

Adult Hepatic Progenitor Cell Niche: How it affects the Progenitor Cell Fate

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ABSTRACT

The hepatic progenitor cell (HPC) niche is a special microenvironment composed of different cell types, extracellular matrix (ECM) components, growth factors and cytokines released by the niche cells that help to maintain the characteristics of HPCs and the balance between their activation, proliferation and differentiation. Composition of this special microenvironment, created in response to specific liver damage, together with critical interactions between different partners of the HPC niche can determine the fate decision and differentiation pathways of HPCs. A number of recent studies have shed light on factors and signals from the HPC niche that determines the choice of HPCs differentiation towards a specific cell type depending on the nature of the liver injury and resultant microenvironment created by this injury. This paper seeks to provide an in-depth review, through a literature review and the authors' experiences, of the most recent findings on the role of the HPC niche in fate choice option of HPCs toward either hepatocytes or bile duct epithelial cells and its clinical relevance.

KEYWORDS

Hepatic progenitor cells; Hepatic progenitor cell niche; Molecular cross-talk

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INTRODUCTION

The concept of a stem cell niche was first proposed by biologist Raymond Schofield in 1978 when he found that despite bone marrow, the spleen cannot maintain hematopoietic stem cell (HSCs) behavior.¹ Since then, this microscopic niche has clearly been defined in numerous organs, particularly the bone marrow, hair follicles and intestine.²⁻⁴ The niche is a special microenvironment composed of different cells, extracellular matrix (ECM) components, growth factors and cytokines released by the niche cells that help to maintain the characteristics of stem cells and the balance between activation/differentiation.⁵ Composition of this special microenvironment together with critical interactions between different components of the stem cell niche is the key element to control stem cell behavior and the balance between stem cell activation, proliferation and differentiation. Significance of the microenvironment in the control of hepatic progenitor cell (HPCs) behavior and fate has nicely been shown in studies where transplantation of non-functional cells from cirrhotic rats into non-cirrhotic livers resulted in the full metabolic and proliferative functional recovery of the engrafted cells.⁶ Despite stem cell niches of adult organs such as the intestine,

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hair follicles and bone marrow, the stem/progenitor cell niche in the adult liver is less understood. The role of Notch and Wnt-beta catenin signaling pathways in regulating stem cell self-renewal in hair follicles and the intestine are well documented.^{7,8} In the HSC niche in the bone marrow, an interaction between CXCR4 and its ligand CXCL12 stromal-cell-derived factor 1 (SDF1) released by bone marrow endothelial and stromal cells is crucial for maintenance and localization of HSCs.⁹ The Wnt-beta catenin (canonical Wnt) signaling pathway is also important for the maintenance of HSCs in an undifferentiated state.¹⁰ The role of the non-canonical Wnt pathway in long-term maintenance of quiescent HSCs has been recently proposed in an interesting study conducted by Sugimura et al. who have also observed that non-canonical Wnt can antagonize the canonical Wnt signaling pathway.¹¹

In the field of HPCs, a handful of studies in recent years have shed light on factors and signals from the HPC niche that determine the choice of HPC differentiation towards a specific cell type dependent on the type and severity of the liver injury and resultant environment created by it. This paper seeks to provide an in-depth review, through a literature review and the authors' experiences, of the most recent finding on the role of the HPC niche in fate choice option of HPCs and its clinical significance.

Liver regeneration: A two-tier cell system

The liver's anatomical position in the body and its diverse critical physiologic functions challenge the liver with a wide variety of toxins and insults. Therefore, it is not surprising that the liver has an amazing capacity to regenerate after different types of injuries.¹² Depending on the nature and severity of the liver damage, different pathways and cell types are involved in the process of regeneration and are not exclusively independent of each other.¹³⁻¹⁶

After mild-to-moderate acute liver damage or following partial hepatectomy, which is the classic model of hepatocyte-mediated liver regeneration, hepatocytes shoulder the burden of liver regeneration. However, when the regenerative capability of

mature proliferating hepatocytes is compromised (e.g., in acute or chronic liver failure), a reserve cell compartment called HPCs (oval cells in rodents) is activated.¹⁷⁻¹⁹ Accordingly, HPCs have been identified in a wide variety of human liver diseases in which a certain degree of hepatocyte damage with impaired regeneration of remaining hepatocytes and/or bile duct epithelial cells exist.²⁰⁻²² Upon activation and proliferation, HPCs are able to differentiate towards both hepatocellular and biliary type cells. Contribution of HPCs to liver regeneration in severe acute liver damage is well described. Sequential histological observations of the native liver in patients with acute liver failure and massive loss of hepatocytes who underwent auxiliary partial orthotopic liver transplantation (APOLT) have shown full regeneration of the liver from resident HPCs. Indirect contribution of HPCs to liver regeneration in chronic human liver diseases has also been shown.²³⁻²⁹

