Published in final edited form as:
Prog Mol Biol Transl Sci. 2010 ; 97: 229–249. doi:10.1016/B978-0-12-385233-5.00008-8.

Biology of the Adult Hepatic Progenitor Cell: "Ghosts in the Machine"

Houda Darwiche[☼] and **Bryon E. Petersen**[†]

[☼]Department of Pathology, Immunology, and Laboratory Medicine, University of Florida, Gainesville, Florida, USA

[†]Program in Stem Cell Biology and Regenerative Medicine, College of Medicine, University of Florida, Gainesville, Florida, USA

Abstract

This chapter reviews some of the basic biological principles governing adult progenitor cells of the liver and the mechanisms by which they operate. If scientists were better able to understand the conditions that govern stem cell mechanics in the liver, it may be possible to apply that understanding in a clinical setting for use in the treatment or cure of human pathologies. This chapter gives a basic introduction to hepatic progenitor cell biology and explores what is known about progenitor cell-mediated liver regeneration. We also discuss the putative stem cell niche in the liver, as well as the signaling pathways involved in stem cell regulation. Finally, the isolation and clinical application of stem cells to human diseases is reviewed, along with the current thoughts on the relationship between stem cells and cancer.

I. Introduction: The Oval Cell

The putative liver stem cell, usually referred to as the "oval cell," is a heterogeneous population of small portal zone cells with a high nuclear/cytoplasmic ratio and an ovoid nucleus.[1] First observed in the rat liver, these cells can proliferate extensively when activated. Although the exact mechanism of their activation has yet to be determined, one condition for their stimulation is that hepatocyte proliferation must be severely impaired.

Once activated, oval cells migrate into the liver lobule, where they can differentiate into hepatocytes and biliary cells. Also during the activation process, a myriad of cell types including progenitors, mature duct cells, activated stellate cells, and fibroblasts emerge nearby; therefore, it is unclear whether oval cells that arise in different species or as a result of different insults are truly comparable. Liver progenitor cells that are observed in chronic conditions of impaired hepatocyte proliferation or differentiation in human pathologies are referred to as intermediate hepatobiliary cells; these cells bear a very strong resemblance to their more extensively studied rodent analogs. However, for the purposes of this chapter, we will use the term "oval cell" and "oval cell response" to describe liver progenitors and the cellular changes that occur upon their activation in all species.

Researchers are working on identification of these cells via the use of cellular surface markers, and it is very probable that descriptions of the different hepatic cell types will include surface marker designations in the future.[2] However, regardless of the final nomenclature, the preponderance of available data suggests that the precursors to oval cells are not mature hepatocytes.[3] In fact, the most likely location for oval cell precursors in the adult liver is the Canal of Hering, and it is widely believed that oval cells are a bipotential transient amplifying population, derived from normally quiescent stem cells that reside in this offshoot of the biliary tree.[4,5] In normal liver tissue, oval cell numbers are so limited

that they are almost beyond detection; however, oval cell activation leads to the profuse replication of these cells in the periportal regions of the liver. Morphologically, oval cells are small in size, only about 10 μm in diameter, with a large nuclear to cytoplasmic ratio and an ovoid nucleus, giving them their name.

Oval cells possess characteristics similar to ductular cells in their distinct isoenzyme profiles, expressing markers such as cytokeratin 19 (CK-19) and γ-glutamyltranspeptidase (GGT), and also have been shown to express α-fetoprotein (AFP).[6,7] Characterization of these progenitors can be achieved via the utilization of monoclonal antibodies such as OV-6 and thymus cell antigen 1 (Thy-1), at least in some species.[8–10] Oval cells are thought to be capable of generating both hepatocytes and biliary epithelial cells, thus qualifying them as bipotential progenitor cells in adult livers.[11,12] It has been shown that delta-like protein (Dlk) can be used to isolate AFP-positive cells from fetal and adult regenerating rat liver.[13] More recently, evidence has come to light showing that cells positive for epithelial cell adhesion molecule (EpCAM) are also capable of repopulating the liver after injury, and that these cells express the classic oval cell markers such as AFP, CK19, and OV-6.[14]

While most of the experimental evidence for oval cell bipotentiality has come from *in vitro* differentiation of immortal liver cell lines upon oval cell activation,[11,12] recent genetic lineage tracing studies performed *in vivo* provided evidence for the presence of a bipotential precursor during oval cell activation.[15] Periportal Foxl1-Cre marked cells yielded both hepatocytes and cholangiocytes, and although the study did not conclusively address whether a single cell could give rise to both cell types, it corroborates other data published in the literature.[16]

Murine oval cells have been found to differ from their rat and human counterparts in their expression profiles. In fact, until recently there was only one oval cell-specific antibody, referred to as A6, available for detection of mouse oval cells.[17] A panel of antibodies raised against cells present in the mouse oval cell response has shown pervasive antigenic heterogeneity among hepatic cells activated by DDC, as at least two distinct classes of proliferating cells, distinguished by antigen expression and ductal versus periductal localization, were identified.[2]

In the rat model, oval cells can be isolated based on expression of the surface marker OV6; furthermore, there are subpopulations with distinct biological behaviors.[18] Oval cells induced by carcinogen exposure in the rat have been shown to express genes typically associated with hematopoietic stem cells, such as stem cell factor (SCF) and its receptor, c-kit. During the early stages after partial hepatectomy in the 2-acetylaminofluorene/partial hepatectomy model for oval cell activation, c-kit expression is observed in AFP-positive oval cells, but SCF is expressed in both oval and stellate cells.[19] While previous research has shown that the hematopoietic marker Thy-1 is also highly expressed on rat oval cells,[10,20] recent studies suggest that Thy-1 may actually be a marker of myofibroblasts[21]; however, these data are inconclusive with regards to the immunohistochemical profiles of these cells.

The expression of hematopoietic progenitor markers in oval cells is not unique to the rat, as human liver oval cells can express CD34 as well as the bile duct marker CK19.[22] Nonhematopoietic cells expressing c-kit have been shown to be present in pediatric liver diseases such as extrahepatic biliary atresia (EHBA) and fulminant hepatic failure (FHF).[22] Finally, DDC-activated mouse oval cells have been reported to express very high levels of the hematopoietic progenitor marker Sca-1.[23] Therefore, multiple independent studies support the concept that a percentage of hepatic oval cells, but not regenerating hepatocytes, can express genes also found in hematopoietic stem cells. These findings have contributed to

the hypothesis that oval cell precursors might be found in the bone marrow, and further emphasize the heterogeneity of oval cell populations.

As a result of these conflicting reports, attempts to uncover hepatic stem cells in adult normal liver are still underway. A recent report demonstrated that normal murine liver-derived cells in the nonparenchymal cell (NPC) fraction isolated through cell aggregate formation contained bipotential progenitor cells.[24] Also, colony formation assays using rat adult liver cells revealed that 0.043% of the NPC fraction formed bipotential colonies and, furthermore, that the frequency of such colonies did not significantly differ whether the cells were obtained from normal intact liver or liver after partial hepatectomy.[25] These results suggest that hepatic stem/progenitor cells are in fact present in normal adult liver.

