Cell Stem Cell
Review

Stem/Progenitor Cells in Liver Development, Homeostasis, Regeneration, and Reprogramming

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The liver is a central organ for homeostasis with unique regenerative capacities. Mature hepatocytes possess a remarkable capacity to proliferate upon injury, challenging efforts to discern the role of adult liver stem cells in this process. In contrast, stem/progenitor cells in the developing liver have been extensively characterized, and these investigations have informed efforts to produce functional hepatocytes in vitro for cell therapy and drug screening. In this Review, we describe recent advances in the characterization of liver stem cells and discuss evidence supporting and refuting whether self-renewable and bipotential liver stem cells exist in development, homeostasis, regeneration, and disease.

Introduction

Stem cells are defined, in general, by their ability to self-renew and differentiate into multiple lineages. Functionally, stem cell activity can be defined by several methods. Clonogenicity and multilineage differentiation in vitro are classical and convenient assays to demonstrate stemness and have been widely used to assess stem cell activity in various tissues. Genetic lineage tracing and long-term label-retaining assays have been used to identify and characterize stem cells in vivo. These assays have allowed extensive characterization of several tissue-specific stem cells, including intestinal stem cells, dermal stem cells, and hair follicle stem cells (Barker et al., 2012; Blanpain and Fuchs, 2009; Fuchs, 2009). Alternatively, long-term repopulation upon transplantation of a single sorted cell has been long regarded as the gold standard of stem cell activity in the hematopoietic compartment (Oguro et al., 2013; Smith et al., 1991). The stem cell for the liver has been defined as the cell that gives rise to both hepatocytes and biliary epithelial cells (cholangiocytes), the two types of liver epithelial cells. Although there are many reports describing liver stem cells, the measures used to define liver stem cells have not necessarily been adequate in many cases. In this Review, we describe the defining characteristics used to describe liver stem cells and discuss the evidence supporting and refuting whether self-renewable and bipotential liver stem cells exist in development, homeostasis, and regeneration.

Liver Functions and Architecture

The liver is a central organ for homeostasis and carries out a wide range of functions, including metabolism, glycogen storage, drug detoxification, production of various serum proteins, and bile secretion. Since liver functions are essential for homeostasis, liver diseases, such as hepatitis, fibrosis, and cirrhosis, often result in morbidity and mortality. The liver is unique in its extraordinary capacity to regenerate from various injuries. Strikingly, the liver's ability to recover its original mass after surgical removal of a significant portion makes it possible to transplant liver tissue from a living donor. The basic architectural unit of the liver is the liver lobule, which is described in detail in Figure 1.

Most of the metabolic and synthetic functions of the liver are carried out by hepatocytes, which account for approximately 60% of total liver cells and 80% of the volume of the organ. Hepatocytes are highly polarized epithelial cells and form cords. Their basolateral surfaces face fenestrated sinusoidal endothelial cells, facilitating the exchange of materials between hepatocytes and blood vessels. Tight junctions formed between hepatocytes create a canaliculus that surrounds each hepatocyte. Bile secreted from mature hepatocytes is exported sequentially through bile canaliculi surrounded by the apical membrane of neighboring hepatocytes, intrahepatic bile ducts, extrahepatic bile ducts, and, finally, the duodenum. The bile duct is formed by a specialized type of epithelial cell called a cholangiocyte.

Hepatoblasts as the Liver Progenitor Cell in Development

The onset of mouse liver development begins at embryonic day (E) 8.5 from the foregut endoderm, which is derived from medial and lateral domains of developing ventral foregut (Tremblay and Zaret, 2005). The commitment of endoderm cells to the liver is dictated by two crucial cytokines: fibroblast growth factor (FGF) from the developing heart (Gualdi et al., 1996; Jung et al., 1999) and bone morphogenetic protein (BMP) from the septum transversum mesenchyme (STM) (Rossi et al., 2001). The foregut endoderm cells destined for hepatic fate begin to express transcription factors Hex and HNF4α as well as the liver-specific genes α-fetoprotein (AFP) and albumin (ALB) and migrate as cords into the surrounding STM. These cells are common progenitor cells, which give rise to both hepatocytes and cholangiocytes, and are called "hepatoblasts" during liver development. Historically, immunohistochemical analysis had been used to characterize hepatoblasts; however, specific cell surface markers for prospective isolation of hepatoblasts were not identified until more recently. Because the fetal liver is a

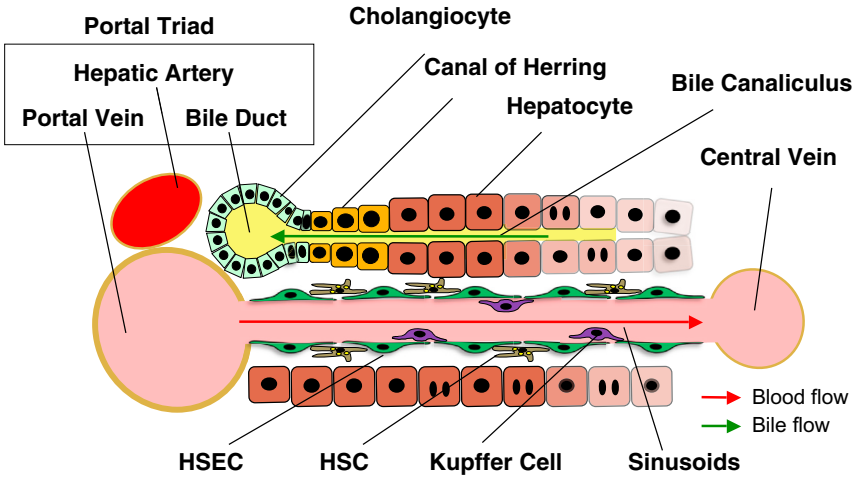

Figure 1. Schematic Overview of Liver Lobule

The portal triad consists of the portal vein, hepatic artery, and bile ducts. Blood from the portal vein and the hepatic artery flows toward the central vein between hepatocytes through the sinusoids surrounded by fenestrated hepatic sinusoidal endothelial cells (HSECs). Bile produced by hepatocytes is collected into bile ducts via the bile canaliculi. Kupffer cells, resident macrophages of the liver, are located at the luminal side of sinusoids, while hepatic stellate cells (HSCs) are positioned in close proximity to HSECs at the "space of Disse," a location between hepatocytes and a sinusoid. The canal of Hering is the junctional region between hepatocytes and bile ducts.

major hematopoietic organ and blood cells occupy the majority of liver cells, a combination of negative selection by CD45 (common leukocyte antigen) and TER119 (erythroid cell antigen) and positive selection by some cell surface markers has been utilized to successfully isolate hepatoblasts. In many cases, the sorted cells from fetal liver were evaluated by the expression of liver-specific genes such as AFP and ALB, clonogenicity and bipotency in vitro, and their ability to repopulate adult liver upon transplantation. Suzuki et al. (2000) developed a single-cell-based assay called the hepatic colony-forming unit in culture (H-CFU-C) and showed that the CD45$^-$ TER119$^-$ c-Kit$^-$ CD29$^+$ CD49f$^+$ fraction of E13.5 mouse liver contained colony-forming cells with the potential to differentiate into hepatocytic and cholangiocytic lineages. Since then, sorting for c-Kit^{low} (Minguet et al., 2003), c-Kit$^-$ c-Met$^+$ CD49f$^{+/low}$ (Suzuki et al., 2003), CD13$^+$ (Kakinuma et al., 2009), or CD13$^+$ c-Kit$^-$ CD49f$^{-/low}$ CD133$^+$ (Kamiya et al., 2009) in combination with CD45$^-$ and TER119$^-$ has been applied to isolate the hepatoblast compartment. Alternatively, positive selection with a single specific marker has also been reported to isolate hepatoblasts. Delta-like 1 homolog (Dlk1), also known as Pref-1, is expressed in liver buds as early as E9.0 in mouse embryo and can also be used to isolate bipotential cells. Dlk1 expression is gradually decreased in the liver by the neonatal stage and becomes undetectable in adult liver. Dlk1$^+$ cells isolated from E14.5 livers form highly proliferative colonies composed of the hepatocyte and cholangiocyte lineages in vitro (Tanimizu et al., 2003). E-cadherin, an epithelial-specific marker, was also utilized to isolate hepatoblasts (Nitou et al., 2002; Nierhoff et al., 2005). E12.5 liver epithelial cells were shown to specifically express E-cadherin, Dlk1, and Liv2, a unique marker for epithelial cells in the E9.5–E12.5 fetal liver (Watanabe et al., 2002), and sorted E-cadherin$^+$ cells repopulated the liver after transplantation. Nierhoff et al. (2007) also identified additional markers, CD24a and Neighbor of Punc E11 (Nope), to isolate hepatoblasts by comparing the gene expression profiles of purified E13.5 E-cadherin$^+$ liver cells and adult liver. Epithelial cell adhesion molecule (EpCAM) was expressed in HNF4α^+ hepatoblasts of liver buds as early as E9.5 in mice. The EpCAM$^+$ Dlk1$^+$ cell population sorted from E11.5 liver contained in vitro colony-forming cells, indicating that hepatoblasts are present in this population at this early stage of liver development (Tanaka et al., 2009) (Figure 2). However, clonogenic mouse hepatic cells were found in EpCAM$^-$ Dlk1$^+$ cells at E13.5, suggesting that hepatoblasts likely change their characteristics during the course of liver development. Alternatively, fate mapping by dye labeling revealed that there are distinct endodermal regions that give rise to hepatoblasts (Tremblay and Zaret, 2005), thus it might be possible that regionally distinct hepatoblast descendants could have different properties. Gadue et al. (2009) generated two monoclonal antibodies, ENDM1 and ENDM2, that show remarkable specificity for mouse foregut ventral endoderm. Interestingly, the endoderm population recognized by those antibodies has the potential to generate cells of the hepatic lineage, although these markers are downregulated by specification to the hepatic fate. These antibodies may be useful to further study the characteristics of endoderm progenitor cells (Xu et al., 2011). The expression profile of representative cell surface markers during liver development is illustrated in Figure 2, and the characterization of hepatoblasts by prospective isolation is summarized in Table 1.

Engraftment of in vitro expanded hepatoblasts in adult mouse liver injury models can be used to demonstrate some aspects of stem cell activity. However, unlike long-term repopulation of a single sorted hematopoietic stem cell, which demonstrates self-renewal and multidifferentiation potential in mice, engraftment of hepatoblasts requires a large number of cells, with more than 5×10^4 cells needed to engraft a single mouse or rat (Kakinuma et al., 2009; Nierhoff et al., 2005; Oertel et al., 2008; Suzuki et al., 2000; Tanimizu et al., 2003). In addition, engraftment of transplanted hepatoblasts to bile ducts has not necessarily been convincingly demonstrated, most likely because of the lack of an appropriate bile duct injury model to assess engraftment. Thus, transplantation studies using hepatoblasts do not provide sufficient evidence to fulfill the stringent criteria of "stemness" that is used for other stem cell types, such as hematopoietic stem cells.