The hepatic progenitor cell (HPC) niche and immunophenotype of HPCs

The Canals of Hering and bile ductules localized in the portal tract and the periportal parenchyma are believed to be the niche for adult human HPCs.³⁰ Canals of Hering are partially lined by small hepatocytes and partially by bile duct epithelial cells. They are the physiologic link between the hepatocyte canalicular system and the biliary tree.³⁰ This anatomical position of HPCs nicely fits within their physiological role following activation.

It is generally accepted that oval cells are derived from oval cell precursors in the bile duct epithelium. Strong evidence for the existence of the HPC niche has been presented from elegant label retaining studies in which small label [5-bromo-2'-deoxyuridine (BrdU)]-retaining epithelial cells were found in the portal and periportal areas of mice livers where a sublethal dose of acetaminophen was administered coincident with BrdU.³¹ In addition to label retention studies, lineage-tracing studies in a mouse model of oval cell-mediated liver injury from a methionine- and choline-deficient diet supplemented with 0.15% ethionine (MCDE)

showed a continuous cell supply from the Sox9-expressing progenitor zone within the portal area which provided further support for the existence of the HPC niche in the portal-periportal area.³²

HPCs are heterogeneous small epithelial cells with a large oval shaped nucleus and scanty cytoplasm. They are immunoreactive for a different panel of markers that include adult biliary markers (K7, K19 and K14), adult and fetal hepatocyte markers (K8, K18, C-met, α -fetoprotein and albumin) and adult hematopoietic markers (c-Kit, CXCR4 and Sca-1). These cells also express neural cell adhesion molecule (NCAM), prominin1 (CD133), telomerase and epithelial cell adhesion molecule (EPCAM).³⁰⁻³⁴ As previously mentioned, the capability of HPCs to differentiate into both cholangiocytes and hepatocytes is dependent upon the epithelial cell-type which incurs the most damage. If hepatocytes are the most important type of damaged cells as in severe viral hepatitis or massive loss of hepatocytes in acute liver failure, HPCs will differentiate towards hepatocytes to restore lost liver mass and function.^{22,29,35} However if cholangiocytes are the primary damaged cell type such as in primary biliary cirrhosis or primary sclerosing cholangitis, then HPCs differentiate towards biliary epithelial cells.^{34,36} Hence HPCs are heterogeneous dynamic cell populations that constantly change their phenotype according to the liver's impaired cell type and its consequent differentiation stage. Therefore, a unique HPC marker specific for these cells does not exist.

Composition of the hepatic progenitor cell (HPC) niche: How cross-talk (cell-cell and cell-matrix) in the HPC niche influences HPCs activation and differentiation.

Role of inflammation

Few data exist on the composition of the HPC niche and its cross-talk in the normal liver.³⁷ In the injured liver, different cell types such as cholangiocytes, hepatocytes, hepatic stellate cells/myofibroblasts, endothelial cells, macrophages and other inflammatory cells can potentially interact with HPCs. Our current knowledge in this field is pre-

dominantly derived from animal models of liver injury where oval cell activation and differentiation are observed. These models suggest a role for macrophages and lymphocytes as important cell components of the HPC niche in stimulating and initiating a regenerative response.³⁸ A critical balance between inflammatory cells, their products and HPCs is an important determinant of this regenerative process.³⁸⁻⁴⁰ A correlation between the degree of HPCs activation and the severity of liver disease has been shown in both acute and chronic human liver diseases.^{18,41-43} It has also been shown that HPCs are surrounded with inflammatory infiltrate in chronic viral hepatitis.⁴² These findings suggest that products of inflammatory infiltrate may function as growth or chemotactic factors for HPCs to initiate the regenerative process. For instance, cytokine TNF-like weak inducer of apoptosis (TWEAK), a member of the pro-inflammatory TNF family, exclusively plays an important role in HPCs proliferation.⁴⁴ TWEAK which is produced by monocytes, T lymphocytes and macrophages can stimulate HPCs proliferation directly through its specific receptor, fibroblast growth factor-inducible 14 (Fn14).⁴⁴ TWEAK receptor Fn14 is also overexpressed in many chronic human liver diseases including non-alcoholic cirrhosis and viral hepatitis, which suggests the potential role of the TWEAK/Fn14 axis in initiation of HPCs proliferation.⁴⁵