Protocols for the isolation, culture, and propagation of facultative hepatic progenitor cells have existed for several decades. The phenotype of hepatic progenitors remains controversial, and the relevance of hepatic progenitors after liver injury, particularly after exposure to chemicals and carcinogens and as a source of transplantable cells, is not clear. Intrahepatic progenitor cells from nondamaged fetal and adult liver offer a more clinically relevant population. Recent data show that bipotential, epithelial colony-forming cells can be successfully isolated from fetal and adult mouse liver by immunoselection for epithelial cell adhesion molecule (EpCAM).[26,27] This is an encouraging development, as EpCAM-positive cells are putative hepatic progenitors in fetal and adult human livers, which are also capable of forming colonies in culture.[28]

In our laboratory, we have found that approximately 60%of EpCAM-positive cells also express Thy-1, and of that population about 20% are positive for Dlk-1 (H. Darwiche, N. Steiger and BE Petersen, unpublished observations). Although it remains questionable if EpCAM-positive cells in mice and humans represent cells of comparable functional phenotype, inferences made from rodent studies using cells of the same immunohistochemical marker could be extrapolated to facilitate the production of clinically usable material.

II. Canals of Hering: The Putative Oval Cell Niche

A stem cell niche is described as the cellular and extracellular microenvironment which supports stem cells and contributes to sustain self-renewal.[29] In fact, the interaction of stem cells with other cell types in this microenvironment is thought to be essential for regulating stem cell maintenance, as a plethora of different types of signaling and adhesion molecules exist within the niche. Indeed, the stem cell niche has been attributed functions such as (i) the maintenance of stem cell quiescence and (ii) the provision of proliferation or differentiation-inducing signals when numerous progenitor cells are required to give rise to transit-amplifying cells committed to producing mature cell lineages.[29–32]

In the stem cell niches of many organs, that is, bone marrow, intestine, and brain, signaling pathways such as Wnt, Notch, and Hedgehog function in concert. The presence of these signals is thought to regulate the maintenance of stem cell quiescence, to control proliferation, and to govern cell fate decisions. In particular, Notch and Wnt signals overlap to control the detailed pattern of cell fate choices as stem cells divide and give rise to differentiated progeny.[32]

The hepatic stem cell niche is thought to exist in the biliary tree at the level of the Canals of Hering. As is the case with niches in other organs, it is composed of numerous cell types, including portal myofibroblasts, hepatic stellate cells, endothelial cells, hepatocytes, cholangiocytes, Kupffer cells, pit cells, and immune cells. Any of these cell types can

interact and cross-talk with oval cells, thereby influencing their proliferative and differentiative capacities from within the niche itself.[33]

Wnt secretion, whether autocrine or paracrine, is clearly involved in the stem cell response seen in mice, rodents, and humans.[34–36] Also, Hedgehog signaling is essential for survival of progenitor cells, as evidenced by the activation of the specific receptor Patched (PTC) expressed by progenitors.[37] Furthermore, inflammatory cells present in response to injury are responsible for producing a range of cytokines and chemokines that could potentially influence the progenitor cell response to liver injury.[33] For example, T cells express a tumor necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK) which can stimulate progenitor cell proliferation by engaging its specific receptor, Fn14.[38] Hepatic progenitors can also potentially be stimulated by other various components of the inflammatory response, such as lymphotoxin-β, IFNγ, TNF-α, and even histamine.[38,39] Furthermore, it has been proposed that resistance to transforming growth factor (TGF)-β, which under normal circumstances works to decrease proliferative capacity, allows progenitors to divide under conditions that would normally inhibit hepatocyte proliferation.[40]

Recent research has also clarified the relationship between progenitor cells and hepatic stellate cells.[41–44] Stellate cells serve as a key source of growth factors, such as TGF-α, hepatocyte growth factor (HGF), and acidic fibroblast growth factor (aFGF). Because of the wide panel of molecular intermediates they may produce and secrete, particularly after their sustained activation in a disease state, hepatic stellate cells are definitely involved in various liver pathologies, in addition to their well-known role in fibrosis and extracellular matrix remodeling. Specifically, there appears to be an association between the degree of hepatic injury, progenitor cell expansion, and fibrosis in many human liver diseases such as viral hepatitis, steatohepatitis, and primary biliary cirrhosis.

It is possible that stellate cells can be activated directly by hepatic progenitors or that activated stellate cells could promote progenitor cell expansion and drive their differentiation into mature lineages. It is also feasible that activation of both cell types could occur separately but simultaneously, through similar mediators or stimuli.[45] A recent study using murine models indicates that hepatic progenitors need a support matrix, thought to be provided by stellate cells or myofibroblasts, to aid in migration and anchorage, as well as progenitor cell differentiation and repopulation of the damaged liver.[46]

Moreover, studies performed in our laboratory have demonstrated that when activation of stellate cells was inhibited, the oval cell response to 2-aceytlaminofluorene (2AAF)–PH treatment was drastically reduced, as determined by expression levels of OV-6 and AFP.[44] These observations reinforce the theory that niche compounds play a key role in progenitor proliferation and differentiation. Further studies are necessary in order to delineate the mechanisms for cross talk between progenitors, stellate cells, and the extracellular matrix in human liver diseases.

Perhaps most significantly, there is little known about the functions of specific growth factors and hormones present in the stem cell niche during the expansion of hepatic progenitors. For example, numerous previous studies have demonstrated that cholangiocyte proliferation during liver injury can be regulated by several factors such as steroid hormones (i.e., estrogens, progesterone), growth factors (i.e., insulin-like growth factor 1, IGF1; vascular endothelial growth factor, VEGF), and neurotransmitters (i.e., acetylcholine).[47–49] Moreover, cholangiocytes have been shown to secrete both autocrine and paracrine factors that regulate proliferation as well as activate fibrogenic response of portal myofibroblasts and hepatic stellate cells. Studies in our laboratory have shown that loss of such factors as SDF-1 and IGFBP-3, which are produced by stellate cells, can lead to loss of activation of

the oval cell compartment.[50,51] Furthermore, cholangiocytes might be able to undergo epithelial–mesenchymal transition and thereby increase the number of fibrogenic cells in the portal triad.[47]

One of the key factors in determining the developmental potential of a stem cell is its environment. It has been demonstrated in a rat model that hematopoietic stem cells can give rise to oval cells, which retain hematopoietic markers such as Thy-1 but also gain expression of liver-specific markers such as AFP. Hematopoietic stem cells obtained from adult peripheral blood retain a tremendous developmental plasticity.[7,10,23] Taken together, this indicates that hematopoietic stem cells and oval cells may share a common developmental origin or may even arise directly from the same cell.

The role of these substances in the regulation of the stem cell niche, as well as possible cross talk between the progenitors and cholangiocytes, need to be further elucidated. Understanding of the signaling pathways that regulate progenitor cell proliferation during the progression of liver diseases and/or injury could open the possibility for the development of therapeutic strategies. The hepatic progenitor compartment could represent an important target for new therapeutic approaches to liver diseases through a correct pharmacological modulation. Regardless of origin, however, all stem cells execute their developmental programs by regulating gene expression. Determining which signals are responsible for such processes as induction of differentiation, self-renewal and/or maintenance of pluripotentiality will lead to a better understanding of the biology of oval cells and their role in the liver.