Stem Cell Properties of Fetal Liver Cells

Several reports using culture systems have demonstrated the presence of a potential liver "stem cell" in the fetal liver, which has the capacity for unlimited proliferation and multilineage

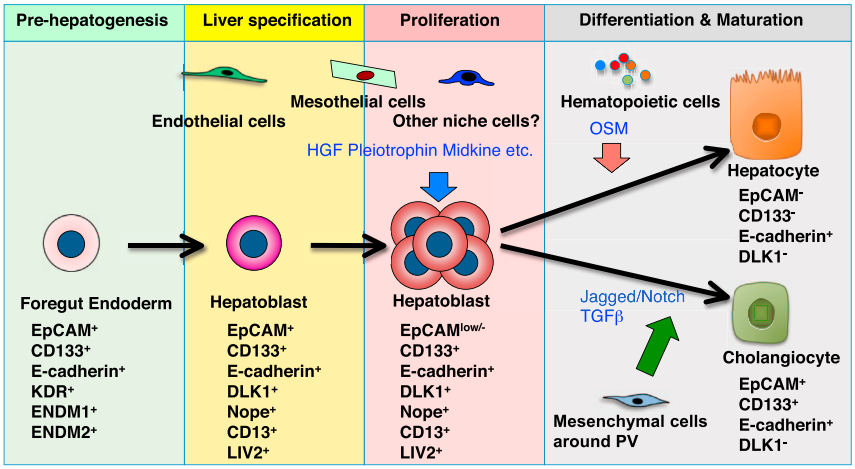

Figure 2. Schematic Model of Regulatory Mechanisms and Cell Surface Markers for Hepatic Epithelial Cells during Mouse Liver Development

The representative cell surface markers are shown. While EpCAM is transiently downregulated in hepatoblasts at the midgestational stage, its expression is restored in cholangiocytes, but not in hepatocytes, later on. DLK1 is also expressed in hepatoblasts but is not expressed in mature hepatocytes or cholangiocytes.

differentiation. Dlk1$^+$ cells in mouse fetal liver contain clonogenic cells named "HPPL" that continuously proliferate on laminin-coated plates and differentiate to both hepatocytes and cholangiocytes under differentiation conditions (Tanimizu et al., 2004). Similar bipotential cell lines exhibiting the properties of liver stem cells were also obtained after a long latency period in culture of fetal liver cells, though their specific cellular origin was unknown (Strick-Marchand and Weiss, 2002; Tsuchiya et al., 2005). One of the cell lines, referred to as BMEL (Strick-Marchand and Weiss, 2002), expresses hepatocytic transcription factors such as HNF1α, HNF4α, and GATA4, but not ALB. Because BMEL cell lines can be generated reproducibly, the bipotential progenitors provide a useful experimental model to study the mechanism of differentiation.

Dan et al. (2006) established cells similar to those mouse cells—multipotent progenitor cells from human fetal liver cells in long-term culture named "hFLMPCs," which exhibited capacities of self-renewal, multipotent differentiation, and repopulation in a mouse liver injury model. Intriguingly, the hFLMPCs expressed several stem-cell-related markers, such as CD90, c-Kit, CD44h, and EpCAM, but neither AFP nor ALB. Schmelzer et al. (2007) reported that EpCAM$^+$ cells isolated from human fetal liver contained multipotent precursors of hepatoblasts, which expressed stem cell markers and could be expanded in culture. The cultured EpCAM$^+$ cells, which they called hepatic stem cells (hHpSCs), expressed ALB weakly, but no AFP. Transplantation of freshly isolated EpCAM$^+$ cells or hHpSCs expanded in culture into NOD/SCID mice resulted in mature liver tissue expressing human-specific proteins. Recently, Goldman et al. (2013) reported that mouse and human fetal liver cells distinct from endothelial cells expressed KDR, also known as VEGFR2/Flk1, and expressed low levels of ALB. A lineage-tracing experiment using the *Kdr-Cre* mouse revealed that KDR$^+$ cells were present in the endoderm in E8.0 embryos prior to hepatic specification and that the labeled hepatic progenitor gave rise to hepatocytes and cholangiocytes, suggesting that KDR$^+$ ALB$^-$ AFP$^-$ hepatic progenitors are a precursor of hepatoblasts (Figure 2). Ultimately, the absence of liver-specific ALB or AFP in "fetal liver-derived stem-like cells" expanded in vitro suggests that fetal liver stem cells may be present as hepatoblast precursors, such as foregut endoderm stem cells, rather than hepatoblasts in vivo. In other words, if the liver stem cell were defined as a persistently self-renewable cell, most hepatoblasts would be unlikely to fall into this category. Because liver development from foregut endoderm is a continuous process, it seems likely that the cell surface characteristics of hepatoblasts change over time. Whether or not fetal liver cells with self-renewal capacity persist throughout an individual's life span remains an open question.

Mechanisms Underlying the Proliferation and Differentiation of Hepatoblasts

Proliferation and differentiation of hepatoblasts are supported and coordinated by various cell types in the liver during embryogenesis. Here, we focus on the cell-to-cell interactions relevant to the growth and differentiation of hepatoblasts into hepatocytes (Figure 2). At the earliest stage of liver development, hepatoblasts emerge from foregut endoderm and proliferate to form the liver bud in a process that requires Flk1$^+$ endothelial cells in STM (Matsumoto et al., 2001). At later stages, mesothelial cells (MCs), which form the mesothelium that covers the parenchyma and prevents adhesion with other tissues, express high levels of various growth factors for hepatocytes such as HGF, Midkine, and Pleiotrophin (Onitsuka et al., 2010). Fetal MCs enhance proliferation of Dlk1$^+$ hepatoblasts/immature hepatocytes in a coculture system. Wilms tumor 1 (WT1) is a transcription factor essential for development of MCs and the lack of WT1 impairs liver growth (Ijpenberg et al., 2007). Together, these results indicate that immature MCs contribute to hepatoblast proliferation.

By contrast, Thy1$^+$ mesenchymal cells in the murine fetal liver promote the maturation of CD49f$^+$ hepatic progenitor cells by direct cell-to-cell contact in a coculture system (Hoppo et al., 2004). Interestingly, human embryonic stem cell (ESC)-derived KDR$^+$ AFP$^-$ hepatic progenitors were shown to promote the maturation of KDR$^-$ AFP$^+$ hepatic cells in vitro, suggesting the possibility that a small population of hepatoblasts itself provides a niche for hepatic maturation (Goldman et al., 2013). Hematopoietic cells start to colonize the liver in midgestation, and the fetal liver serves as the major hematopoietic tissue until the perinatal stage when hematopoiesis shifts to the bone marrow. Hepatoblasts/immature hepatocytes are closely associated with hematopoietic precursor cells and support hematopoiesis in fetal liver. Reciprocally, cytokines secreted from blood cells play a pivotal role for functional and morphological maturation of hepatocytes; e.g., Oncostatin M (OSM), an interleukin-6 family member, produced by hematopoietic cells promotes hepatocyte

Table 1. Identification of Hepatoblasts by Cell Sorting Using Cell Surface Markers

Species	Developmental Stage Used for Assays	Used Markers for Cell Sorting	Afp or Alb Expression	Clonogenicity	Bipotency in Clonogenic Analysis	Repopulating Activity In Vivo	References
Mouse	E13.5	$CD45^-/TER119^-/c\text{-}Kit^-/CD29^+/CD49f^+$	+	+	+	+	(Suzuki et al., 2000)
	E12.5	$E\text{-}cad^+$	+	NT	hepatocytic	NT	(Nitou et al., 2002)
	E11	$CD45^-/TER119^-/c\text{-}Kit^{low}$	+	+	+	chimeric fetal liver organoids	(Minguet et al., 2003)
	E14.5	$Dlk1^+$	+	+	+	+	(Tanimizu et al., 2003)
	E13.5	$CD45^-/TER119^-/c\text{-}Kit^-/c\text{-}Met^+/CD49f^{+/low}$	+	+	+	NT	(Suzuki et al., 2003)
	E12.5	$E\text{-}cad^+$, $Liv2^+$	+	+	NT	+	(Nierhoff et al., 2005)
	E13.5	$Nope^+$, $CD24a^+$	+	NT	NT	NT	(Nierhoff et al., 2007)
	E11.5	$Epcam^+/Dlk1^+$	+	+	NT	NT	(Tanaka et al., 2009)
	E13.5	$CD45^-/TER119^-/CD13+$	+	+	+	+	(Kakinuma et al., 2009)
	E13.5	$CD45^-/TER119^-/c\text{-}Kit^-/CD13+/CD49f^{-/low}/CD133^+$	+	+	+	NT	(Kamiya et al., 2009)
Rat	E13	$RT1A^{l-}OX18^{low}/ICAM\text{-}1^+$	+	+	+	NT	(Kubota and Reid, 2000)
	E16–E18	$(OX43/OX44)^-/Thy\text{-}1^+$	+	NT	NT	NT	(Fiegel et al., 2003)
	E14	$Dlk\text{-}1^+$	+	NT	+	+	(Oertel et al., 2008)
Human	18–22 week gestation age	$EpCAM^+$	+	+	+	+ (NOD/SCID mice)	(Schmelzer et al., 2007)

NT, not tested.

maturation in vitro by inducing the expression of several metabolic enzymes and the formation of adherens junctions (Kamiya et al., 1999, 2002; Matsui et al., 2002). Hematopoiesis shifts from fetal liver to the bone marrow around birth, and metabolic and synthetic functions of the liver drastically change as reflected by the altered expression of various genes. Typically, xenobiotic-metabolizing cytochrome P450 genes are highly upregulated in neonatal stages. However, the metabolic shift is not a uniform process. Even fully mature hepatocytes constitute heterogenous cell populations depending on their location within a hepatic lobule, which is referred to as "metabolic zonation." A number of genes related to metabolism are differentially expressed in either the periportal or pericentral zone. Despite several studies investigating the involvement of Wnt/β-catenin signaling in metabolic zonation (Benhamouche et al., 2006; Burke et al., 2009; Sekine et al., 2006), the responsible Wnt ligand remains unclear.

Mechanisms Regulating the Differentiation of Hepatoblasts into Cholangiocytes

In contrast to hepatocyte maturation, bile ducts are formed specifically around the portal vein, indicating that there must be a regionally specific cue for the induction of cholangiocytes from hepatoblasts. Although intrahepatic bile ducts are not visible by E16 in mouse liver, the specification of cholangiocyte lineage from hepatoblasts occurs around E15 in the vicinity of portal vein branches. Alagille syndrome is a human autosomal-dominant developmental disorder that is characterized by defects in multiple organs including impaired differentiation of intrahepatic bile ducts. NOTCH2 and JAGGED1, a ligand for Notch family receptors, have been identified as the genes that cause this disorder (Alagille et al., 1987; Li et al., 1997; McDaniell et al., 2006; Oda et al., 1997). Consistently, Notch2 is expressed in hepatoblasts, whereas Jagged1 is expressed in periportal mesenchymal cells (Kodama et al., 2004), and a mouse model with the double mutations of Notch2 and Jagged1 exhibited the paucity of intrahepatic bile ducts that is observed in humans (McCright et al., 2002). Activation and/or inactivation of Notch signaling in vivo and in vitro have demonstrated that biliary duct mophogenesis and/or differentiation are affected by Notch signaling in the periportal region (Hofmann et al., 2010; Tanimizu and Miyajima, 2004; Zong et al., 2009). These results indicate that Notch signaling plays a critical role for bile duct development. Another important signaling pathway regulating biliary differentiation is TGF-β/Activin. TGF-β2 and TGF-β3 are predominantly expressed in the portal region (Antoniou et al., 2009), and HNF6 (OC-1) and OC-2, onecut transcription factors controlling biliary tract development, modulate expression of α2-macroglobulin and follistatin, which are inhibitors of the TGF-β/Activin pathway, in the parenchymal region (Clotman et al., 2005). Collectively these studies revealed that various types of fetal liver cells constitute the niche for proliferation and differentiation of hepatoblasts (Figure 2).

Programming and Reprogramming Hepatic Development In Vitro

Reproduction of liver development from embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) in vitro provides a proof of principle for liver organogenesis and also paves the way for practical use of hepatocytes in drug screening and transplantation settings. Liver transplantation is the only established effective treatment for end-stage liver diseases; however, a shortage of donors limits this treatment. As alternatives to organ

transplantation, hepatocyte transplantation and creation of bioartificial livers with functional hepatocytes may provide potentially effective treatments. For such practical uses, a large quantity of hepatocytes will be needed and accordingly, efforts have been made toward generating hepatocytes from pluripotent stem cells (PSCs). Because freshly isolated hepatocytes from adult liver rapidly lose their function in culture, generation of fully functional hepatocytes from PSCs in vitro is a difficult task. Nevertheless, progress has been made toward this goal based on our understanding of liver development. By recapitulating developmental processes, step-wise protocols to differentiate hepatocytes from PSCs have been established. Typically, PSCs are induced to become definitive endoderm with Activin A in a monolayer culture, BMP4 and FGF2 are added to induce hepatic specification, HGF to induce differentiation into immature hepatocytes, and OSM with or without dexamethasone (Dex) to generate mature hepatocytes (Si-Tayeb et al., 2010). Aggregation culture of human ESC (hESC)-derived hepatocytes together with cAMP further enhanced maturation (Ogawa et al., 2013). While there is still room for improvement in terms of maturation, human iPSCs (hiPSCs) can be used for modeling inherited diseases. Rashid et al. (2010) generated iPSCs from patients with α1-anti-trypsin deficiency, familial hypercholesterolemia, and glycogen storage disease and showed that hepatocytes derived from patient-derived iPSCs recapitulate pathological features of the diseases. Furthermore, targeted gene correction of α1-anti-trypsin deficiency in iPSCs restored structure and function of α1-anti-trypsin in iPSC-derived hepatocytes (Yusa et al., 2011).