Up regulation of chemokine SDF-1 (CXCL12) in human chronic liver diseases is well documented.⁴⁶ SDF-1 binds to its receptor, CXCR4 which is expressed by HPCs and numerous other cells. This chemokine may play a role in the migration and recruitment of CXCR4-positive T lymphocytes around the bile ducts.⁴⁶ Attracted CXCR4-positive T cells, in turn, express TWEAK which stimulates HPCs expansion according to the aforementioned mechanism.^{47,48} Other key elements of the inflammatory response in oval cell activation in rodents can also stimulate HPCs and include the IL6 family [leukemia inhibitory factor (LIF) and oncostatin M (OSM)], lymphotoxin- β , interferon gamma (IFN γ), and TNF α .^{14,49-51}

Role of the extracellular matrix (ECM)

The ECM is one of the most important drivers in the HPC niche. The ECM is a structural network that consists of different types of collagens, proteoglycans and glycoproteins such as laminin and has an important role in many biological functions including cell proliferation, migration and differentiation.⁵² It has been shown that α -SMA-positive matrix producing cells increase in number along with an increase in the number of oval cells during liver injury.³⁷ Van Hul et al. in a choline-deficient, ethionine-supplemented (CDE) model of oval cell proliferation found that ECM deposition and activation of matrix-producing cells (α -SMA-positive myofibroblast-like cells) occurred prior to oval cell expansion and in front of them along the porto-venous gradient of lobular invasion.⁵³ Their finding has confirmed the hypothesis that HPCs need a support matrix for migration and anchor in order to differentiate for reconstitution of a damaged liver.⁵⁴ The significance of cross-talk between ECM components and oval cells/HPCs in the expansion and differentiation of these cells has also been suggested by other groups.^{55,56} In a wide variety of human chronic liver diseases, HPCs and ECM producing activated hepatic stellate cells/myofibroblasts appear to proliferate in close proximity to each other and correlate in terms of numbers.^{37,57,58} Recently, it has been shown that in patients with chronic hepatitis B and C, HPCs co-localize with laminin, endothelial cells, myofibroblasts and macrophages.³⁷ A key role has been revealed for the ECM component, laminin, in the maintenance and expansion of HPCs in an undifferentiated biliary state similar to what is found on the role of the laminin matrix in expansion of oval cells in vitro.⁵⁵ Further in vivo detailed studies of laminin inhibition during oval cell mediated liver regeneration in mice confirm the theory that laminin signaling is implicated in maintaining HPCs in an undifferentiated/biliary state and that inhibition of laminin can aid differentiation towards hepatocytes.⁵⁹

Role of Hedgehog (Hh), Wnt and Notch signaling pathways

Hedgehog (Hh), Wnt/beta catenin and Notch

signaling pathways are involved in HPC activation and differentiation in both rodents and animals.⁶⁰⁻⁶⁴ It has been demonstrated that both Hh ligands and the Hh receptor, Patched, are simultaneously expressed within the oval cell compartment which suggests that liver progenitors maintain their viability throughout life via autocrine and/or paracrine Hh signaling.⁶² Studies in patients with alcoholic steatohepatitis and primary biliary cirrhosis have also shown that HPCs and their neighboring hepatic stellate cells (HSCs) are able to both produce and respond to Hh ligands.^{63,64}

In a recent study by our group, laser micro dissected HPCs and their niche have been compared in hepatocytic (hepatitis C cirrhosis) and cholangiocytic (primary biliary cirrhosis) liver diseases. Gene-expression analysis and immunohistochemistry/-fluorescence of micro-dissected material revealed that activation of the Wnt/beta catenin signaling pathway plays a significant role in HPCs expansion, while in biliary diseases the Notch pathway is implicated in differentiation of HPCs towards biliary epithelial cells.⁶⁰ A meticulous, recent study by Boulter et al. has reported the functional role of Notch and Wnt signaling pathways and the importance of injury-specific HPC niche changes for appropriate delineation of bipotential HPC fate (hepatocellular versus biliary).⁶¹ During biliary liver damage which is typically observed in primary biliary cirrhosis and primary sclerosing cholangitis, there is an increase in periportal α -SMA-positive myofibroblasts which express Jagged 1 and also produce collagen matrix. Oval cells/HPCs in both mouse and human biliary liver diseases express the receptors Notch1 or Notch2, respectively, which are activated through interaction with their ligand Jagged 1.^{60,61} Notch/Jagged interactions activate the Notch signaling pathway leading to downstream expression on Notch target genes. However, the Notch pathway is critically inhibited during hepatocytic differentiation of HPCs. In parenchymal (hepatocytic) diseases in both humans and rodents, there is an increased number of infiltrating macrophages in the periportal areas.^{37,60,61} Macrophages express Wnt3a in response to the phagocytosis of