III. Oval Cell-Mediated Liver Regeneration

The liver is one of the few organs that are known to be capable of rapid regeneration. This process usually takes place in response to liver injury resulting from surgical resection or exposure to destructive agents. Although this ability is referred to as regeneration, what is actually occurring is a process of compensatory growth or hyperplasia. Rather than the resected liver tissue growing back, the portions of the liver remaining, for example, after a two-thirds partial hepatectomy, increase in size to make up for the loss of tissue.[52] This expansion continues until the mass of the “regenerated” liver reaches within approximately 10% of the original liver mass. Completion of this process usually takes approximately 2 weeks in rodents, but can take up to 2 months in humans. While the original mass of the liver may be restored, the original anatomical form of the liver is not, indicating that regenerative growth after partial hepatectomy is a closely regulated process synergistic with the body’s demand for liver functionality, independent of its anatomical form.[53]

There have been many studies investigating the possibility that hepatic progenitor cells can modulate the regeneration process following partial hepatectomy; however, no experimental evidence exists to confirm this hypothesis. In fact, based on data that has been published over the last century, hepatocytes irrefutably are the cells responsible for regeneration after partial hepatectomy.[53] However, in the last decade, a clear distinction has been made between regeneration after partial hepatectomy alone and regeneration after partial hepatectomy combined with inhibition of hepatocyte proliferation. Many studies have shown that oval cells can be activated by suppressing mature hepatocyte proliferation. For example, administration of 2-acetylaminofluorine blocks hepatocyte proliferation by interfering with their ability to divide properly. When followed by partial hepatectomy or CCl_4 treatment, the liver mounts an active regenerative response. As the mature hepatocytes are unable to divide, the oval cell compartment is activated and oval cells become the dominant cellular source for regeneration.

Oval cell replication is important in many models of liver injury including carcinogenesis induced by azo dyes and choline-deficient/ethionine-containing diets (CDE diet), injury caused by D-galactosamine, and injury produced by 2AAF, dipin, or CCl_4 treatment in combination with PH.[54,55] In fact, oval cells may constitute more than 70% of the liver mass during regeneration caused by administration of 2AAF/PH; it is thought that the cells form a transit-amplifying compartment that includes undifferentiated progenitors, medially differentiated transit cells, and newly differentiated hepatocytes.[53] Recent work has also indicated that oval cells may have a role in carcinogenesis. The mechanisms controlling progenitor activation are under intense investigation, and whilst a number of inflammatory cytokines and growth factors have been described in rodent models, recent studies have indicated an important role for Hedgehog signaling in progenitor activation in alcoholic steatohepatitis in mice and humans.[56,57]

Multiple growth factors (i.e., TGF-α, EGF, HGF, and SDF-1) can stimulate oval cell growth,[53] and in fact, oval cells can produce and respond to several of their own cytokines. NPCs, such as stellate cells, can enhance oval cell growth and differentiation via secretion of growth factors, and also by direct cell–cell interactions.[16] Oval cells are similar to hepatocytes in that both require growth factors for cell cycle progression and both cell types also require a “priming” process in order to respond to these stimuli[58]; however, global gene expression patterns, as well as expression of specific markers, are different between the two cell types. For example, expression of genes related to the interferon-γ signaling network is greatly increased in oval cells when they are mediating liver regeneration, but these genes are not activated in the regenerating liver after partial hepatectomy alone.[58]

IV. Molecular Regulation of the Oval Cell Response

There are four distinct components of the oval cell response: activation, proliferation, migration, and differentiation.[59] Some of the factors involved in regulation of the oval cell response have been identified through genetic studies using murine models, as well as by *in vitro* studies of oval cell lines in culture. As previously indicated, there are a multitude of epithelial, hematopoietic, and mesenchymal cell types present during hepatic progenitor activation, and therefore in most cases it is still unclear which cells transmit and which receive important molecular signals. This is made even more complicated by the fact that the signals impacting these processes can do so either directly (by acting on the proliferating epithelial cells), or indirectly by signaling to a nonepithelial cell type which can, in turn, act on oval cells.

In addition to describing cellular topology, future studies must examine the temporal sequence of signaling events. As previously stated, the involvement of interferon-γ in oval cell activation appears to be critical. Mice that are deficient for interferon-γ have an attenuated oval cell response, but increased liver regeneration potential after partial hepatectomy.[60,61] On the contrary, the addition of interferon-γ to TNF and lipopolysaccharide (LPS), both growth factors known to be present during liver growth, leads to an arrest in proliferation of cultured hepatocytes but appears to promote oval cell activation.[62]

Another pathway has been associated with oval cell activation even in the absence of injury. Transgenic mice expressing TWEAK in hepatocytes actually exhibit a spontaneous oval cell response. Furthermore, the resulting proliferating ducts also express the TWEAK receptor Fn14.[38] TWEAK-Fn14 signaling in the liver appears to be progenitor specific, as Fn14 is absent from hepatocytes, and hepatocyte proliferation occurs at normal levels despite TWEAK expression.

Recent evidence has indicated an important role for the hedgehog signaling pathway in liver progenitor activation, particularly in alcoholic fatty liver disease. In mice and humans, alcoholic steatohepatitis induces proliferation of immature duct cells in the portal tracts.[56,57] This is accompanied by transcriptional upregulation of Hedgehog family members (*Sonic hedgehog* and *Indian hedgehog*) and expression of Hedgehog target genes in the proliferating ducts as well as the adjacent myofibroblasts.[56] Hedgehog inhibitors can impair progenitor proliferation.[57] Indirect evidence suggests that activation of hedgehog signaling might be downstream of TGF-β (i.e., the inflammatory response).

It has also been demonstrated that TGF-β can induce differential regulation of mature hepatocytes and immature oval cells. TGF-β is an activator of apoptosis in mature hepatocytes but does not affect oval cells or biliary epithelia.[56] Thus, one of the emerging themes of hepatic progenitor activation is that inflammatory cytokines have opposite effects on hepatocytes and progenitors. Another molecule involved in the oval cell response is interleukin-6, which appears to regulate progenitor activation and proliferation. Mice lacking interleukin-6 and given a choline-deficient diet have a significantly reduced level of progenitor activation.[63] The absence of TNF-α has also been shown to impair oval cell activation,[64] although mice deficient in the TNF receptor seem to have a normal oval cell response.

Acting as a downstream effector molecule for TGF-β is connective tissue growth factor, or CTGF. CTGF is a secretory protein that is known to act on many cell types to stimulate fibrotic processes, promote fibroblast proliferation, migration, and the production of extracellular matrix.[65]We found that CTGF upregulation occurred concomitantly with increases in TGF-β. Furthermore, inhibition of CTGF was associated with a significant decrease in oval cell proliferation as well as a lower level of expression of AFP, showing that CTGF induction is essential for a robust oval cell response following 2AAF–PH in the rat.[65,66]

Among the large variety of cytokines secreted by stellate cells, TGF-β likely represents that with the highest impact on collagen overproduction and accumulation in liver fibrosis. But, liver fibrosis is also characterized by important changes in extracellular matrix remodeling, a process that is critical for proper regeneration of the liver lobule. Quiescent and early activated stellate cells are involved in extracellular matrix degradation by producing metalloproteinases, in other words, by exhibiting a matrix-degrading phenotype. It is well known that animals deficient for critical metalloproteinases have defective regeneration capabilities. During perpetuation of injury, aside from enhanced degradation of normal liver matrix, stellate cells overproduce tissue inhibitors of fibrillary collagen degradation, namely tissue inhibitor of metalloproteinase-1, -2 (TIMPs) that maintain fibrosis.[67]

V. From Bench to Bedside: Isolation of Hepatic Progenitors and Their Use in Medicine

The existence of a hepatic stem cell compartment gives rise to expectations regarding its clinical applications. With the increasing interest in characterizing oval cells with respect to their origins, questions arise regarding the mechanism of their recruitment and their differentiation potential. Given the biological properties of stem/progenitor cells, isolation and transplantation of hepatic progenitors could represent a novel source of clinically applicable therapies for many human liver diseases, ranging from congenital metabolic diseases to end-stage cirrhosis to hepatocellular carcinoma (HCC).[68–70]