During the course of the differentiation process from PSCs, hepatoblasts with the potential to differentiate to both hepatocytes and cholangiocytes are expected to emerge. Accordingly, hepatoblast-like cells expressing both hepatocyte and cholangiocyte markers were shown to emerge from PSCs, and they were able to differentiate to hepatocytes and form cysts of cholangiocytes in vitro (Yanagida et al., 2013; Zhao et al., 2009). The liver progenitors were expanded on stromal cells in vitro, and transplantation into mouse liver injury models, such as fumarylacetoacetate-hydrolase (Fah) deficiency, allowed engraftment and subsequent differentiation to hepatocytes. In other protocols, by transient adenoviral-induced expression of Hex, a homeotic gene essential for hepatic differentiation, PSC-derived liver progenitors were generated (Takayama et al., 2013). The Hex-induced cells proliferated and maintained the potential for bidirectional differentiation in vitro and were capable of engraftment in a CCl_4-injured liver. Recently, induced multipotent progenitor cells (iMPCs), which were generated from partially reprogrammed human fibroblasts, were shown to differentiate to hepatocytes (Zhu et al., 2014). A similar approach has also been recently used to generate endoderm-like cells capable of differentiating into pancreatic lineages (Li et al., 2014). Importantly, iMPC-derived endoderm progenitors proliferated extensively, and their differentiation was induced with both small molecules that are known to promote hepatocyte differentiation from PSCs and inhibitors against TGF-β and Notch signaling pathways that are known to promote biliary differentiation, resulting in more differentiated hepatocyte phenotypes as compared to iPSC-derived hepatocytes. Nonetheless, iMPC-hepatocytes more closely resembled human fetal hepatocytes. Upon transplantation, iMPC-hepatocytes proliferated extensively in an immunodeficient *Fah*$^{-/-}$ mouse; however, human serum albumin was not detected for 2 months, indicating that the transplanted cells are still immature and require a substantial maturation period in mouse.

Because the development and differentiation of hepatocytes are supported by various nonparenchymal cells such as endothelial cells and mesenchymal cells, it is likely that addition of those cells to differentiation culture of hepatocytes from ESCs/iPSCs would improve hepatic functions. Recently, Takebe et al. (2013) developed a coculture system of human iPSC-derived hepatic specified definitive endoderm with human umbilical vein endothelial cells (HUVECs) and mesenchymal cells, in which 3D cell clusters are formed. Hepatocytes in the cell clusters expressed many hepatic enzymes. Upon transplantation of the cell clusters into mice, they became vascularized and expressed liver proteins, resulting in improved survival of mice in a toxin injury model. These characteristics suggest that the cell clusters developed in vitro may be considered as liver organoids. However, the cells do not reconstitute the full liver or exhibit long-term function, and their differentiation to cholangiocytes as well as their formation of biliary architecture remains to be demonstrated. Goldman et al. (2013) recently reported an intriguing observation that hESC-derived endoderm cells do not express KDR, but generate KDR$^+$ hepatic progenitors and KDR$^-$ hepatic cells when cultured in media supporting hepatic differentiation. KDR$^+$ progenitors are supportive cells for the maturation of committed hepatic cells and hepatic cells generated from KDR$^+$ progenitors support HCV infection. Taken together, these studies demonstrate the benefits of including supporting cells in PSC-derived hepatocyte culture.

Hepatocyte-like cells were also derived from cells of nonhepatic lineages, such as human mesenchymal cells in bone marrow or CD105$^+$ mesenchymal cells in adipose tissue, by being incubated with FGF and HGF and then OSM and Dex (Banas et al., 2007; Lee et al., 2004). More recently, mouse fibroblasts were directly reprogrammed by combined expression of transcription factors, including Hnf4α plus Foxa1, Foxa2, or Foxa3 (Sekiya and Suzuki, 2011), and Gata4, Hnf1α, and Foxa3 together with inactivation of p19^{Arf} (Huang et al., 2011). Those reprogrammed cells, called iHep cells, closely resemble hepatocytes and express hepatic genes. Notably, transplanted iHep cells repopulated the livers of *Fah*$^{-/-}$ mice and rescued the lethal phenotype. Importantly, human fibroblasts can also be directly reprogrammed to hepatocytes that exhibit key metabolic functions by expression of FOXA3, HNF1A, and HNF4A (Huang et al., 2014) or a combination of HNF1A, HNF4A, and HNF6 together with the maturation factors ATF1, PROX1, and CEBPA (Du et al., 2014). It is notable that different sets of transcription factors can reprogram fibroblasts to hepatocytes. This plasticity is quite reminiscent of the flexible and self-sustaining cross-regulatory network that operates during liver organogenesis (Kyrmizi et al., 2006), whose incipient activation by any of the sets of reprogramming factors could be sufficient to ignite the self-organizing developmental program to achieve functionally differentiated hepatocytes. Alternatively, it could reflect some difference in fibroblasts and/or culture conditions used for reprogramming. The characteristics of the reprogrammed hepatocytes may not necessarily be the same among these

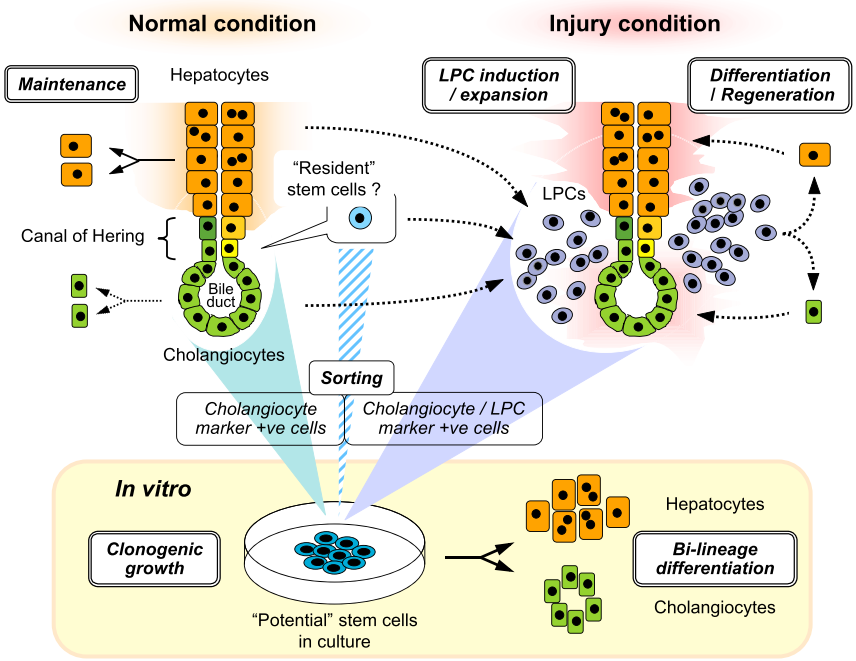

Figure 3. Stem/Progenitor Cells in Adult Liver under Normal and Injured Conditions

Under normal physiological conditions, homeostatic maintenance of hepatocytes is achieved predominantly by proliferation of mature hepatocytes. Upon certain types of injury condition, unique epithelial cell populations with an immature phenotype, called adult liver progenitor cells (LPCs), emerge and expand, and are thought to contribute to the regeneration process due to their bilineage differentiation potential to both hepatocytes and cholangiocytes. Although the origin of LPCs is still not clear, the dominant theory is that they are derived from the canal of Hering, which may harbor putative "resident" stem cells as the exact cell of origin. Purification by cell sorting and subsequent culture experiments have demonstrated that the cholangiocyte marker-positive population from normal liver, as well as the cholangiocyte/LPC marker-positive subset from injured liver, contains a "potential" liver stem cell population defined in vitro by clonogenicity and bilinegae differentiation potential.

studies and further evaluation of their variance and underlying mechanisms should lead to improvement in reprogramming strategies. The direct reprogramming strategy has also been used to successfully produce hepatoblast-like cells, called induced hepatic stem cells (iHepSCs), from mouse embryonic fibroblasts by introducing Hnf1β and Foxa3 (Yu et al., 2013). iHepSCs are capable of showing bilineage differentiation in vitro as well as in vivo, and they may potentially offer an expandable source for production of functional hepatocytes. While these efforts to generate functional hepatocytes or progenitor cells from pluripotent/multipotent stem cells or by reprogramming somatic cells are encouraging, practical use of the cells will require a more sophisticated understanding of their terminal differentiation.

Stem Cells in the Normal Adult Liver

Although careful consideration is required as to whether hepatoblasts can truly be called "stem cells" in the fetal liver as mentioned earlier, they nevertheless can be regarded as a bona fide progenitor cell population with bilineage differentiation potential in vivo. The situation regarding stem/progenitor cells, however, is further complicated in the adult liver. The nature and role of tissue stem cells in adult organs/tissues can be considered, for simplicity, in the context of two distinct (though closely related) processes; namely, homeostatic maintenance (or tissue turnover) under normal physiological conditions, and tissue repair/regeneration under pathological conditions upon various types of injury. In many tissues/organs, such as the hematopoietic system, intestine, and epidermis, mature differentiated cells have a short life span and their continuous replenishment from the stem cell compartment is critical to maintain structural and functional integrity of the tissue/organ. In contrast, the normal turnover of mature hepatocytes slowly occurs over a period of more than several months (MacDonald, 1961; Magami et al., 2002), and it is therefore questionable whether any stem cell is required for liver maintenance, at least under normal conditions. As such, a widely accepted view in the field argues that maintenance of hepatic tissue under normal physiological conditions is achieved by proliferation of mature hepatocytes that occurs throughout the liver parenchyma (Figure 3).

The slow turnover rate of hepatocytes makes it practically difficult to apply the long-term label-retaining assay to identify stem cells in vivo in the adult liver. Instead, many recent studies have employed Cre/loxP-mediated genetic marking and lineage tracing systems to characterize the mode of tissue maintenance in the adult mouse liver. A long-lasting, yet still inconclusive, model for hepatocyte turnover is the so-called "streaming liver hypothesis." Based on radiolabeled nucleotide-incorporation assays in the rat liver, Zajicek et al. (1985) claimed that new produced hepatocytes appear in the periportal area (where bile ducts exist) and flow along the hepatic cord toward the pericentral region to continuously replenish the tissue, thus implying the existence of a possible stem cell compartment for homeostatic maintenance of the adult liver. Since then, much evidence has accumulated both in favor and against this hypothesis. The most powerful, and rather surprising, evidence supporting this theory was provided by a genetic lineage tracing study based on the cholangiocyte marker Sox9 (Furuyama et al., 2011). Using a knockin (KI) mouse strain where the inducible recombinase CreERT2 is inserted into the Sox9 locus, cholangiocytes, but not hepatocytes, were lineage-labeled in the normal adult liver. The label gradually spread out to hepatocytes from the periportal toward pericentral regions and eventually occupied nearly the whole parenchyma after 1 year. The labeled cells also remained present in bile ducts, indicating that the Sox9-expressing cholangiocytes can continuously supply mature hepatocytes for normal tissue turnover while possessing self-renewing activity as well.

