditions in vitro into hepatocytes and biliary epithelial cells. There are strong associations between DRs and changes in the extracellular matrix, but understanding further the mechanisms underlying this interaction may help in the development of novel therapies to improve regeneration and reduce fibrosis.

Defining HPCs/DRs and the Stem Cell Niche in the Liver

Oval cells, a population of small cells with an ovoid nucleus and a high nuclear-to-cytoplasmic ratio, were initially described in the portal areas of rat livers after chemical injury.[5,6] These cells have been noted to coexpress markers of hepatocytes (albumin) and biliary epithelial cells (keratin-19). Cells with similar but not identical characteristics have also been seen in humans and mice and have been called liver progenitor cells or HPCs. Three-dimensional reconstructions in human liver suggest that they arise from the interface between the hepatocyte canalicular system and the biliary tree, known as the canals of Hering.[7] Attempts to identify putative HPCs have assessed the ability of cells to differentiate towards both hepatocytic and biliary lineages as well as their clonogenic capacity.[8]

Alternatively, the regenerative capacity of HPCs can be shown in vivo using liver repopulation assays.[9,10] A number of HPC markers have been proposed, but none are completely specific.[11] Although the epithelial component of the DR has a predominantly biliary phenotype, it is a heterogeneous population and contains a range of cell types from primitive progenitors to more hepatocyte-like cells.[12] DRs show distinct polarity, with hepatocytic and biliary differentiation at either end.[13]

In addition to these epithelial progenitors, there has been significant debate regarding the possible contribution of mesenchymal cells to adult liver regeneration through the processes of mesenchymal-epithelial or epithelial-mesenchymal transition.[14] Potential contributions from both glial fibrillary acidic protein–expressing cells[15] and more recently α–smooth muscle actin—expressing cells[16] to hepatocytes and biliary cells have been shown. However, a recent lineage tracing report that marked nearly all HSCs showed that HSCs have no epithelial progenitor function across a wide range of liver injury models, including the 3,5-diethoxycarbonyl-1,4-dihydro-collidine and methionine choline-deficient, ethionine-supplemented diet models that provoke ductular reactions.[17] A further recent study that achieved similarly high recombination efficiencies in activated myofibroblasts also did not find any contribution to

Abbreviations used in this paper: 2-AAF, 2-acetylaminofluorene; CDE, choline-deficient ethionine-supplemented; DR, ductular reaction; EpCAM, epithelial cell adhesion molecule; HPC, hepatic progenitor cell; MMP, matrix metalloproteinase; TGF, transforming growth factor.

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epithelial populations in the liver (personal communication, November 2013).[18]

Animal Models

A number of models have been used to induce HPCs in rodents. In the rat, combining partial hepatectomy with chemical inhibition of hepatocyte proliferation using 2-acetylaminofluorene (2-AAF) or retrorsine induces a robust HPC response.[19] A similar ductular response is seen in response to D-galactosamine[20] and allyl alcohol.[21] In mice, partial hepatectomy and 2-AAF fail to produce convincing activation of HPCs.[22] Instead, several dietary or toxin models of mouse HPC activation have been described: the choline-deficient, ethionine-supplemented (CDE) diet,[23] the 3,5-diethoxycarbonyl-1,4-dihydro-collidine–supplemented diet,[9] and phenobarbital/cocaine.[24] HPCs have also been shown after genetic induction of senescence in hepatocytes.[25]

Relative Contributions of Hepatocytes and HPCs to Liver Regeneration

Mature hepatocytes represent the main source of regeneration in normal liver turnover and acute injury.[26] In cirrhosis, however, hepatocytes express markers of cell cycle arrest, suggesting an impaired ability to contribute to regeneration.[27] This is supported by rodent models of fibrosis and cirrhosis, where there is reduced hepatocyte DNA synthesis in response to partial hepatectomy[28,29] or mitogens.[30]