The many types of hepatic progenitor cells offer a cellular basis of stem cell therapy for the treatment of liver disease. Stem cell-derived hepatocytes can be used for drug screening and

disease modeling, human bioartificial liver construction, and potentially transplantation therapy. The ability to use human cell types in the pharmaceutical industry not only allows expedition of novel human drug development but also takes into account variability in drug metabolism because of cytochrome P450 polymorphisms. Phase I trials employing a bioartificial liver device have already shown promise as patients exhibited improvement in neurological state and hemodynamics, but these are currently very limited by the use of xenogeneic materials. These devices permit some restoration of liver metabolic function and are likely to act as a bridge to liver transplantation in patients with advanced or end-stage liver failure.[71,72]

There may be several advantages to using stem/progenitor cells as opposed to mature hepatocytes for transplantation. Numerous studies utilizing rat models of primary hepatocyte transplantation revealed that transplantation leads to influential donor chimerism that can rescue animals from lethal hepatic failure.[73–75] Hepatocyte transplantation has been performed formore than 15 years in humans with varied degrees of success.[76–79] The main problems with using hepatocytes for cell-based therapies include the limited number of donor organs as well as the fact that the cells being used are usually not in optimal condition. Attempts of immortalization of primary hepatocytes are currently only partially successful as proliferation and hepatocellular function appear mutually exclusive.[80] The recently developed human hepatoma cell line HepaRG[81] retains some attributes of primary hepatocytes, but nonetheless remains a cancer cell line, and therefore is restricted in its application to *in vitro* modeling of human drug toxicity and incorporation in extracorporeal support devices.

In two clinical trials, hepatocyte transplantation was shown to be effective for the treatment of liver-based congenital metabolic disorders. Transplantation of allogeneic hepatocytes into the liver of a patient with Crigler–Najjar syndrome was shown to be successful in some measure, as the patient survived for 11 months and his hyperbilirubinemia was partially corrected.[82] Furthermore, it has been reported that transplantation of cryopreserved hepatocytes into patients with inherited factor VII deficiency led to a gradual reduction in their requirement for recombinant factor VIIa until, after 8–10 weeks, patients were receiving only about 20% of their original dose of factor VIIa.[83]

The ability of hepatocyte transplantation to contribute to the long-term rescue of hepatic metabolic disorders remains unclear. Moreover, *in vitro* expansion of mature hepatocytes is not feasible because long-term cultivation of hepatocytes results in hypofunction of hepatocytic metabolism. Therefore, transplantation of mature hepatocytes in the setting of acute liver failure should only be viewed as a bridge to liver transplantation, as it can temporarily improve the metabolic status of extremely ill patients.[76,77]

Transplantation of stem/progenitor cells could make for better long-term repopulation and sustained metabolic activity, due to the constant propagation of new hepatocytes; however, numerous issues remain to be addressed before progenitor cells can be used in clinical practice. Perhaps most significantly, isolation of hepatic progenitor cells from human material has proven to be very difficult. Although hepatic progenitor cells are a distinct population based on the expression of several markers, indisputable isolation of a pure fraction has been a major obstacle in liver progenitor cell research.[84] Hepatic progenitors represent a dynamic cellular compartment that continuously changes morphology, phenotype, and cell surface marker expression, making it difficult to isolate and maintain uniform progenitor populations. Moreover, many of the described markers are not completely specific, thereby limiting their use for isolation of viable cells.[85,86]

Two different clinically relevant populations of hepatic progenitors have recently been isolated from nondamaged fetal or adult human livers using epithelial cell adhesion molecule (EpCAM) or Thy-1 as markers.[87,88] It was demonstrated that purified $EpCAM^+$ cells from fetal or postnatal livers are able to engraft the livers of immunodeficient adult mice (with or without prior injury) and to give rise to mature human liver parenchymal cells. The efficiency of the isolation of $EpCAM^+$ cells from human livers, the demonstration of their pluripotency, and the evidence that they can generate mature liver tissue after transplantation are encouraging for clinical use.[87] Similar results have been obtained by Weiss and colleagues through the isolation of $Thy\text{-}1^+$ cells from adult human livers and their transplantation in a mouse model.[88] It should be noted that the engraftment efficiencies in these studies using these surface markers for isolation are quite low, approximately 5% for Thy-1-positive cells and 2% for EpCAM-positive cells.

The exact phenotype of the isolated hepatic progenitor cells remains unclear, which presents a problem for their isolation and subsequent transplantation. Differential expression of AFP was thought to be a potential mechanism for distinguishing between adult progenitors and hepatoblasts, but this is controversial as this marker is shared by hepatic progenitor cells. Moreover, Thy-1, initially introduced as a marker of progenitor cells, is not expressed by the progenitors themselves but by the neighboring stellate cells or progenitors of the mesenchymal lineage.[84] Until scientists are better able to determine the relationships between differentiation state and expression of these markers, as well as the overlap between surface marker expression, transplantation efficiency based on cell sorting with any surface marker as a basis will remain relatively low.

Activation of progenitor cells has been demonstrated in several disorders of the liver; this is not surprising, as the vast majority of human liver diseases are characterized by various levels of damage, loss, and impaired regeneration of mature hepatocytes and/or biliary epithelial cells. In fact, there are only a handful of human liver diseases in which activation of hepatic progenitors does not occur. For example, there is no documentation of progenitor cells after acute and complete extrahepatic biliary obstruction, most commonly caused by bile stones. The ductular reaction seen in these cases is the result of proliferation of mature biliary epithelial cells, and is thereby fundamentally different than what is seen in other liver diseases, where progenitor cell proliferation/differentiation is known to occur.[89–91]

Following acute massive necrosis of hepatocytes, there is a massive induction of proliferation in the progenitor compartment in the liver. In addition, a significant progenitor activation has been exhibited in the advanced stages of a majority of chronic human liver diseases.[92–94] A threshold of a 50% loss of hepatocytes, together with a significant decrease in proliferation of the remaining mature hepatocytes, is required for an extensive activation of hepatic progenitors. Under these conditions, activation of progenitor cell proliferation occurs within 1 week, and their differentiation into mature hepatocytes and biliary epithelial cells begins within 2 weeks. Furthermore, there is a positive correlation between progenitor cell activation and clinical parameters of disease severity, such as the Model for End Stage Liver Disease (MELD) score, as well as an influence on the prognosis of the pathological condition in question.[94]

Activation of hepatic progenitor cells is also associated with inhibition of hepatocyte replication in long-term chronic liver diseases. In the majority of chronic liver pathologies, progenitor cell proliferation is determined by the extent of fibrosis.[95] Activation and localization of progenitors can also correlate with the localization of inflammatory infiltrate, as seen in chronic viral hepatitis. In cases of moderate-to-severe lobular inflammation, progenitor cells were haphazardly dispersed throughout the parenchyma and surrounded by intermediate hepatocyte-like cells, suggestive of progenitor migration away from the

periportal regions and into the lobular parenchyma, where their differentiation into mature lineages finally occurs.[95]

Another avenue for the clinical use of stem cells presents itself with the advent of bioengineered organs and tissues. The development of tissue scaffolds for the seeding of stem cells has immense potential, but until recently the clinical applications of these scaffolds have been limited. The most clinically relevant engineered tissue has been the cartilage.[96] The injection of tissue-engineered cartilage into osteoarthritic as well as nonarthritic knees and other joints were reported to have greatly improved joint stability and motion; however, further long-term studies must be made to determine the stability and long-term effects of these grafts.[97] The growth of autologous cells on decellularized human heart valves and subsequent implantation of these valves has also been clinically worthwhile.[98] Another engineered tissue that has been evaluated in a clinical study is the bladder. Here, patients received bladders engineered with autologous urothelial and muscle cells; these patients demonstrated clinical benefits from the implanted tissue for up to 5 years postimplantation.[99] The successes seen with bladders, heart valves, and cartilage demonstrate the possibilities for the clinical use of stem cells.