However, subsequent studies by other groups employing different types of genetic lineage tracing systems have together

Table 2. Identification and Characterization of Adult Liver Stem/Progenitor Cells by Cell Sorting

Species	Markers and/or the Mouse Lineage Tracing Model Used	Liver Sample/Disease Model Used	Clonogenicity	Bipotency in Clonogenic Analysis	Repopulating Activity In Vivo	References
Mouse	$CD133^+/CD45^-$	normal	+	+	NT	(Rountree et al., 2007)
		ANIT	+	+	NT	(Rountree et al., 2007)
		CCl_4 (chronic)	+	+	NT	(Rountree et al., 2007)
		DDC	+	+	NT	(Rountree et al., 2007)
	$CD133^+/D45^-$/ $TER119^-$	normal	± (small colonies only)	cholangiocytic	NT	(Suzuki et al., 2008)
		DDC	+ (both large and small colonies)	+	+ (*Fah*$^{-/-}$ mice)	(Suzuki et al., 2008)
	$CD45^-/TER119^-/c\text{-}Kit^-$/ $Sca1^-/CD13^+/CD49f^+$/ $CD133^+$	normal	+	+	+ (nude mice, Rs + PHx)	(Kamiya et al., 2009)
	$Epcam^+$	normal	+	+	NT	(Okabe et al., 2009)
		DDC	+	+	NT	(Okabe et al., 2009)
	$CD24^+/CD45^-/TER119^-$	normal	NT	NT	+ (*Fah*$^{-/-}$ mice)	(Qiu et al., 2011)
	MIC1-1C3$^+$/$CD133^+$/ $CD26^-/CD45^-/CD11b^-$/ $CD31^-$	normal	+	+	± (*Fah*$^{-/-}$ mice) *1	(Dorrell et al., 2011)
		DDC	+	+	± (*Fah*$^{-/-}$ mice) *1	(Dorrell et al., 2011)
	Sox9-CreERT2 BAC Tg (R26R-YFP reporter$^+$)/ $CD45^-/CD11b^-/CD31^-$	DDC	+	NT	NT	(Dorrell et al., 2011)
	Foxl1-Cre Tg (R26R-YFP reporter$^+$)/$CD45^-$	DDC	+	+	NT	(Shin et al., 2011)
	Lgr5-LacZ KI ($LacZ^+$)	CCl_4 (acute)	+ (organoids)	+	+ (FRG mice)	(Huch et al., 2013)
Rat	$EpCAM^+$	D-gal	NT	NT	+ (Rs + PHx)	(Yovchev et al., 2008)
Human	$EpCAM^+$	neonates (0–1 year); pediatric (2–13 years); adult (19–81 years)	+	+	+ (NOD/SCID mice)	(Schmelzer et al., 2007)
	$ALDH^+$ (enzyme activity)	(unknown)	+	hepatocytic	NT	(Dollé et al., 2012)

NT, not tested. ANIT, α-naphthylisothiocyanate; DDC, 3,5-diethoxycarbonyl-1,4-dihidro-collidine; D-gal, D-galactosamine. Rs + PHx, retrorsine + partial hepatectomy; FRG, *Fah*$^{-/-}$ *Rag2*$^{-/-}$ *Il2rg*$^{-/-}$. *1 < 0.1% of the total liver in 2 out of 20 *Fah*$^{-/-}$ mice (Huch et al., 2013).

provided rather conflicting results with the above report. Studies using a different *Sox9-CreERT2* strain (BAC transgenic) (Carpentier et al., 2011) or *osteopontin* (*OPN*)*-CreERT2* (Español-Suñer et al., 2012) to label cholangiocytes did not observe the flow of lineage-labeled cells out of the bile duct. Meanwhile, in studies using a complimentary approach where hepatocytes were initially labeled by a Cre-expressing adeno-associated viral (AAV) vector, contribution of label-negative, nonhepatocytic lineage cells (including cholangiocytes) for the maintenance of parenchymal tissue was not observed (Malato et al., 2011; Yanger et al., 2013). Overall, the results strongly argue against the streaming liver hypothesis, although they do not completely refute the possibility that the cell of origin for the "stream" still exists among periportal hepatocytes, rather than cholangiocytes. Given that cholangiocytes are indeed capable of contributing to parenchymal regeneration under certain types of liver injury conditions, even in the *OPN-CreERT2* system (Español-Suñer et al., 2012), it seems likely that cholangiocytes in the *Sox9-CreERT2* KI mouse liver (Furuyama et al., 2011) are somehow biased to differentiate to hepatocytes due to genetic and/or environmental factors, the natures of which are of potential interest. Further studies are needed to resolve this discrepancy and elucidate the exact mode and the underlying mechanisms for physiological maintenance of the liver.

Notably, another study by Iverson et al. (2011) used a unique "in vivo chronometer" system based on *Alb-Cre*-mediated fluorescent color conversion. The result suggested that 0.076% of all hepatocytes in steady-state adult mouse liver were newly born within the previous 4 days from *Alb-Cre*$^-$ cell populations, which could potentially contribute to homeostatic maintenance of liver parenchyma under normal conditions. Because the *Alb-Cre* activity is induced in hepatoblasts at the fetal stage and continues during their maturation to adult hepatocytes, this result implicates a contribution of nonepithelial lineage cells for hepatocyte maintenance, an intriguing possibility that needs to be further evaluated in other experimental settings.

Notwithstanding this complicated situation regarding in vivo identification, many groups have succeeded in isolating "liver stem cell" populations from the adult liver based on marker gene expression and flow cytometric cell purification followed by in vitro cultivation (Table 2 and Figure 3). As is the case with the fetal liver cells, the cells in question can be defined as "stem cells" because they are (1) clonogenic with high growth potential, (2) able to inducibly differentiate to both hepatocyte

and cholonagiocyte lineages under appropriate culture conditions, and, in some cases, (3) capable of repopulating the liver upon transplantation. Although these cells can be considered as ''potential'' liver stem cells because they can be functionally defined in culture, it remains unclear whether and where they exist in situ in living organisms and how they behave under physiological conditions. It should be noted that potential liver stem cell populations are usually identified as those that are positive for cholangiocyte markers including EpCAM (Okabe et al., 2009), CD133 (also known as prominin 1) (Kamiya et al., 2009), and the MIC1-1C3 antigen (Dorrell et al., 2011), implicating a possible role of the biliary system as a compartment harboring ''resident'' liver stem cells, if they exist (Figure 3). In support of this theory, $EpCAM^+$ cells isolated from postnatal human livers, as well as from fetal livers as mentioned earlier, have also been found to contain hepatic stem cells (hHpSCs) that can be defined based on their in vitro function (Schmelzer et al., 2007). Moreover, multipotential stem/progenitor cells that can give rise to hepatocytes, cholangiocytes, and pancreatic islets have been identified in humans within the peribiliary glands, which are unique epithelial structures in the extrahepatic biliary tree (Cardinale et al., 2011; Carpino et al., 2012). The corresponding stem/progenitor cells with similar characteristics and anatomical localization may also be present in mice (Dipaola et al., 2013; Irie et al., 2007), although they have not been fully characterized.

Stem/Progenitor Cells in Liver Regeneration

The characteristic feature of the liver is its high regenerative capacity, and there has also been debate, with some skepticism, as to whether stem cells are involved in this process. This skepticism is primarily because partial hepatectomy (PHx), the surgical removal of (a) particular lobe(s) of the organ, has long been regarded as the paradigm for experimental analysis of the mechanisms underlying liver regeneration (Michalopoulos, 2007; Michalopoulos and DeFrances, 1997). The PHx protocol does not cause any injury to the remnant hepatic tissue, and the subsequent regenerative process is considered to be achieved by hypertrophy and proliferation of mature hepatocytes (Miyaoka et al., 2012), without apparent involvement of any immature stem cell population. Indeed, the results of recent genetic lineage tracing studies in mice support this notion (Español-Suñer et al., 2012), although a small yet significant proportion of newborn hepatocytes generated from cellular sources other than preexisting hepatocytes has also been suggested (Furuyama et al., 2011; Iverson et al., 2011; Malato et al., 2011). Nevertheless, the robust regenerative capacity of hepatocytes manifested upon PHx is quite striking and thus might have contributed to a widespread prejudice that the liver does not require any stem/progenitor cells for its regeneration.

While PHx is truly an excellent model to study the process of compensatory growth of the liver and provides useful information relevant to living donor liver transplantation, it does not faithfully recapitulate pathological situations in many human liver diseases, which often involve hepatocyte death and concomitant induction of inflammatory and fibrogenic responses. Under many pathological conditions, such as chronic viral hepatitis, alcoholic liver disease, and nonalcoholic fatty liver disease, unique epithelial cell populations emerge and expand that are not usually observed in a normal liver (Figure 3). These cells typically exhibit immature and intermediate phenotypes between hepatocytes and cholangiocytes as determined by morphology and molecular marker expression, and they are considered to be bipotential progenitor cell populations (Fausto, 2004; Roskams et al., 2003, 2004; Turányi et al., 2010). Such cell populations have been termed in several different ways, such as ''ductular hepatocytes,'' ''atypical ductal cells,'' ''intermediate hepatobiliary cells,'' or ''hepatic/liver progenitor cells (HPCs/LPCs).'' The term ''oval cells,'' which was originally coined to describe a specific, ovoid cell population observed in a rat model of liver carcinogenesis (Farber, 1956), is also often used, particularly in rodent models. Some researchers consider such disease-activated progenitor cell populations as ''liver stem cells'' and, indeed, the term ''oval cells'' is sometimes introduced in literature as a synonym for liver stem cells.

Oval cells are the prototype for adult liver stem/progenitor cell populations, which emerge when the liver is injured under conditions causing defective hepatocyte proliferation (Fausto, 2004). The most established and reliable protocol currently used to induce oval cells is the 2-acetylaminofluorene (2-AAF)/PHx system in rats, where hepatocyte proliferation is blocked by 2-AAF prior to application of PHx (Evarts et al., 1987). Ever since their first description by Farber in 1956 (Farber, 1956), oval cells have been extensively characterized histologically, which cumulatively suggests that they have bipotential differentiation capability toward both hepatocytes and cholangiocytes. Unfortunately, the lack of genetic lineage tracing systems in rats (as well as the absence of oval-cell-specific markers) has hindered efforts to verify this theory. It is thus quite natural for liver biologists to examine whether oval cells exhibit stem cell activity in mouse models. Although the 2-AAF/PHx system is not applicable in mice, several other liver injury models have been used for ''oval cell'' induction, among which the administration of a 3,5-diethoxycarbonyl-1,4-dihidro-collidine (DDC)-containing diet (Preisegger et al., 1999) or a choline-deficient ethionine-supplemented diet (CDE) (Akhurst et al., 2001) are the most extensively used. However, it seems that the blockade of hepatocyte proliferation in these mouse models is not as complete as in the rat 2-AAF/PHx model. Moreover, the injuries sustained in those mouse models are markedly different from that of the 2-AAF/PHx rat model. The DDC-induced injury is considered to be targeted primarily to the biliary compartment and serves as a model for sclerosing cholangitis and biliary fibrosis (Fickert et al., 2007), while the CDE protocol induces fatty liver and is sometimes used as a model for nonalcoholic steatohepatitis. As the phenotypic and mechanistic differences among these models as well as other ''oval cell'' induction protocols have become more and more recognized, there is an increasing consensus that the cells induced therein are not exactly the same. Thus, applying the terminology ''oval cell'' in mouse injury models seems to have caused substantial confusion and should be avoided. We suggest that ''LPC'' (liver progenitor cell) is a more appropriate term to broadly describe all the various disease-activated, putative stem/progenitor cell populations that have been observed in the liver regardless of species or injury model.

Characterization of LPCs by Molecular Markers

Despite their potentially different characteristics and ontogeny based on disease etiology, various common markers, such as

CK19, EpCAM, and CD133, have been identified to describe LPCs in mice, rats, and humans (Okabe et al., 2009; Rountree et al., 2007; Suzuki et al., 2008; Yovchev et al., 2007). Sox9, osteopontin, and the MIC1-1C3 antigen are also regarded as equivalent LPC markers, at least in mice (Carpentier et al., 2011; Dorrell et al., 2008, 2011; Matsuo et al., 2011). Using flow-cytometry-based cell separation methods in conjunction with cell surface markers (e.g., EpCAM, CD133, or MIC1-1C3) or fluorescent reporters (e.g., Sox9-dependent YFP reporter), LPCs can be viably isolated and subjected to in vitro culture to be evaluated for their proliferation and differentiation potentials. Collectively, such isolation and culture methods have demonstrated that LPCs contained cells that are clonogenic in vitro and can differentiate into both hepatocytic and cholangiocytic lineages under certain culture conditions (Table 2 and Figure 3). These results strongly suggest that LPCs, when strictly defined by clonogenicity and in vitro differentiation potential, indeed contain bipotential stem/progenitor cells in the liver. Importantly, cholangiocytes isolated from the normal liver based on the same molecular markers also qualitatively exhibit the same potential in vitro. Thus, it remains uncertain whether in vitro assays adequately and specifically evaluate the potential of disease-activated LPCs distinct from the "potential" liver stem cells residing in the biliary compartment under normal conditions.