apoptotic hepatocytes and other biological debris. Secretion of Wnt3a by macrophages induces neighboring oval cells to express Notch pathway inhibitor Numb, which is activated by β -catenin and suppress transcription of Notch target genes, resulting in biliary differentiation inhibition. In confirmation of these observations, macrophage ablation during hepatocyte regeneration has been shown to block differentiation of HPCs towards hepatocytes; instead, it stimulated cholangiocytic differentiation of HPCs.^{61,65} Taken together, these data indicate that this is the cell-cell and cell-matrix cross-talk which is observed in a special type of liver damage that will ultimately direct the HPCs fate. Understanding such interplay at the HPC niche is necessary to develop new therapies to enhance liver regeneration for different types of human liver diseases.

Hepatic progenitor cells (HPCs) and fibrosis

A correlation between the degree of HPCs activation and the severity of liver fibrosis has been shown across a wide range of chronic human liver diseases.^{56,58} A recent study suggested a fibrogenic role for HPCs in fibrotic rat liver via the TWEAK signaling pathway.⁶⁶ This has raised the question whether HPCs are important not only in liver regeneration but also in liver fibrogenesis. The relative timing of HPCs activation and ECM deposition can be one approach to answer this question. Van Hul et al. in their study of oval cell proliferation have found that ECM deposition and activation of matrix-producing cells occur prior to (day 3) oval cell expansion (day 7) and in front of this expansion⁵³ which suggests that matrix deposition and HPCs proliferation are, at least partly, independent of one another. Further evidence to support the influence of ECM on HPCs rather than HPCs influence of the ECM has been derived from a study in which iloprost administration reduced HPCs activation and increased hepatocyte differentiation.⁵⁹ Nevertheless it cannot be concluded from these studies that matrix deposition and HPCs proliferation/expansion are entirely mutually exclusive.

Hepatic progenitor cells (HPCs) and cancer

HPCs are activated in a wide variety of chronic

human liver diseases such as chronic viral hepatitis and alcoholic/nonalcoholic steatohepatitis in which the risk of development of hepatocellular carcinomas (HCCs) are high.⁶⁷ In addition, immunophenotyping and histological studies report that some of the HCCs show markers of HPCs such as CK7 and CK19. Therefore, it is suggested that HPCs are implicated in the development of HCCs (HPCs differentiation arrest model).^{67,68} However, as with HPCs, there is no unique liver cancer progenitor cell marker specific to this cell population for the isolation of these cells and their consequent molecular and functional characterizations. On the other hand, recent investigations have shown that deregulation of Wnt signaling pathways can lead to the development of HCCs.⁶⁹ Overexpression of Notch can result in the development of cholangiocarcinomas from hepatocytes which is similar to Notch activation in differentiation of HPCs toward cholangiocytes.^{70,71} Accurate, detailed double and triple immunostaining and confocal labeling is needed to clearly identify and label positive HPCs after which serial transplantation capability can be demonstrated in these cells,⁶⁸ although the identification of specific gene products associated with HPCs or with HCCs remains challenging.

CONCLUSION

New insights into the HPC niche and mechanisms that govern properties of adult HPCs have several different applications. For example, the knowledge of a laminin-rich niche of HPCs and its pivotal role in maintenance of their undifferentiated proliferative state have led to the design and development of new synthetic and biologic scaffolds that have been shown to maintain the bipotential nature of these cells. This novelty has important regenerative and drug testing applications, particularly in the context of cancer stem cells where the challenge is to identify hepatic progenitor cancer cells and show their direct link to HCCs. In vitro modeling of the malignant behavior/transformation of HPCs, if any, can ultimately lead to the development of new, more efficacious modalities in the treatment of HCCs. In addition, the knowledge of complex injury-specific

interactions between different elements of the HPC niche hold great promise to target niche cells in different types of liver diseases in vivo, enhancing tremendous liver regenerative capabilities. Such insights will also enable researchers to develop new cell based therapeutic strategies for the treatment of end stage liver diseases by targeting niche cells in vitro.

CONFLICT OF INTEREST

The authors declare no conflict of interest related to this work.

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