However, in order for stem/progenitor cells to make the jump “from bench to bedside,” several obstacles will need to be overcome. For example, the maintenance of progenitors in culture has surfaced as a major hurdle. Developing this technique is critical because any therapeutic use of these cells will require the expansion of a small population of cells *ex vivo* prior to transplantation. The second impediment has been selectively directing the differentiation of oval cells down a hepatocyte or cholangiocyte committed pathway as needed. It is likely that the signals mediating these differentiation processes will be sufficiently complex as to disallow their exact replication *in vitro*. Factors governing oval cell differentiation may include contact dependence, extracellular matrix contact, or exposure to soluble signaling proteins in the serum. To circumvent the need for overcoming this second hurdle, cells could be transplanted in their precursor form with the natural microenvironment of the liver dictating their differentiation, but this approach has yet to be validated.

Finally, the suitability of hepatic progenitor cells for hepatic repopulation in the clinical setting still needs to be assessed. In fact, a major obstacle in the transplantation of hepatic progenitor cells in human patients and in their application in clinical trials is represented by the production of these cells in the respect of strict guidelines and laws. As recently suggested, the standardization of the processes from basic research to the development of clinical trials will be essential to ensure cell-based therapies for chronic liver diseases.[70] This process will include the use of standardized methodologies to characterize the cells to be grafted using a common and validated set of phenotypic markers, as well as a standard set of *in vitro* functional studies. Animal models should be used for preclinical assessment of the repopulation ability of hepatic progenitor cells or their capability to differentiate into a progeny able to functionally replace liver cell loss. Finally, clinical trials using hepatic stem/progenitor cells or their differentiated progeny should be planned, with the development of standardized protocols for validated procedures to define the nature of cells, the patients enrolled, the transplantation procedure and pretreatment of the liver, as well as standard data collection regarding efficacy, and possible side effects.[70]

VI. Hepatic Stem Cells and Liver Cancer

It has been suggested that human hepatic stem cells may be able to give rise to HCC as well as cholangiocarcinomas (CCC).[5,76] In these models, a periportal population of small “primitive” oval epithelial cells proliferate either in association with or before hepatocyte

multiplication. Multiple studies have demonstrated that a substantial number of HCCs contain a progenitor cell population. Furthermore, detailed immunophenotyping of HCCs indicated that 28–50% of HCCs express markers of progenitor and biliary epithelial cells such as CK7 and CK19. These tumors also consist of cells that have an intermediate phenotype between progenitors and mature hepatocytes. In fact, HCCs that express hepatocyte and biliary cell markers such as albumin, CK7, and CK19 carry a significantly poorer prognosis and higher recurrence after surgical resection.[77] Nevertheless, these coordinative studies have not yet proven that HCC is derived from or driven by oval cells.

One of the goals in this type of research is the identification of markers specific to cancer stem cells in the liver as well as other organs. However, the problem with this lies with the ability to isolate these cells from normal liver or from HCC samples, and the subsequent functional and molecular characterization. Strict double and triple immunohistochemical and confocal labeling is necessary to identify marker-positive stem cells. Such rigorous labeling, however, has not been consistently adhered to. Furthermore, definitive experiments showing serial transplantability of marker-positive-cells has yet to be demonstrated. Moreover, it is difficult to identify gene products that are specifically associated with putative progenitors or with HCC. The challenge lies in defining the markers specific to these cells at varying stages of differentiation, in HCC, and the elusive liver cancer stem cell.[100]

The observation that many reagents that activate oval cells in rodents are carcinogenic supports the idea that alterations in the frequency or proliferative capacity of oval cells can contribute to liver cancer. In mice and rats, some HCCs and CCCs have been proposed to be of oval cell origin.[101,102] The detection of c-myc, Ras, and AFP after oval cell activation is consistent with this hypothesis.[103,104] In humans, oval cells/intermediate hepatobiliary cells have also been observed in samples from patients with liver cancer or chronic diseases.[92] HCCs sometimes express markers of the bile duct lineage, such as CK7 and CK19, despite a predominantly hepatocytic phenotype.[105]

Recent studies have suggested CK19 as a prognostic marker for early neoplastic lesions. These studies are also indicative of a progenitor cell derivation for HCC in a rat RH (resistant hepatocyte) model. CCCs, conversely, have been observed to yield intermediate hepatocytes.[78] These tumors often incorporate oval cell activation in the form of a ductular reaction. Thus, progenitor cells might have a role in the emergence and maintenance of some liver tumors. Elucidating the molecular processes of oval cell activation may lead to novel strategies to be employed in the field of cancer therapeutics.

VII. Conclusions

Currently, liver progenitor cells represent a key area in the development of new therapeutic strategies for liver disease. These cells represent a “stand-by” stem cell compartment that can be activated and driven to differentiate into mature lineages through modulation of cytokines and growth factors. Signals that govern progenitor cell proliferation and differentiation into mature parenchymal liver cells are still not clarified. Likewise, the role of the niche in the modulation of the resident progenitor cell compartment remains to be elucidated. Moreover, the shortage of liver donors and the well-known limits of liver transplantation underscore the need and the possibility of applying a stem cell therapy to end-stage chronic hepatic diseases and to acute massive liver injury.

It is pivotal to elucidate the underlying disease pathophysiology, identify key developmental factors, and optimize tissue culture environments if the true potential of stem cell derivatives is to be realized. Future studies should observe guidelines recently published by a collaborative workforce to standardize progenitor cell definition, preclinical animal models,

and endpoint parameters. One of the next essential steps will be to define the parameters of safety and efficacy that need to be attained before the possible application of cellular liver therapies.[70]