Several studies have identified possible LPC-specific markers that can distinguish LPCs form cholangiocytes in the normal liver. Trop2 (Tacstd2), a transmembrane molecule that is structurally related to and is a paralog of EpCAM, has been found to be expressed exclusively in LPCs under the DDC-induced injury condition, but not in cholangiocytes in the normal liver (Okabe et al., 2009). Thus, Trop2 may serve as a genuine LPC-specific marker and if so, it would be advantageous for further characterization of LPCs. The clonogenicity and differentiation potential of the Trop2$^+$ cells as well as experiments tracing their fate have not yet been examined. The transcription factor Foxl1 has been identified as another potential LPC-specific marker, in that a transgenic (Tg) mouse line expressing Cre recombinase under the control of the *Foxl1* promoter has been used to demonstrate that both hepatocytes and cholangiocytes were derived from Foxl1$^+$ LPCs under certain injury conditions (Sackett et al., 2009). The Cre recombinase used in the *Foxl1-Cre* Tg mouse is constitutively expressed and cannot be temporally regulated, so it remains undetermined whether single Foxl1$^+$ LPCs can clonally differentiate into these two lineages or whether the Foxl1$^+$ population contains distinct hepatocytic and cholangiocytic progenitors. More recently, Lgr5, a well-established marker for stem cells in the intestine as well as several other tissues/organs, has also been demonstrated to be expressed specifically in damage-induced LPCs in the liver using *Lgr5-LacZ* and *Lgr5-CreERT2* KI mice (Huch et al., 2013). Lgr5$^+$ cells can be clonally expanded as transplantable organoids in a 3D culture system, and those organoids retain many characteristics of the original epithelial architecture. The relationship between Lgr5$^+$ cells and those expressing the authentic LPC markers such as CK19 and EpCAM has not been clearly determined. In addition, a major drawback for Foxl1 and Lgr5 is that expression of the endogenous genes/proteins in LPCs has not been clearly demonstrated thus far, and assays currently rely on the use of those particular Tg and KI mouse lines. It should be emphasized that the Foxl1 and Lgr5 markers are not expressed prior to damage and thus cannot be used to prelabel the cells that arise during regeneration. Nevertheless, further characterization of such cell populations should provide unique and more versatile LPC-specific markers and genetic tools, with which clonal analyses of LPCs may be achieved in vivo.

Origin of LPCs

LPCs are apparently a "facultative" stem/progenitor cell population that emerge and are recognized only under damage conditions, and the cell of origin for this subset of cells is of significant interest. Based on the histological analyses of rat oval cells, it has long been proposed that the canal of Hering, the junctional structure connecting bile canaliculi formed by hepatocytes with bile ducts lined by cholangiocytes, is the origin of these cells (Paku et al., 2001) (Figure 3). This notion is consistent with the fact that LPCs almost always emerge from the periportal area. Furthermore, given its anatomical location between cholangiocyte and hepatocyte populations, it seems reasonable to assume that this structure may serve as a niche for putative progenitor cell populations that are destined to differentiate toward these two cell lineages. Hence, the concept that the canal of Hering may serve as the origin of LPCs is widely accepted, though it is not formally proven to be applicable to such progenitors in general. This notion further suggests that the canal of Hering serves as a niche for a specialized precursor cell population for LPCs, which some researchers would prefer to denote as "liver stem cells." Unfortunately, however, direct proof for this idea is still hampered by lack of any specific marker for such cells as well as the cells constituting the canal of Hering. As mentioned earlier, in vitro assays have clearly detected the presence of potential liver stem cells with clonogenicity and bilineage differentiation potential in the biliary compartment, even in the normal liver. It is of considerable interest to determine whether they actually correspond to and play a role as the reserve stem cells in vivo to produce LPCs once the liver is injured.

While much effort is still underway to identify putative, specific markers for the cell of origin for LPCs, Cre/loxP-mediated genetic lineage tracing studies in mice have been done by labeling "mature" liver epithelial cells based on well-established differentiation markers. Consistent with the fact that most of the molecular markers for LPCs are also expressed in cholangiocytes, lineage tracing studies employing cholangiocyte-specific Cre driver strains have collectively indicated that LPCs identified under liver injury conditions can be derived from cholangiocytes in the normal liver (Dorrell et al., 2011; Español-Suñer et al., 2012; Furuyama et al., 2011). It is not clear, however, whether most if not all cholangiocytes can equally or similarly behave as precursors for LPCs, or if there is a certain type of specialized "LPC cell-of-origin" subpopulation located somewhere among cholangiocytes. For the latter possibility, the cholangiocytes adjacent to hepatocytes that form the canal of Hering would be a plausible candidate. Notably, the potential contribution of hepatocytes as an origin for LPCs can also be considered. In a study using mice with chimeric livers generated from the transplantation of wild-type (*Fah*$^{+/+}$) mouse-derived hepatocytes to *Fah*$^{-/-}$ recipients, which resulted in more than 90% repopulation, almost all of the LPCs induced upon subsequent application of

the DDC injury were found to be of host (*Fah*$^{-/-}$) origin and not derived from repopulated (wild-type) hepatocytes, suggesting that hepatocytes are not likely to act as the cell of origin for LPCs (Wang et al., 2003). However, more recent studies employing lineage tracing approaches have demonstrated that hepatocytes can be reprogrammed to LPCs under certain liver injury conditions including the DDC model (Yanger et al., 2013). Again, it is unclear whether most if not all mature hepatocytes possess such plasticity or whether there are specific subsets of hepatocytes, such as those lining a part of the canal of Hering, that can be converted to LPCs.

In addition to the phenotypic and ontogenic characterization of LPCs, many recent studies focus on elucidating the cellular and molecular frameworks for their regulatory mechanisms and have identified several key signaling pathways. Similar to the various extrahepatic tissue stem/progenitor cells, a set of developmental "toolkit" genes, including Wnt (Apte et al., 2008; Boulter et al., 2012; Hu et al., 2007; Itoh et al., 2009; Yang et al., 2008), Notch (Boulter et al., 2012; Fiorotto et al., 2013; Kitade et al., 2013), and FGF (Takase et al., 2013), have been shown to play a relevant role in LPC regulation. Consistent with the notion that chronic liver injury conditions where LPCs are activated usually accompany inflammatory responses, involvement of several inflammatory cytokines, such as tumor necrosis factor (TNF)-alpha, interleukin-6, and interferon-gamma, has also been reported (Akhurst et al., 2005; Knight et al., 2000; Yeoh et al., 2007). Among those factors, TNF-related WEAK inducer of apoptosis (TWEAK) and FGF7 are of particular interest because they are capable of inducing de novo activation of LPCs. Forced expression of either of these factors in the normal liver can lead to induction and expansion of cells reminiscent of LPCs, even in the absence of any liver injury regimen (Jakubowski et al., 2005; Takase et al., 2013). This phenomenon strongly suggests that the cell of origin for LPCs is responsive to these extracellular signals. Other growth factors, such as HGF and EGF, have also been implicated in regulating proliferation and/or differentiation of LPCs (Ishikawa et al., 2012; Kitade et al., 2013). These humoral factors may be of potential use in therapeutic strategies to counter liver disease by enhancing the inherent regenerative capacity within the organ.

Cancer Stem Cells in the Liver and Their Origins

Tumors exhibit considerable heterogeneity even though they arise clonally. This heterogeneity is thought to result from hierarchical organization of the tumor cells by a subset of cells with stem cell features, i.e., cancer stem cells (CSCs) with the potential for self-renewal and differentiation (Kreso and Dick, 2014). Because elimination of CSCs is necessary for eradication of tumors, much attention has been given to the identification of CSCs for developing effective therapeutic drugs. Besides such a practical point of view, the origin of CSCs, an abnormal stem cell type, is of considerable interest in stem cell biology. Liver cancer is an aggressive disease with a poor outcome. Among primary liver cancers, hepatocellular carcinoma (HCC) accounts for 70%–85% of the cases and intrahepatic cholangiocarcinoma (ICC) is the second most frequent type of liver cancer. Both HCC and ICC are heterogenous in their cellular morphology and clinical outcome. Mixed HCC-cholangiocellular carcinoma (HCC-CCC) is a rare form of liver cancer exhibiting both hepatocellular and cholangiocellular features, reminiscent of liver stem/progenitor cells. Intriguingly, forced expression of polycomb group protein Bmi1 or β-catenin in hepatoblasts allowed their expansion in vitro, and transplantation of clonally expanded cells produced tumors with histological features of combined HCC-CCC, suggesting that liver stem/progenitor cells may be the origin of such tumors (Chiba et al., 2007). However, the vast majority of human HCCs arise from chronic infection with hepatitis virus with HCC subsequently developing after a long latency, suggesting that HCC is derived from mature hepatocytes. HCC exhibits considerable heterogeneity and often contains cells expressing liver stem/progenitor markers, e.g., EpCAM, CD133, CD44, CD24, and DLK1 (reviewed in Yamashita and Wang, 2013). Consistently, recent studies have identified the oncofetal gene SALL4, which encodes a transcription factor functioning in fetal liver development (Oikawa et al., 2009), as a prognostic marker for a progenitor subclass of hepatocellular carcinoma with an aggressive phenotype (Oikawa et al., 2013; Yong et al., 2013; Zeng et al., 2014). Interestingly, some cell lines derived from HCC such as Huh7 also show heterogeneity and a subpopulation expressing some of these stem/progenitor cell markers or excreting Hoechst dye (side population) were shown to develop tumors in immunodeficient mice, indicating that they are tumor initiating cells, a key characteristic of CSCs (Chiba et al., 2006; Haraguchi et al., 2010). However, because most of previous studies showing tumor-initiating activity were based on cell lines, isolation of fresh cells with stem cell markers from patient tissues and demonstration of tumor-initiating activity is necessary for precise identification of liver CSCs. Because stem/progenitor cell markers are often expressed in HCC, mature hepatocytes may regress to an immature stage during tumorigenesis. Alternatively, there may be immature hepatocytes infected with virus, which then give rise to tumors. Intriguingly, recent studies using cell fate tracing of hepatocytes demonstrated that ICC originates from hepatocytes rather than cholangiocytes and that Notch and Akt signaling cooperate to convert hepatocytes into biliary cells that act as precursors of ICCs (Fan et al., 2012; Sekiya and Suzuki, 2012). While it remains unknown whether stem/progenitor cells emerge transiently during the conversion, the plasticity of hepatocytes provides insight into the mechanism for pathogenesis as a consequence of defective liver regeneration.

Conclusion

Hepatocytes and cholangiocytes are two types of liver epithelial cells and the cell type that eventually gives rise to both epithelial cells has been considered to be the liver stem cell. In fetal, adult, and diseased livers, there are clearly some cells with the potential to proliferate and differentiate to both cell types in vitro, indicating the presence of "potential" stem cells in liver. However, if the liver stem cell is defined by the ability for continuous self-renewal and contribution to both type of epithelial cells in vivo, it is questionable whether liver stem cells are present in the body. Hepatoblasts in fetal liver proliferate but also change their phenotype throughout development, so they are more appropriately defined as progenitor cells. In normal adult liver, the presence of a resident self-renewable stem cell still remains to be demonstrated. In injured and regenerating livers, proliferating cells around the portal vein are also better referred to as progenitor cells rather than stem cells until any solid evidence showing

their clonal differentiation to both hepatocytes and cholangiocytes in vivo is provided. Because there is substantial variability among definitions and identification strategies, extreme caution and consideration should be employed when comparing results from the literature that refer to "liver stem cells," as the characteristics of these cells may vary according to the way they are defined in each study.

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REFERENCES

Akhurst, B., Croager, E.J., Farley-Roche, C.A., Ong, J.K., Dumble, M.L., Knight, B., and Yeoh, G.C. (2001). A modified choline-deficient, ethionine-supplemented diet protocol effectively induces oval cells in mouse liver. Hepatology *34*, 519–522.

Akhurst, B., Matthews, V., Husk, K., Smyth, M.J., Abraham, L.J., and Yeoh, G.C. (2005). Differential lymphotoxin-beta and interferon gamma signaling during mouse liver regeneration induced by chronic and acute injury. Hepatology *41*, 327–335.

Alagille, D., Estrada, A., Hadchouel, M., Gautier, M., Odièvre, M., and Dommergues, J.P. (1987). Syndromic paucity of interlobular bile ducts (Alagille syndrome or arteriohepatic dysplasia): review of 80 cases. J. Pediatr. *110*, 195–200.