There is now compelling evidence from animal models using genetic lineage tracing that HPCs can contribute, albeit modestly, to functional hepatocytes during injury. Using an osteopontin-linked Cre to label HPCs, it was shown that up to 3.26% of all hepatocytes were derived from progenitors.[31] This same study found a negligible contribution from HPCs to hepatocytes during normal liver homeostasis after partial hepatectomy or carbon tetrachloride injury. Another lineage tracing study labeled hepatocytes using an adenoviral-associated vector–linked Cre. This showed that following after carbon tetrachloride injury, 1.3% of hepatocytes were derived from a nonhepatocyte source, presumed to represent HPCs.[32] Again, there was little contribution to homeostasis after acute resection or toxic injury. Although these numbers represent a minor proportion of mature hepatocytes, the injury models used are mild and short-term compared with chronic liver disease in humans. Further lineage tracing or transplantation experiments are required to determine the true functional regenerative capacity of HPCs over prolonged, repeated, or severe liver injury that closely models human disease.

In humans, there is indirect evidence to suggest a lineage connection between ductular reactions and hepatocytes. Intraseptal hepatocytes in cirrhosis are strongly associated with keratin-19–positive ductular reactions and can be shown by 3-dimensional reconstructions to link to the biliary tree.[33] However, in this static tissue analysis, a definitive product-precursor relationship cannot be proven and it could be argued that hepatocytes may give rise to ductular cells. However, hepatocytes that are positive for epithelial cell adhesion molecule (EpCAM) associate close to ductular reactions and have a longer telomere length than EpCAM-negative hepatocytes, suggesting their origin from a slow-cycling stem/progenitor cell.[34]

Extracellular Matrix and Cell Behavior

The extracellular matrix, initially considered an inert scaffold for cells, is now recognized as dynamic and widely variable between tissues during both development and disease, with an important role in regulating cell behavior.[35] In particular, the behavior of stem cells is critically influenced by their microenvironment.[36,37] Individual matrix components can influence cells directly by binding to cell surface receptors, resulting in intracellular signal transduction. Changes in the matrix content may also affect cells indirectly by changing local concentrations of growth factors and altering physical properties of the tissue such as stiffness.

HPCs and the Niche

HPCs occur in close association with a niche composed of other cells, including hepatic stellate cells and macrophages, and extracellular matrix (Figure 1).[38] Recent work has provided evidence of how cell-cell signaling from both stellate cells and macrophages can influence progenitor cell fate via the Wnt and Notch pathways.[39] There is also activation of the hedgehog pathway during liver injury,[40,41] which can influence HPC behavior in vitro.

Both niche cell types, stellate cells and macrophages, influence the composition of the extracellular matrix. Hepatic stellate cells (and portal fibroblasts) are the main source of matrix in the liver[42,43] and also produce matrix metalloproteinases (MMPs) and their inhibitors that regulate matrix degradation.[44] Macrophages have a dual role in fibrosis; depletion of macrophages during injury ameliorates fibrosis, but depletion during the recovery phase causes persistence of fibrosis.[45] Although this appears to be mediated at least partly via changes in myofibroblast numbers, hepatic macrophages are also a source of MMPs.[46] The process is complex, however. In chronic hepatitis C, macrophages expressing MMP-9 were confined to a portal subpopulation and increased with increasing portal fibrosis.[47] In addition to the contribution of the niche cells, ductular epithelial cells/HPCs themselves appear to be capable of matrix synthesis, at least in vitro,[38] and produce MMPs.[48]

HPCs/DRs and Fibrosis

In human liver disease, the DR correlates closely with the severity of fibrosis across a range of liver pathologies, including chronic hepatitis C,[49] alcoholic and nonalcoholic steatohepatitis,[50,51] recurrence of viral hepatitis after liver transplantation (where florid DRs with accompanying fibrosis occur in fibrosing cholestatic hepatitis[52]), and genetic hemochromatosis.[53] This has raised the question as to whether the fibrosis is in some way beneficial for HPC-mediated regeneration or whether fibrosis is unintentionally exacerbated by the progenitor reaction.[54] Clearly, this issue has major implications for the development of either antifibrotic or proregenerative therapies.