REFERENCES

1. Shinozuka H, Lombardi B, Sell S. Early histological and functional alterations of ethionine liver carcinogenesis in rats fed a choline-deficient diet. Cancer Res. 1978; 38:1092–1098. [PubMed: 76508]
2. Dorrell C, Erker L, Lanxon-Cookson KM. Surface markers for the murine oval cell response. Hepatology. 2008; 48:1282–1291. [PubMed: 18726953]
3. Wang X, foster M, Al-Dhalimy M. The origin and liver repopulating capacity of murine oval cells. Proc Natl Acad Sci USA. 2003; 100:11881–11888. [PubMed: 12902545]
4. Alison MR, Golding M, Sarraf CE. Liver damage in the rat induces hepatocyte stem cells from biliary epithelial cells. Gastroenterology. 1996; 110:1182–1190. [PubMed: 8613008]
5. Theise ND, Saxena R, Portmann BC. The canals of Hering and hepatic stem cells in humans. Hepatology. 1978; 38:1092–1098.
6. Fausto N, Campbell JS. The role of hepatocytes and oval cells in liver regeneration and repopulation. Mech Dev. 2003; 120:117–130. [PubMed: 12490302]
7. Petersen BE, Bowen WC, Patrene KD, Mars WM, Sullivan AK, Murase N, et al. Bone marrow as a potential source of hepatic oval cells. Science. 1999; 284:1168–1170. [PubMed: 10325227]
8. Faris RA, Monfils BA, Dunsford HA, Hixson DC. Antigenic relationship between oval cells and a subpopulation of hepatic foci, nodules and carcinomas induced by the resistant hepatocyte model system. Cancer Res. 1991; 51:1308–1317. [PubMed: 1997169]
9. Crosby HA, Nijjar SS, de Goyet JD, Kelly DA, Strain AJ. Progenitor cells of the biliary epithelial cell lineage. Semin Dev Biol. 2002; 13:397–403.
10. Petersen BE, Goff JP, Greenberger JS, Michalopoulos GK. Hepatic oval cells express the hematopoietic stem cell marker Thy-1 in the rat. Hepatology. 1998; 27:433–445. [PubMed: 9462642]
11. Spagnoli FM, Amicone L, Tripodi M. Identification of a bipotential precursor cell in hepatic cell lines derived from transgenic mice expressing cyto-Met in the liver. J Cell Biol. 1998; 143:1101–1112. [PubMed: 9817765]
12. Strick-Marchand H, Morosan S, Charneau P, Kremsdorf D, Weiss MC. Bipotential mouse embryonic liver stem cell lines contribute to liver regeneration and differentiate as bile ducts and hepatocytes. Proc Natl Acad Sci USA. 2004; 101:8360–8365. [PubMed: 15155906]
13. Jensen CH, Jauho EI, Santoni-Rugiu E, Holmskov U, Teisner B, Tygstrup N, et al. Transit-amplifying ductular (oval) cells and their hepatocytic progeny are characterized by a novel and distinctive expression of delta-like protein/preadipocyte factor 1/fetal antigen 1. Am J Pathol. 2004; 164:1347–1359. [PubMed: 15039222]
14. Yovchev MI, Grozdanov PN, Zhou H. Identification of adult hepatic progenitor cells capable of repopulating injured rat liver. Hepatology. 2008; 47:636–647. [PubMed: 18023068]
15. Sackett SD, Li Z, Hurtt R. Foxl1 is a marker of bipotential hepatic progenitor cells in mice. Hepatology. 2009; 49:920–929. [PubMed: 19105206]
16. Paku S, Schnur J, Nagy P, Thorgeirsson SS. Origin and structural evolution of the early proliferating oval cells in rat liver. Am J Pathol. 2001; 158:1313–1323. [PubMed: 11290549]
17. Faktor VM, Engel'gardt NV, Iazova AK. Common antigens of oval cells and cholangiocytes in the mouse: their detection by using monoclonal antibodies. Ontogenez. 1990; 21:625–632. [PubMed: 2095484]
18. Sanchez A, Factor VM, Schroeder IS. Activation of NF-kappaB and STAT3 in rat oval cells during 2-acetylaminofluorene/partial hepatectomy-induced liver regeneration. Hepatology. 2004; 39:376–385. [PubMed: 14767990]
19. Fujio K, Evarts RP, Hu Z. Expression of stem cell factor and its receptor, c-kit, during liver regeneration from putative stem cells in adult rat. Lab Invest. 1994; 70:511–516. [PubMed: 7513770]

20. Spangrude GJ, Heimfeld S, Weissman IL. Purification and characterization of mouse hematopoietic stem cells. Science. 1988; 241:58–62. [PubMed: 2898810]
21. Dezso K, Jelnes P, Laszlo V. Thy-1 is expressed in hepatic myofibroblasts and not oval cells in stem cell-mediated liver regeneration. Am J Pathol. 2007; 17:1529–1537. [PubMed: 17884967]
22. Baumann U, Crosby HA, Ramani P. Expression of the stem cell factor receptor c-kit in normal and diseased pediatric liver: identification of a human hepatic progenitor cell? Hepatology. 1999; 30:112–117. [PubMed: 10385646]
23. Petersen BE, Grossbard B, Hatch H. Mouse A6-positive hepatic oval cells also express several hematopoietic stem cell markers. Hepatology. 2003; 37:632–640. [PubMed: 12601361]
24. Azuma H, Hirose T, Fujii H, Oe S, Yasuchika K, Fujikawa T. Enrichment of hepatic progenitor cells from adult mouse liver. Hepatology. 2003; 37:1385–1394. [PubMed: 12774018]
25. Yamamoto H, Togo S, Zheng YW, Kubota T, Taniguchi H, Shimada H. Adult rat hepatic bipotent progenitor cells remain dormant even after extensive hepatectomy. Wound Repair Regen. 2007; 15:422–429. [PubMed: 17537130]
26. Tanaka M, Okabe M, Suzuki K, Kamiya Y, Tsukahara Y, Saito S, et al. Mouse hepatoblasts at distinct developmental stages are characterized by expression of EpCAM and DLK1: drastic change of EpCAM expression during liver development. Mech Dev. 2009; 126:665–676. [PubMed: 19527784]
27. Okabe M, Tsukahara Y, Tanaka M, Suzuki K, Saito S, Kamiya Y, et al. Potential hepatic stem cells reside in EpCAM+ cells of normal and injured mouse liver. Development. 2009; 136:1951–1960. [PubMed: 19429791]
28. Schmelzer E, Zhang L, Bruce A, Wauthier E, Ludlow J, yao HL, et al. Human hepatic stem cells from fetal and postnatal donors. J Exp Med. 2007; 204:1973–1987. [PubMed: 17664288]
29. Moore KA, Lemischka IR. Stem cells and their niches. Science. 2006; 311:1880–1885. [PubMed: 16574858]
30. Zhang L, Theise N, Chua M. The stem cell niche of human livers: symmetry between development and regeneration. Hepatology. 2008; 48:1598–1607. [PubMed: 18972441]
31. Naveiras O, Daley GQ. Stem cells and their nice: a matter of fate. Cell Mol Life Sci. 2006; 63:760–766. [PubMed: 16568245]
32. Crosnier C, Stamataki D, Lewis J. Organizing cell renewal in the intestine: stem cells, signals and combinatorial control. Nat Rev Genet. 2006; 7:349–359. [PubMed: 16619050]
33. Alison MR, Islam S, Lim S. Stem cells in liver regeneration, fibrosis and cancer: the good, the bad, and the ugly. J Pathol. 2009; 217:282–298. [PubMed: 18991329]
34. Yang W, Yan HX, Chen L. Wnt/beta-catenin signaling contributes to activation of normal and tumorigenic liver progenitor cells. Cancer Res. 2008; 68:4287–4295. [PubMed: 18519688]
35. Hu M, Kurobe M, Jeong YJ. Wnt/beta-catenin signaling in murine hepatic transit amplifying progenitor cells. Gastroenterology. 2007; 133:1579–1591. [PubMed: 17983805]
36. Apte U, Thompson MD, Cui S. Wnt/beta-catenin signaling mediates oval cell response in rodents. Hepatology. 2008; 47:288–295. [PubMed: 17929301]
37. Sicklick JK, Li YX, Melhem A. Hedgehog signaling maintains resident hepatic progenitors throughout life. Am J Physiol Gastrointest Liver Physiol. 2006; 290:G859–G870. [PubMed: 16322088]
38. Jakubowski A, Ambrose C, Parr M. TWEAK induces liver progenitor cell proliferation. J Clin Invest. 2005; 115:2330–2340. [PubMed: 16110324]
39. Francis H, Glaser S, Demorrow S. Small mouse cholangiocytes proliferate in response to H1 histamine receptor stimulation by activation of the IP3/CaMK I/CREB pathway. Am J Physiol Cell Physiol. 2008; 295:C499–C513. [PubMed: 18508907]
40. Nguyen LN, Furuya MH, Wolfraim LA. Transforming growth factor-beta differentially regulates oval cell and hepatocyte proliferation. Hepatology. 2007; 45:31–41. [PubMed: 17187411]
41. Santoni-Rugiu E, Jelnes P, Thorgeirsson SS. Progenitor cells in liver regeneration: molecular responses controlling their activation and expansion. APMIS. 2005; 113:876–902. [PubMed: 16480456]