Antoniou, A., Raynaud, P., Cordi, S., Zong, Y., Tronche, F., Stanger, B.Z., Jacquemin, P., Pierreux, C.E., Clotman, F., and Lemaigre, F.P. (2009). Intrahepatic bile ducts develop according to a new mode of tubulogenesis regulated by the transcription factor SOX9. Gastroenterology *136*, 2325–2333.

Apte, U., Thompson, M.D., Cui, S., Liu, B., Cieply, B., and Monga, S.P. (2008). Wnt/beta-catenin signaling mediates oval cell response in rodents. Hepatology *47*, 288–295.

Banas, A., Teratani, T., Yamamoto, Y., Tokuhara, M., Takeshita, F., Quinn, G., Okochi, H., and Ochiya, T. (2007). Adipose tissue-derived mesenchymal stem cells as a source of human hepatocytes. Hepatology *46*, 219–228.

Barker, N., van Oudenaarden, A., and Clevers, H. (2012). Identifying the stem cell of the intestinal crypt: strategies and pitfalls. Cell Stem Cell *11*, 452–460.

Benhamouche, S., Decaens, T., Godard, C., Chambrey, R., Rickman, D.S., Moinard, C., Vasseur-Cognet, M., Kuo, C.J., Kahn, A., Perret, C., and Colnot, S. (2006). Apc tumor suppressor gene is the "zonation-keeper" of mouse liver. Dev. Cell *10*, 759–770.

Blanpain, C., and Fuchs, E. (2009). Epidermal homeostasis: a balancing act of stem cells in the skin. Nat. Rev. Mol. Cell Biol. *10*, 207–217.

Boulter, L., Govaere, O., Bird, T.G., Radulescu, S., Ramachandran, P., Pellicoro, A., Ridgway, R.A., Seo, S.S., Spee, B., Van Rooijen, N., et al. (2012). Macrophage-derived Wnt opposes Notch signaling to specify hepatic progenitor cell fate in chronic liver disease. Nat. Med. *18*, 572–579.

Burke, Z.D., Reed, K.R., Phesse, T.J., Sansom, O.J., Clarke, A.R., and Tosh, D. (2009). Liver zonation occurs through a beta-catenin-dependent, c-Myc-independent mechanism. Gastroenterology *136*, 2316–2324, e1–e3.

Cardinale, V., Wang, Y., Carpino, G., Cui, C.B., Gatto, M., Rossi, M., Berloco, P.B., Cantafora, A., Wauthier, E., Furth, M.E., et al. (2011). Multipotent stem/progenitor cells in human biliary tree give rise to hepatocytes, cholangiocytes, and pancreatic islets. Hepatology *54*, 2159–2172.

Carpentier, R., Suñer, R.E., van Hul, N., Kopp, J.L., Beaudry, J.B., Cordi, S., Antoniou, A., Raynaud, P., Lepreux, S., Jacquemin, P., et al. (2011). Embryonic ductal plate cells give rise to cholangiocytes, periportal hepatocytes, and adult liver progenitor cells. Gastroenterology *141*, 1432–1438, e1–e4.

Carpino, G., Cardinale, V., Onori, P., Franchitto, A., Berloco, P.B., Rossi, M., Wang, Y., Semeraro, R., Anceschi, M., Brunelli, R., et al. (2012). Biliary tree stem/progenitor cells in glands of extrahepatic and intraheptic bile ducts: an anatomical in situ study yielding evidence of maturational lineages. J. Anat. *220*, 186–199.

Chiba, T., Kita, K., Zheng, Y.W., Yokosuka, O., Saisho, H., Iwama, A., Nakauchi, H., and Taniguchi, H. (2006). Side population purified from hepatocellular carcinoma cells harbors cancer stem cell-like properties. Hepatology *44*, 240–251.

Chiba, T., Zheng, Y.W., Kita, K., Yokosuka, O., Saisho, H., Onodera, M., Miyoshi, H., Nakano, M., Zen, Y., Nakanuma, Y., et al. (2007). Enhanced self-renewal capability in hepatic stem/progenitor cells drives cancer initiation. Gastroenterology *133*, 937–950.

Clotman, F., Jacquemin, P., Plumb-Rudewiez, N., Pierreux, C.E., Van der Smissen, P., Dietz, H.C., Courtoy, P.J., Rousseau, G.G., and Lemaigre, F.P. (2005). Control of liver cell fate decision by a gradient of TGF beta signaling modulated by Onecut transcription factors. Genes Dev. *19*, 1849–1854.

Dan, Y.Y., Riehle, K.J., Lazaro, C., Teoh, N., Haque, J., Campbell, J.S., and Fausto, N. (2006). Isolation of multipotent progenitor cells from human fetal liver capable of differentiating into liver and mesenchymal lineages. Proc. Natl. Acad. Sci. USA *103*, 9912–9917.

Dipaola, F., Shivakumar, P., Pfister, J., Walters, S., Sabla, G., and Bezerra, J.A. (2013). Identification of intramural epithelial networks linked to peribiliary glands that express progenitor cell markers and proliferate after injury in mice. Hepatology *58*, 1486–1496.

Dollé, L., Best, J., Empsen, C., Mei, J., Van Rossen, E., Roelandt, P., Snykers, S., Najimi, M., Al Battah, F., Theise, N.D., et al. (2012). Successful isolation of liver progenitor cells by aldehyde dehydrogenase activity in naïve mice. Hepatology *55*, 540–552.

Dorrell, C., Erker, L., Lanxon-Cookson, K.M., Abraham, S.L., Victoroff, T., Ro, S., Canaday, P.S., Streeter, P.R., and Grompe, M. (2008). Surface markers for the murine oval cell response. Hepatology *48*, 1282–1291.

Dorrell, C., Erker, L., Schug, J., Kopp, J.L., Canaday, P.S., Fox, A.J., Smirnova, O., Duncan, A.W., Finegold, M.J., Sander, M., et al. (2011). Prospective isolation of a bipotential clonogenic liver progenitor cell in adult mice. Genes Dev. *25*, 1193–1203.

Du, Y., Wang, J., Jia, J., Song, N., Xiang, C., Xu, J., Hou, Z., Su, X., Liu, B., Jiang, T., et al. (2014). Human hepatocytes with drug metabolic function induced from fibroblasts by lineage reprogramming. Cell Stem Cell *14*, 394–403.

Español-Suñer, R., Carpentier, R., Van Hul, N., Legry, V., Achouri, Y., Cordi, S., Jacquemin, P., Lemaigre, F., and Leclercq, I.A. (2012). Liver progenitor cells yield functional hepatocytes in response to chronic liver injury in mice. Gastroenterology *143*, 1564–1575.e7.

Evarts, R.P., Nagy, P., Marsden, E., and Thorgeirsson, S.S. (1987). A precursor-product relationship exists between oval cells and hepatocytes in rat liver. Carcinogenesis *8*, 1737–1740.

Fan, B., Malato, Y., Calvisi, D.F., Naqvi, S., Razumilava, N., Ribback, S., Gores, G.J., Dombrowski, F., Evert, M., Chen, X., and Willenbring, H. (2012). Cholangiocarcinomas can originate from hepatocytes in mice. J. Clin. Invest. *122*, 2911–2915.

Farber, E. (1956). Similarities in the sequence of early histological changes induced in the liver of the rat by ethionine, 2-acetylamino-fluorene, and 3′-methyl-4-dimethylaminoazobenzene. Cancer Res. *16*, 142–148.

Fausto, N. (2004). Liver regeneration and repair: hepatocytes, progenitor cells, and stem cells. Hepatology *39*, 1477–1487.

Fickert, P., Stöger, U., Fuchsbichler, A., Moustafa, T., Marschall, H.U., Weiglein, A.H., Tsybrovskyy, O., Jaeschke, H., Zatloukal, K., Denk, H., and Trauner, M. (2007). A new xenobiotic-induced mouse model of sclerosing cholangitis and biliary fibrosis. Am. J. Pathol. *171*, 525–536.

Fiegel, H.C., Kluth, J., Lioznov, M.V., Holzhüter, S., Fehse, B., Zander, A.R., and Kluth, D. (2003). Hepatic lineages isolated from developing rat liver show different ways of maturation. Biochem. Biophys. Res. Commun. *305*, 46–53.

Fiorotto, R., Raizner, A., Morell, C.M., Torsello, B., Scirpo, R., Fabris, L., Spirli, C., and Strazzabosco, M. (2013). Notch signaling regulates tubular morphogenesis during repair from biliary damage in mice. J. Hepatol. *59*, 124–130.

Fuchs, E. (2009). The tortoise and the hair: slow-cycling cells in the stem cell race. Cell *137*, 811–819.

Furuyama, K., Kawaguchi, Y., Akiyama, H., Horiguchi, M., Kodama, S., Kuhara, T., Hosokawa, S., Elbahrawy, A., Soeda, T., Koizumi, M., et al. (2011). Continuous cell supply from a Sox9-expressing progenitor zone in adult liver, exocrine pancreas and intestine. Nat. Genet. *43*, 34–41.

Gadue, P., Gouon-Evans, V., Cheng, X., Wandzioch, E., Zaret, K.S., Grompe, M., Streeter, P.R., and Keller, G.M. (2009). Generation of monoclonal antibodies specific for cell surface molecules expressed on early mouse endoderm. Stem Cells *27*, 2103–2113.

Goldman, O., Han, S., Sourrisseau, M., Dziedzic, N., Hamou, W., Corneo, B., D'Souza, S., Sato, T., Kotton, D.N., Bissig, K.D., et al. (2013). KDR identifies a conserved human and murine hepatic progenitor and instructs early liver development. Cell Stem Cell *12*, 748–760.

Gualdi, R., Bossard, P., Zheng, M., Hamada, Y., Coleman, J.R., and Zaret, K.S. (1996). Hepatic specification of the gut endoderm in vitro: cell signaling and transcriptional control. Genes Dev. *10*, 1670–1682.

Haraguchi, N., Ishii, H., Mimori, K., Tanaka, F., Ohkuma, M., Kim, H.M., Akita, H., Takiuchi, D., Hatano, H., Nagano, H., et al. (2010). CD13 is a therapeutic target in human liver cancer stem cells. J. Clin. Invest. *120*, 3326–3339.

Hofmann, J.J., Zovein, A.C., Koh, H., Radtke, F., Weinmaster, G., and Iruela-Arispe, M.L. (2010). Jagged1 in the portal vein mesenchyme regulates intrahepatic bile duct development: insights into Alagille syndrome. Development *137*, 4061–4072.

Hoppo, T., Fujii, H., Hirose, T., Yasuchika, K., Azuma, H., Baba, S., Naito, M., Machimoto, T., and Ikai, I. (2004). Thy1-positive mesenchymal cells promote the maturation of CD49f-positive hepatic progenitor cells in the mouse fetal liver. Hepatology *39*, 1362–1370.

Hu, M., Kurobe, M., Jeong, Y.J., Fuerer, C., Ghole, S., Nusse, R., and Sylvester, K.G. (2007). Wnt/beta-catenin signaling in murine hepatic transit amplifying progenitor cells. Gastroenterology *133*, 1579–1591.

Huang, P., He, Z., Ji, S., Sun, H., Xiang, D., Liu, C., Hu, Y., Wang, X., and Hui, L. (2011). Induction of functional hepatocyte-like cells from mouse fibroblasts by defined factors. Nature *475*, 386–389.

Huang, P., Zhang, L., Gao, Y., He, Z., Yao, D., Wu, Z., Cen, J., Chen, X., Liu, C., Hu, Y., et al. (2014). Direct reprogramming of human fibroblasts to functional and expandable hepatocytes. Cell Stem Cell *14*, 370–384.

Huch, M., Dorrell, C., Boj, S.F., van Es, J.H., Li, V.S., van de Wetering, M., Sato, T., Hamer, K., Sasaki, N., Finegold, M.J., et al. (2013). In vitro expansion of single Lgr5+ liver stem cells induced by Wnt-driven regeneration. Nature *494*, 247–250.

Ijpenberg, A., Pérez-Pomares, J.M., Guadix, J.A., Carmona, R., Portillo-Sánchez, V., Macías, D., Hohenstein, P., Miles, C.M., Hastie, N.D., and Muñoz-Chápuli, R. (2007). Wt1 and retinoic acid signaling are essential for stellate cell development and liver morphogenesis. Dev. Biol. *312*, 157–170.

Irie, T., Asahina, K., Shimizu-Saito, K., Teramoto, K., Arii, S., and Teraoka, H. (2007). Hepatic progenitor cells in the mouse extrahepatic bile duct after a bile duct ligation. Stem Cells Dev. *16*, 979–987.

Ishikawa, T., Factor, V.M., Marquardt, J.U., Raggi, C., Seo, D., Kitade, M., Conner, E.A., and Thorgeirsson, S.S. (2012). Hepatocyte growth factor/c-met signaling is required for stem-cell-mediated liver regeneration in mice. Hepatology *55*, 1215–1226.

Itoh, T., Kamiya, Y., Okabe, M., Tanaka, M., and Miyajima, A. (2009). Inducible expression of Wnt genes during adult hepatic stem/progenitor cell response. FEBS Lett. *583*, 777–781.

Iverson, S.V., Comstock, K.M., Kundert, J.A., and Schmidt, E.E. (2011). Contributions of new hepatocyte lineages to liver growth, maintenance, and regeneration in mice. Hepatology *54*, 655–663.

Jakubowski, A., Ambrose, C., Parr, M., Lincecum, J.M., Wang, M.Z., Zheng, T.S., Browning, B., Michaelson, J.S., Baetscher, M., Wang, B., et al. (2005). TWEAK induces liver progenitor cell proliferation. J. Clin. Invest. *115*, 2330–2340.

Jung, J., Zheng, M., Goldfarb, M., and Zaret, K.S. (1999). Initiation of mammalian liver development from endoderm by fibroblast growth factors. Science *284*, 1998–2003.

Kakinuma, S., Ohta, H., Kamiya, A., Yamazaki, Y., Oikawa, T., Okada, K., and Nakauchi, H. (2009). Analyses of cell surface molecules on hepatic stem/progenitor cells in mouse fetal liver. J. Hepatol. *51*, 127–138.

Kamiya, A., Kinoshita, T., Ito, Y., Matsui, T., Morikawa, Y., Senba, E., Nakashima, K., Taga, T., Yoshida, K., Kishimoto, T., and Miyajima, A. (1999). Fetal liver development requires a paracrine action of oncostatin M through the gp130 signal transducer. EMBO J. *18*, 2127–2136.

Kamiya, A., Kojima, N., Kinoshita, T., Sakai, Y., and Miyaijma, A. (2002). Maturation of fetal hepatocytes in vitro by extracellular matrices and oncostatin M: induction of tryptophan oxygenase. Hepatology *35*, 1351–1359.

Kamiya, A., Kakinuma, S., Yamazaki, Y., and Nakauchi, H. (2009). Enrichment and clonal culture of progenitor cells during mouse postnatal liver development in mice. Gastroenterology *137*, 1114–1126, e1–e14.

Kitade, M., Factor, V.M., Andersen, J.B., Tomokuni, A., Kaji, K., Akita, H., Holczbauer, A., Seo, D., Marquardt, J.U., Conner, E.A., et al. (2013). Specific fate decisions in adult hepatic progenitor cells driven by MET and EGFR signaling. Genes Dev. *27*, 1706–1717.

Knight, B., Yeoh, G.C., Husk, K.L., Ly, T., Abraham, L.J., Yu, C., Rhim, J.A., and Fausto, N. (2000). Impaired preneoplastic changes and liver tumor formation in tumor necrosis factor receptor type 1 knockout mice. J. Exp. Med. *192*, 1809–1818.

Kodama, Y., Hijikata, M., Kageyama, R., Shimotohno, K., and Chiba, T. (2004). The role of notch signaling in the development of intrahepatic bile ducts. Gastroenterology *127*, 1775–1786.

Kreso, A., and Dick, J.E. (2014). Evolution of the Cancer Stem Cell Model. Cell Stem Cell *14*, 275–291.

Kubota, H., and Reid, L.M. (2000). Clonogenic hepatoblasts, common precursors for hepatocytic and biliary lineages, are lacking classical major histocompatibility complex class I antigen. Proc. Natl. Acad. Sci. USA *97*, 12132–12137.

Kyrmizi, I., Hatzis, P., Katrakili, N., Tronche, F., Gonzalez, F.J., and Talianidis, I. (2006). Plasticity and expanding complexity of the hepatic transcription factor network during liver development. Genes Dev. *20*, 2293–2305.

Lee, K.D., Kuo, T.K., Whang-Peng, J., Chung, Y.F., Lin, C.T., Chou, S.H., Chen, J.R., Chen, Y.P., and Lee, O.K. (2004). In vitro hepatic differentiation of human mesenchymal stem cells. Hepatology *40*, 1275–1284.

Li, L., Krantz, I.D., Deng, Y., Genin, A., Banta, A.B., Collins, C.C., Qi, M., Trask, B.J., Kuo, W.L., Cochran, J., et al. (1997). Alagille syndrome is caused by mutations in human Jagged1, which encodes a ligand for Notch1. Nat. Genet. *16*, 243–251.

Li, K., Zhu, S., Russ, H.A., Xu, S., Xu, T., Zhang, Y., Ma, T., Hebrok, M., and Ding, S. (2014). Small molecules facilitate the reprogramming of mouse fibroblasts into pancreatic lineages. Cell Stem Cell *14*, 228–236.

MacDonald, R.A. (1961). "Lifespan" of liver cells. Autoradio-graphic study using tritiated thymidine in normal, cirrhotic, and partially hepatectomized rats. Arch. Intern. Med. *107*, 335–343.

Magami, Y., Azuma, T., Inokuchi, H., Kokuno, S., Moriyasu, F., Kawai, K., and Hattori, T. (2002). Cell proliferation and renewal of normal hepatocytes and bile duct cells in adult mouse liver. Liver *22*, 419–425.

Malato, Y., Naqvi, S., Schürmann, N., Ng, R., Wang, B., Zape, J., Kay, M.A., Grimm, D., and Willenbring, H. (2011). Fate tracing of mature hepatocytes in mouse liver homeostasis and regeneration. J. Clin. Invest. *121*, 4850–4860.

Matsui, T., Kinoshita, T., Morikawa, Y., Tohya, K., Katsuki, M., Ito, Y., Kamiya, A., and Miyajima, A. (2002). K-Ras mediates cytokine-induced formation of E-cadherin-based adherens junctions during liver development. EMBO J. *21*, 1021–1030.

Matsumoto, K., Yoshitomi, H., Rossant, J., and Zaret, K.S. (2001). Liver organogenesis promoted by endothelial cells prior to vascular function. Science *294*, 559–563.

Matsuo, A., Yoshida, T., Yasukawa, T., Miki, R., Kume, K., and Kume, S. (2011). Epiplakin1 is expressed in the cholangiocyte lineage cells in normal liver and adult progenitor cells in injured liver. Gene Expr. Patterns *11*, 255–262.

McCright, B., Lozier, J., and Gridley, T. (2002). A mouse model of Alagille syndrome: Notch2 as a genetic modifier of Jag1 haploinsufficiency. Development *129*, 1075–1082.

McDaniell, R., Warthen, D.M., Sanchez-Lara, P.A., Pai, A., Krantz, I.D., Piccoli, D.A., and Spinner, N.B. (2006). NOTCH2 mutations cause Alagille syndrome, a heterogeneous disorder of the notch signaling pathway. Am. J. Hum. Genet. *79*, 169–173.

Michalopoulos, G.K. (2007). Liver regeneration. J. Cell. Physiol. *213*, 286–300.

Michalopoulos, G.K., and DeFrances, M.C. (1997). Liver regeneration. Science *276*, 60–66.

Minguet, S., Cortegano, I., Gonzalo, P., Martínez-Marin, J.A., de Andrés, B., Salas, C., Melero, D., Gaspar, M.L., and Marcos, M.A. (2003). A population of c-Kit(low)(CD45/TER119)- hepatic cell progenitors of 11-day postcoitus mouse embryo liver reconstitutes cell-depleted liver organoids. J. Clin. Invest. *112*, 1152–1163.

Miyaoka, Y., Ebato, K., Kato, H., Arakawa, S., Shimizu, S., and Miyajima, A. (2012). Hypertrophy and unconventional cell division of hepatocytes underlie liver regeneration. Curr. Biol. *22*, 1166–1175.

Nierhoff, D., Ogawa, A., Oertel, M., Chen, Y.Q., and Shafritz, D.A. (2005). Purification and characterization of mouse fetal liver epithelial cells with high in vivo repopulation capacity. Hepatology *42*, 130–139.

Nierhoff, D., Levoci, L., Schulte, S., Goeser, T., Rogler, L.E., and Shafritz, D.A. (2007). New cell surface markers for murine fetal hepatic stem cells identified through high density complementary DNA microarrays. Hepatology *46*, 535–547.

Nitou, M., Sugiyama, Y., Ishikawa, K., and Shiojiri, N. (2002). Purification of fetal mouse hepatoblasts by magnetic beads coated with monoclonal anti-e-cadherin antibodies and their in vitro culture. Exp. Cell Res. *279*, 330–343.

Oda, T., Elkahloun, A.G., Pike, B.L., Okajima, K., Krantz, I.D., Genin, A., Piccoli, D.A., Meltzer, P.S., Spinner, N.B., Collins, F.S., and Chandrasekharappa, S.C. (1997). Mutations in the human Jagged1 gene are responsible for Alagille syndrome. Nat. Genet. *16*, 235–242.

Oertel, M., Menthena, A., Chen, Y.Q., Teisner, B., Jensen, C.H., and Shafritz, D.A. (2008). Purification of fetal liver stem/progenitor cells containing all the repopulation potential for normal adult rat liver. Gastroenterology *134*, 823–832.

Ogawa, S., Surapisitchat, J., Virtanen, C., Ogawa, M., Niapour, M., Sugamori, K.S., Wang, S., Tamblyn, L., Guillemette, C., Hoffmann, E., et al. (2013). Three-dimensional culture and cAMP signaling promote the maturation of human pluripotent stem cell-derived hepatocytes. Development *140*, 3285–3296.

Oguro, H., Ding, L., and Morrison, S.J. (2013). SLAM family markers resolve functionally distinct subpopulations of hematopoietic stem cells and multipotent progenitors. Cell Stem Cell *13*, 102–116.

Oikawa, T., Kamiya, A., Kakinuma, S., Zeniya, M., Nishinakamura, R., Tajiri, H., and Nakauchi, H. (2009). Sall4 regulates cell fate decision in fetal hepatic stem/progenitor cells. Gastroenterology *136*, 1000–1011.

Oikawa, T., Kamiya, A., Zeniya, M., Chikada, H., Hyuck, A.D., Yamazaki, Y., Wauthier, E., Tajiri, H., Miller, L.D., Wang, X.W., et al. (2013). Sal-like protein 4 (SALL4), a stem cell biomarker in liver cancers. Hepatology *57*, 1469–1483.

Okabe, M., Tsukahara, Y., Tanaka, M., Suzuki, K., Saito, S., Kamiya, Y., Tsujimura, T., Nakamura, K., and Miyajima, A. (2009). Potential hepatic stem cells reside in EpCAM+ cells of normal and injured mouse liver. Development *136*, 1951–1960.

Onitsuka, I., Tanaka, M., and Miyajima, A. (2010). Characterization and functional analyses of hepatic mesothelial cells in mouse liver development. Gastroenterology *138*, 1525–1535, e1–e6.

Paku, S., Schnur, J., Nagy, P., and Thorgeirsson, S.S. (2001). Origin and structural evolution of the early proliferating oval cells in rat liver. Am. J. Pathol. *158*, 1313–1323.

Preisegger, K.H., Factor, V.M., Fuchsbichler, A., Stumptner, C., Denk, H., and Thorgeirsson, S.S. (1999). Atypical ductular proliferation and its inhibition by transforming growth factor beta1 in the 3,5-diethoxycarbonyl-1,4-dihydrocollidine mouse model for chronic alcoholic liver disease. Lab. Invest. *79*, 103–109.

Qiu, Q., Hernandez, J.C., Dean, A.M., Rao, P.H., and Darlington, G.J. (2011). CD24-positive cells from normal adult mouse liver are hepatocyte progenitor cells. Stem Cells Dev. *20*, 2177–2188.