42. Kuwahara R, Kofman AV, Landis CS. The hepatic stem cell niche: identification by label-retaining cell assay. Hepatology. 2008; 47:1994–2002. [PubMed: 18454509]
43. Sanchez-Munoz D, Castellano-Megias VM, Romero-Gomez M. Expression of Bcl-2 in ductular proliferation is related to periportal hepatic stellate cell activation and fibrosis progression in patients with autoimmune cholestasis. Dig Liver Dis. 2007; 39:262–266. [PubMed: 17270507]
44. Pintilie DG, Shupe TD, Oh SH, Salganik SV, Darwiche H, Petersen BE. Hepatic stellate cells' involvement in progenitor-mediated liver regeneration. Lab Invest. 2010 E-pub ahead of print.
45. Svegliati-Baroni G, De Minicis S, Marzioni M. Hepatic fibrogenesis in response to chronic liver injury: novel insights on the role of cell-to-cell interaction and transition. Liver Int. 2008; 28:1052–1064. [PubMed: 18783548]
46. Van Hul N, Abarca J, Sempoux C. Relation between liver progenitor cell expansion and extracellular matrix deposition in a CDE-induced murine model of chronic liver injury. Hepatology. 2009 (in press).
47. Glaser SS, Gaudio E, Miller T. Cholangiocyte proliferation and liver fibrosis. Expert Rev Mol Med. 2009; 11:e7. [PubMed: 19239726]
48. Alvaro D, Onori P, Metalli VD. Intracellular pathways mediating estrogen-induced cholangiocyte proliferation in the rat. Hepatology. 2002; 36:297–304. [PubMed: 12143037]
49. Alvaro D, Alpini G, Onori P. Effect of ovariectomy on the proliferative capacity of intrahepatic rat cholangiocytes. Gastroenterology. 2002; 123:336–344. [PubMed: 12105861]
50. Zheng D, Oh SH, Jung Y, Petersen BE. Oval cell response in 2-acetylaminofluorene/partial hepatectomy rat is attenuated by short interfering RNA targeted to stromal cell-derived factor-1. Am J Pathol. 2006; 169:2066–2074. [PubMed: 17148669]
51. Steiger-Luther NC, Darwiche H, Oh SH, Williams JM, Petersen BE. Insulin-like growth factor binding protein-3 is required for the regulation of rat oval cell proliferation and differentiation in the 2AAF/PHx model. Hepatic Med Evid Res. 2009; 1:1–20.
52. Steer CJ. Liver regeneration. FASEB J. 1995; 9:1396–1400. [PubMed: 7589980]
53. Fausto N. Protooncogenes and growth factors associated with normal and abnormal liver growth. Dig Dis Sci. 1991; 36:653–658. [PubMed: 2022167]
54. Sell S. Heterogeneity and plasticity of hepatocyte lineage cells. Hepatology. 2001; 33:738–750. [PubMed: 11230756]
55. Shafritz DA, Dabeva MD. Liver stem cells and model systems for liver repopulation. J Hepatol. 2002; 36:552–564. [PubMed: 11943430]
56. Jung Y, Brown KD, Witek RP, Omenetti A, Yang L, Vandongen M, et al. Accumulation of hedgehog-responsive progenitors parallels alcoholic liver disease severity in mice and humans. Gastroenterology. 2008; 134:1532–1543. [PubMed: 18471524]
57. Fleig SV, Choi SS, Yang L, Jung Y, Omenetti A, Vandongen HM, et al. Hepatic accumulation of hedgehog-reactive progenitors increases with severity of fatty liver damage in mice. Lab Invest. 2007; 87:1227–1239. [PubMed: 17952094]
58. Nagy P, Bisgaard HC, Santoni-Rugiu E, Thorgeirsson SS. In vivo infusion of growth factors enhances the mitogenic response of rat hepatic ductal (oval) cells after administration of 2-acetylaminofluorene. Hepatology. 1996; 23:71–79. [PubMed: 8550051]
59. Erker L, Grompe M. Signaling networks in hepatic oval cell activation. Stem Cell Res. 2008; 1:90–102. [PubMed: 19383389]
60. Sun R, Gao B. Negative regulation of liver regeneration by innate immunity (natural killer cells/interferon-gamma). Gastroenterology. 2004; 127:1525–1539. [PubMed: 15521020]
61. Akhurst B, Matthews V, Husk K. Differential lymphotoxin-beta and interferon gamma signaling during mouse liver regeneration induced by chronic and acute injury. Hepatology. 2005; 41:327–335. [PubMed: 15660390]
62. Brooling JT, Campbell JS, Mitchell C. Differential regulation of rodent hepatocyte and oval cell proliferation by interferon gamma. Hepatology. 2005; 41:906–915. [PubMed: 15799032]
63. Matthews VB, Klinken E, Yeoh GC. Direct effects of interleukin-6 on liver progenitor oval cells in culture. Wound Repair Regen. 2004; 12:650–656. [PubMed: 15555057]

64. Knight B, Yeoh GC, Husk KL. Impaired preneoplastic changes and liver tumor formation in tumor necrosis factor receptor type 1 knockout mice. J Exp Med. 2000; 192:1809–1818. [PubMed: 11120777]

65. Pi L, Oh SH, Shupe T, Petersen BE. Role of connective tissue growth factor in oval cell response during liver regeneration after 2-AAF/PHx in rats. Gastroenterology. 2005; 128:2077–2088. [PubMed: 15940639]

66. Pi L, Ding X, Jorgensen M, Pan JJ, Oh SH, Pintilie D, et al. Connective tissue growth factor with a novel fibronectin binding site promotes cell adhesion and migration during rat oval cell activation. Hepatology. 2008; 46:996–1004. [PubMed: 18167060]

67. Benyon RC, Arthur MJP. Extracellular matrix degradation and the role of hepatic stellate cells. Semin Liv Dis. 2001; 21:373–384.