Rashid, S.T., Corbineau, S., Hannan, N., Marciniak, S.J., Miranda, E., Alexander, G., Huang-Doran, I., Griffin, J., Ahrlund-Richter, L., Skepper, J., et al. (2010). Modeling inherited metabolic disorders of the liver using human induced pluripotent stem cells. J. Clin. Invest. *120*, 3127–3136.

Roskams, T.A., Libbrecht, L., and Desmet, V.J. (2003). Progenitor cells in diseased human liver. Semin. Liver Dis. *23*, 385–396.

Roskams, T.A., Theise, N.D., Balabaud, C., Bhagat, G., Bhathal, P.S., Bioulac-Sage, P., Brunt, E.M., Crawford, J.M., Crosby, H.A., Desmet, V., et al. (2004). Nomenclature of the finer branches of the biliary tree: canals, ductules, and ductular reactions in human livers. Hepatology *39*, 1739–1745.

Rossi, J.M., Dunn, N.R., Hogan, B.L., and Zaret, K.S. (2001). Distinct mesodermal signals, including BMPs from the septum transversum mesenchyme, are required in combination for hepatogenesis from the endoderm. Genes Dev. *15*, 1998–2009.

Rountree, C.B., Barsky, L., Ge, S., Zhu, J., Senadheera, S., and Crooks, G.M. (2007). A CD133-expressing murine liver oval cell population with bilineage potential. Stem Cells *25*, 2419–2429.

Sackett, S.D., Li, Z., Hurtt, R., Gao, Y., Wells, R.G., Brondell, K., Kaestner, K.H., and Greenbaum, L.E. (2009). Foxl1 is a marker of bipotential hepatic progenitor cells in mice. Hepatology *49*, 920–929.

Schmelzer, E., Zhang, L., Bruce, A., Wauthier, E., Ludlow, J., Yao, H.L., Moss, N., Melhem, A., McClelland, R., Turner, W., et al. (2007). Human hepatic stem cells from fetal and postnatal donors. J. Exp. Med. *204*, 1973–1987.

Sekine, S., Lan, B.Y., Bedolli, M., Feng, S., and Hebrok, M. (2006). Liver-specific loss of beta-catenin blocks glutamine synthesis pathway activity and cytochrome p450 expression in mice. Hepatology *43*, 817–825.

Sekiya, S., and Suzuki, A. (2011). Direct conversion of mouse fibroblasts to hepatocyte-like cells by defined factors. Nature *475*, 390–393.

Sekiya, S., and Suzuki, A. (2012). Intrahepatic cholangiocarcinoma can arise from Notch-mediated conversion of hepatocytes. J. Clin. Invest. *122*, 3914–3918.

Shin, S., Walton, G., Aoki, R., Brondell, K., Schug, J., Fox, A., Smirnova, O., Dorrell, C., Erker, L., Chu, A.S., et al. (2011). Foxl1-Cre-marked adult hepatic progenitors have clonogenic and bilineage differentiation potential. Genes Dev. *25*, 1185–1192.

Si-Tayeb, K., Noto, F.K., Nagaoka, M., Li, J., Battle, M.A., Duris, C., North, P.E., Dalton, S., and Duncan, S.A. (2010). Highly efficient generation of human hepatocyte-like cells from induced pluripotent stem cells. Hepatology *51*, 297–305.

Smith, L.G., Weissman, I.L., and Heimfeld, S. (1991). Clonal analysis of hematopoietic stem-cell differentiation in vivo. Proc. Natl. Acad. Sci. USA *88*, 2788–2792.

Strick-Marchand, H., and Weiss, M.C. (2002). Inducible differentiation and morphogenesis of bipotential liver cell lines from wild-type mouse embryos. Hepatology *36*, 794–804.

Suzuki, A., Zheng, Y., Kondo, R., Kusakabe, M., Takada, Y., Fukao, K., Nakauchi, H., and Taniguchi, H. (2000). Flow-cytometric separation and enrichment of hepatic progenitor cells in the developing mouse liver. Hepatology *32*, 1230–1239.

Suzuki, A., Iwama, A., Miyashita, H., Nakauchi, H., and Taniguchi, H. (2003). Role for growth factors and extracellular matrix in controlling differentiation of prospectively isolated hepatic stem cells. Development *130*, 2513–2524.

Suzuki, A., Sekiya, S., Onishi, M., Oshima, N., Kiyonari, H., Nakauchi, H., and Taniguchi, H. (2008). Flow cytometric isolation and clonal identification of self-renewing bipotent hepatic progenitor cells in adult mouse liver. Hepatology *48*, 1964–1978.

Takase, H.M., Itoh, T., Ino, S., Wang, T., Koji, T., Akira, S., Takikawa, Y., and Miyajima, A. (2013). FGF7 is a functional niche signal required for stimulation of adult liver progenitor cells that support liver regeneration. Genes Dev. *27*, 169–181.

Takayama, K., Nagamoto, Y., Mimura, N., Tashiro, K., Sakurai, F., Tachibana, M., Hayakawa, T., Kawabata, K., and Mizuguchi, H. (2013). Long-Term Self-Renewal of Human ES/iPS-Derived Hepatoblast-like Cells on Human Laminin 111-Coated Dishes. Stem Cell Reports *1*, 322–335.

Takebe, T., Sekine, K., Enomura, M., Koike, H., Kimura, M., Ogaeri, T., Zhang, R.R., Ueno, Y., Zheng, Y.W., Koike, N., et al. (2013). Vascularized and functional human liver from an iPSC-derived organ bud transplant. Nature *499*, 481–484.

Tanaka, M., Okabe, M., Suzuki, K., Kamiya, Y., Tsukahara, Y., Saito, S., and Miyajima, A. (2009). Mouse hepatoblasts at distinct developmental stages are characterized by expression of EpCAM and DLK1: drastic change of EpCAM expression during liver development. Mech. Dev. *126*, 665–676.

Tanimizu, N., and Miyajima, A. (2004). Notch signaling controls hepatoblast differentiation by altering the expression of liver-enriched transcription factors. J. Cell Sci. *117*, 3165–3174.

Tanimizu, N., Nishikawa, M., Saito, H., Tsujimura, T., and Miyajima, A. (2003). Isolation of hepatoblasts based on the expression of Dlk/Pref-1. J. Cell Sci. *116*, 1775–1786.

Tanimizu, N., Saito, H., Mostov, K., and Miyajima, A. (2004). Long-term culture of hepatic progenitors derived from mouse Dlk+ hepatoblasts. J. Cell Sci. *117*, 6425–6434.

Tremblay, K.D., and Zaret, K.S. (2005). Distinct populations of endoderm cells converge to generate the embryonic liver bud and ventral foregut tissues. Dev. Biol. *280*, 87–99.

Tsuchiya, A., Heike, T., Fujino, H., Shiota, M., Umeda, K., Yoshimoto, M., Matsuda, Y., Ichida, T., Aoyagi, Y., and Nakahata, T. (2005). Long-term extensive expansion of mouse hepatic stem/progenitor cells in a novel serum-free culture system. Gastroenterology *128*, 2089–2104.

Turányi, E., Dezsö, K., Csomor, J., Schaff, Z., Paku, S., and Nagy, P. (2010). Immunohistochemical classification of ductular reactions in human liver. Histopathology *57*, 607–614.

Wang, X., Foster, M., Al-Dhalimy, M., Lagasse, E., Finegold, M., and Grompe, M. (2003). The origin and liver repopulating capacity of murine oval cells. Proc. Natl. Acad. Sci. USA *100* (*Suppl 1*), 11881–11888.

Watanabe, T., Nakagawa, K., Ohata, S., Kitagawa, D., Nishitai, G., Seo, J., Tanemura, S., Shimizu, N., Kishimoto, H., Wada, T., et al. (2002). SEK1/MKK4-mediated SAPK/JNK signaling participates in embryonic hepatoblast proliferation via a pathway different from NF-kappaB-induced anti-apoptosis. Dev. Biol. *250*, 332–347.

Xu, C.R., Cole, P.A., Meyers, D.J., Kormish, J., Dent, S., and Zaret, K.S. (2011). Chromatin "prepattern" and histone modifiers in a fate choice for liver and pancreas. Science *332*, 963–966.

Yamashita, T., and Wang, X.W. (2013). Cancer stem cells in the development of liver cancer. J. Clin. Invest. *123*, 1911–1918.

Yanagida, A., Ito, K., Chikada, H., Nakauchi, H., and Kamiya, A. (2013). An in vitro expansion system for generation of human iPS cell-derived hepatic progenitor-like cells exhibiting a bipotent differentiation potential. PLoS ONE *8*, e67541.

Yang, W., Yan, H.X., Chen, L., Liu, Q., He, Y.Q., Yu, L.X., Zhang, S.H., Huang, D.D., Tang, L., Kong, X.N., et al. (2008). Wnt/beta-catenin signaling contributes to activation of normal and tumorigenic liver progenitor cells. Cancer Res. *68*, 4287–4295.

Yanger, K., Zong, Y., Maggs, L.R., Shapira, S.N., Maddipati, R., Aiello, N.M., Thung, S.N., Wells, R.G., Greenbaum, L.E., and Stanger, B.Z. (2013). Robust cellular reprogramming occurs spontaneously during liver regeneration. Genes Dev. *27*, 719–724.

Yeoh, G.C., Ernst, M., Rose-John, S., Akhurst, B., Payne, C., Long, S., Alexander, W., Croker, B., Grail, D., and Matthews, V.B. (2007). Opposing roles of gp130-mediated STAT-3 and ERK-1/ 2 signaling in liver progenitor cell migration and proliferation. Hepatology *45*, 486–494.

Yong, K.J., Gao, C., Lim, J.S., Yan, B., Yang, H., Dimitrov, T., Kawasaki, A., Ong, C.W., Wong, K.F., Lee, S., et al. (2013). Oncofetal gene SALL4 in aggressive hepatocellular carcinoma. N. Engl. J. Med. *368*, 2266–2276.

Yovchev, M.I., Grozdanov, P.N., Joseph, B., Gupta, S., and Dabeva, M.D. (2007). Novel hepatic progenitor cell surface markers in the adult rat liver. Hepatology *45*, 139–149.

Yovchev, M.I., Grozdanov, P.N., Zhou, H., Racherla, H., Guha, C., and Dabeva, M.D. (2008). Identification of adult hepatic progenitor cells capable of repopulating injured rat liver. Hepatology *47*, 636–647.

Yu, B., He, Z.Y., You, P., Han, Q.W., Xiang, D., Chen, F., Wang, M.J., Liu, C.C., Lin, X.W., Borjigin, U., et al. (2013). Reprogramming fibroblasts into bipotential hepatic stem cells by defined factors. Cell Stem Cell *13*, 328–340.

Yusa, K., Rashid, S.T., Strick-Marchand, H., Varela, I., Liu, P.Q., Paschon, D.E., Miranda, E., Ordóñez, A., Hannan, N.R., Rouhani, F.J., et al. (2011). Targeted gene correction of α1-antitrypsin deficiency in induced pluripotent stem cells. Nature *478*, 391–394.

Zajicek, G., Oren, R., and Weinreb, M., Jr. (1985). The streaming liver. Liver *5*, 293–300.

Zeng, S.S., Yamashita, T., Kondo, M., Nio, K., Hayashi, T., Hara, Y., Nomura, Y., Yoshida, M., Hayashi, T., Oishi, N., et al. (2014). The transcription factor SALL4 regulates stemness of EpCAM-positive hepatocellular carcinoma. J. Hepatol. *60*, 127–134.

Zhao, D., Chen, S., Cai, J., Guo, Y., Song, Z., Che, J., Liu, C., Wu, C., Ding, M., and Deng, H. (2009). Derivation and characterization of hepatic progenitor cells from human embryonic stem cells. PLoS ONE *4*, e6468.

Zhu, S., Rezvani, M., Harbell, J., Mattis, A.N., Wolfe, A.R., Benet, L.Z., Willenbring, H., and Ding, S. (2014). Mouse liver repopulation with hepatocytes generated from human fibroblasts. Nature *508*, 93–97.

Zong, Y., Panikkar, A., Xu, J., Antoniou, A., Raynaud, P., Lemaigre, F., and Stanger, B.Z. (2009). Notch signaling controls liver development by regulating biliary differentiation. Development *136*, 1727–1739.