68. Burra P, Tomat S, Villa E. Experimental hepatology applied to stem cells. Dig Liver Dis. 2008; 40:54–61. [PubMed: 17997371]

69. Gasbarrini A, Rapaccini GL, Rutella S. Rescue therapy by portal infusion of autologous stem cells in a case of drug-induced hepatitis. Dig Liver Dis. 2007; 39:878–882. [PubMed: 16875890]

70. Sancho-Bru P, Najimi M, Caruso M. Stem and progenitor cells for liver repopulation: can we standardize the process from bench to bedside. Gut. 2008

71. Gerlach JC, Zelinger K, Petzerli JF. Bioartificial liver systems: why, what, whither? Regen Med. 2008; 3:575–595. [PubMed: 18588477]

72. McKenzie TJ, Lillegard JB, Nyberg SL. Artificial and bioartificial liver support. Semin Liver Dis. 2008; 28:210–217. [PubMed: 18452120]

73. Makowka L, Rotstein LE, Falk RE, Falk JA, Nossal NA, Langer B. Allogeneic and xenogeneic hepatocyte transplantation in experimental hepatic failure. Transplantation. 1980; 30:429–435. [PubMed: 7008291]

74. Rajvanshi P, Kerr A, Bhargava KK, Burk RD, Gupta S. Studies of liver repopulation using the dipeptidyl peptidase IV-deficient rat and other rodent recipients: cell size and structure relationships regulate capacity for increased transplanted hepatocyte mass in the liver lobule. Hepatology. 1996; 23:482–496. [PubMed: 8617428]

75. Gagandeep S, Rajvanshi P, Sokhi RP, Slehria S, Palestro CJ, Bhargava KK. Transplanted hepatocytes engraft, survive, and proliferate in the liver of rats with carbon tetrachloride-induced cirrhosis. J Pathol. 2000; 191:78–85. [PubMed: 10767723]

76. Shafritz DA, Raimondo G, Burk RD, Lieberman HM, Dusheiko GM, Kew MC. Molecular biology of hepatocellular carcinoma. J Med Virol. 1987; 21:A110–A112.

77. Wu PC, Lai VCH, Fang JWS, Gerber MA, Lai CL, Lau JYN. Hepatocellular carcinoma expressing both hepatocellular and biliary markers also expresses cytokeratin 14, a marker of bipotential progenitor cells. J Hepatol. 1999; 31:965–966. [PubMed: 10580599]

78. Komuta M, Spee B, Vander Borght S. Clinicopathological study on cholangiocellular carcinoma suggesting hepatic progenitor cell origin. Hepatology. 2008; 47:1544–1556. [PubMed: 18393293]

79. Andersen JB, Loi R, Perra A, Factor VM, Ledda-Columbano GM, Columbano A, et al. Progenitor-derived hepatocellular carcinoma model in the rat. Hepatology. 2010; 51:1401–1409. [PubMed: 20054870]

80. Van de Kerkhove MP, Poyck PP, Deurholt T, Hoekstra R, Chamuleau RA, van Gulik TM. Liver support therapy: an overview of the AMC-bioartificial liver research. Dig Surg. 2005; 22:254–264. [PubMed: 16174982]

81. Turpeinen M, Tolonen A, Chesne C, Guillouzo A, Uusitalo J, Pelkonen O. Functional expression, inhibition and induction of CYP enzymes in HepaRG cells. Toxicol In Vitro. 2009; 23:748–753. [PubMed: 19328226]

82. Fox IJ, Chowdhury JR, Kaufman SS, Goertzen TC, Chowdhury NR, Warkentin PI. Treatment of the Crigler-Najjar syndrome type I with hepatocyte transplantation. N Engl J Med. 1998; 338:1422–1426. [PubMed: 9580649]

83. Dhawan A, Mitry RR, Hughes RD, Lehec S, Terry C, Bansal S. Hepatocyte transplantation for ingerited factor VII deficiency. Transplantation. 2004; 78:1812–1814. [PubMed: 15614156]

84. Yovchev MI, Grozdanov PN, Joseph B. Novel hepatic progenitor cell surface markers in the adult rat liver. Hepatology. 2007; 45:139–149. [PubMed: 17187413]

85. Mancino MG, Carpino G, Onori P. Hepatic “stem” cells: state of the art. Ital J Anat Embryol. 2007; 112:93–109. [PubMed: 17687874]

86. Roskams T. Different types of liver progenitor cells and their niches. J Hepatol. 2006; 45:1–4. [PubMed: 16723168]

87. Schmelzer E, Zhang L, Bruce A. Human hepatic stem cells from fetal and postnatal donors. J Exp Med. 2007; 204:1973–1987. [PubMed: 17664288]

88. Weiss TS, Lichtenauer M, Kirchner S. Hepatic progenitor cells from adult human livers for cell transplantation. Gut. 2008; 57:1129–1138. [PubMed: 18417531]

89. Gaudio E, Carpino G, Cardinale V, Franchitto A, Onori P, Alvaro D. New insights into liver stem cells. Dig Liver Dis. 2009; 41:455–462. [PubMed: 19403350]

90. Roskams TA, Thiese ND, Balabaud C. Nomenclature of the finer branches of the biliary tree: canals, ductules, and ductular reactions in human livers. Hepatology. 2004; 39:1739–1745. [PubMed: 15185318]

91. Zhou H, Rogler LE, Teperman L. Identification of hepatocytic and bile ductular cell lineages and candidate stem cells in bipolar ductular reactions in cirrhotic human liver. Hepatology. 2007; 45:716–724. [PubMed: 17326146]

92. Libbrecht L, Roskams T. Hepatic progenitor cells in human liver diseases. Semin Cell Dev Biol. 2002; 13:389–396. [PubMed: 12468238]

93. Bird TG, Lorenzini S, Forbes SJ. Activation of stem cells in hepatic diseases. Cell Tissue Res. 2008; 331:283–300. [PubMed: 18046579]

94. Katoonizadeh A, Nevens F, Verslype C. Liver regeneration in acute severe liver impairment: a clinicopathological correlation study. Liver Int. 2006; 26:1225–1233. [PubMed: 17105588]

95. Libbrecht L, Desmet V, Van Damme B. Deep intralobular extension of human hepatic “progenitor cells” correlates with parenchymal inflammation in chronic viral hepatitis: can “progenitor cells” migrate? J Pathol. 2000; 192:373–378. [PubMed: 11054721]

96. Hollander AP. Maturation of tissue-engineered cartilage implanted in injured and osteoarthritic human knees. Tissue Eng. 2006; 12:1787–1798. [PubMed: 16889509]

97. Kuo CK, Li WJ, Mauck RL, Tuan RS. Cartilage tissue engineering: its potential and uses. Curr Opin Rheumatol. 2006; 18:64–73. [PubMed: 16344621]

98. Cebotari S. Clinical application of tissue engineered human heart valves using autologous progenitor cells. Circulation. 2006; 114:I132–I137. [PubMed: 16820562]

99. Atala A, Bauer SB, Soker S, Yoo JJ, Retik AB. Tissue-engineered autologous bladders for patients needing cystoplasty. Lancet. 2006; 367:1241–1246. [PubMed: 16631879]

100. Mishra L, Banker T, Murray J, Byers S, Thenappan A, He AR, et al. Liver stem cells and hepatocellular carcinoma. Hepatology. 2009; 49:318–329. [PubMed: 19111019]

101. Sell S, Dunsford HA. Evidence for the stem cell origin of hepatocellular carcinoma and cholangiocarcinoma. Am J Pathol. 1989; 134:1347–1363. [PubMed: 2474256]

102. Sell S, Leffert HL. An evaluation of cellular lineages in the pathogenesis of experimental hepatocellular carcinoma. Hepatology. 1982; 2:77–86. [PubMed: 6172353]

103. Braun L, Mikumo R, Fausto N. Production of hepatocellular carcinoma by oval cells: call cycle expression of c-myc and p53 at different stages of oval cell transformation. Cancer Res. 1989; 49:1554–1561. [PubMed: 2647288]

104. Yaswen P, Goyette M, Shank PR. Expression of c-Ki-Ras, c-Ha-Ras, and c-myc in specific cell types during hepatocarcinogenesis. Mol Cell Biol. 1985; 5:780–786. [PubMed: 2581126]

105. Wu PC, Fang JW, Lau VK. Classification of hepatocellular carcinoma according to hepatocellular and biliary differentiation markers. Am J Pathol. 1996; 149:1167–1175. [PubMed: 8863666]