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Liver stem/progenitor cells: their characteristics and regulatory mechanisms

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Liver stem cells give rise to both hepatocytes and bile duct epithelial cells also known as cholangiocytes. During liver development hepatoblasts emerge from the foregut endoderm and give rise to both cell types. Colony-forming cells are present in the liver primordium and clonally expanded cells differentiate into either hepatocytes or cholangiocytes depending on culture conditions, showing stem cell characteristics. The growth and differentiation of hepatoblasts are regulated by various extrinsic signals. For example, periportal mesenchymal cells provide a cue for bipotential hepatoblasts to become cholangiocytes, and mesothelial cells covering the parenchyma support the expansion of foetal hepatocytes by producing growth factors. The adult liver has an extraordinary capacity to regenerate, and after 70% hepatectomy the liver recovers its original mass by replication of the remaining hepatocytes without the activation of liver stem cells. However, in certain types of liver injury models, liver stem/progenitor-like cells, known as oval cells in rodents, proliferate around the portal vein, while the roles of such cells in liver regeneration remain a matter of debate. Clonogenic and bipotential cells are also present in the normal adult liver. In this minireview we describe recent studies on liver stem/progenitor cells by focusing on extracellular signals.

Keywords: cytokine/development/differentiation/hepatocyte/regeneration.

Abbreviations: 2-AAF, 2-acetylaminofluoren; AFP, alpha-fetoprotein; ALB, albumin; BMEL, bipotential mouse embryonic liver cell; CDE, choline-deficient, ethionine-supplemented; DDC, 3,5-diethoxycarbonyl-1,4-dihydro-collidine; Dlk, Delta-like protein 1; DPPIV, dipeptidyl peptidase IV; EpCAM, epithelial cell adhesion molecule; FGF, fibroblast growth factor; H-CFU-C, hepatic colony-forming unit in culture; MC, mesothelial cell; OSM, oncostatin M; PH, partial hepatectomy; STM, septum transversum mesenchyme; TNF, tumour necrosis factor; Wt1, Wilms' tumour 1.

The liver is a central organ for homeostasis owing to its numerous functions, including carbohydrate metabolism, glycogen storage, biosynthesis of various biochemical components including amino acids and nucleotides, lipid metabolism, urea synthesis, drug detoxification, production of plasma proteins and hormones, and destruction of erythrocytes. Because the liver is such an essential organ, liver diseases are often fatal. Liver insults such as hepatitis viruses, drugs, alcohol and genetic, metabolic and immune disorders can lead to steatosis, hepatitis, fibrosis, cirrhosis and cancer and liver disease is a major cause of death. The liver is also known as a unique organ that can regenerate, making it possible to transplant the liver from a living donor. However, the molecular mechanisms underlying organogenesis, maintenance, pathogenesis and regeneration of the liver are not well understood. As the liver is a large organ with a variety of functions, it has been used for many decades as a source to purify numerous enzymes for biochemical studies. By contrast, much progress has been made relatively recently in the characterization of each type of liver cell and analysis of their interactions. Those studies have been facilitated by new technologies such as genomics, mouse mutants and the development of various tools to isolate the cells of interest. In this review, we describe recent studies on liver stem/progenitor cells together with the environments that support their proliferation and differentiation during development and pathogenesis.

Liver architecture and liver stem cells

The liver is divided into lobules and each lobule consists of plates of hepatocytes lined by sinusoidal capillaries that radiate towards a central efferent vein (Fig. 1). Liver lobules are hexagonal and at each of six corners there is a portal triad of vessels consisting of a portal vein, hepatic artery and bile duct. Sinusoids are composed of liver-specific capillaries with fenestrated endothelial cells, hepatic stellate cells (Ito cells), liver-resident macrophages (Kupffer cells) and large granular lymphocytes (pit cells). The liver has a dual blood supply, namely, via the portal vein and the hepatic artery. The portal vein delivers the venous blood flowing from the intestines, pancreas and spleen. The hepatic artery supplies oxygen to the liver. The blood flows from a portal triad through a sinusoidal capillary to a central efferent vein. Hepatocytes are major parenchymal cells carrying out most of the metabolic functions and account for ∼60% of the total liver cell population and 80% of the volume of the organ. Hepatocytes are highly polarized epithelial cells and

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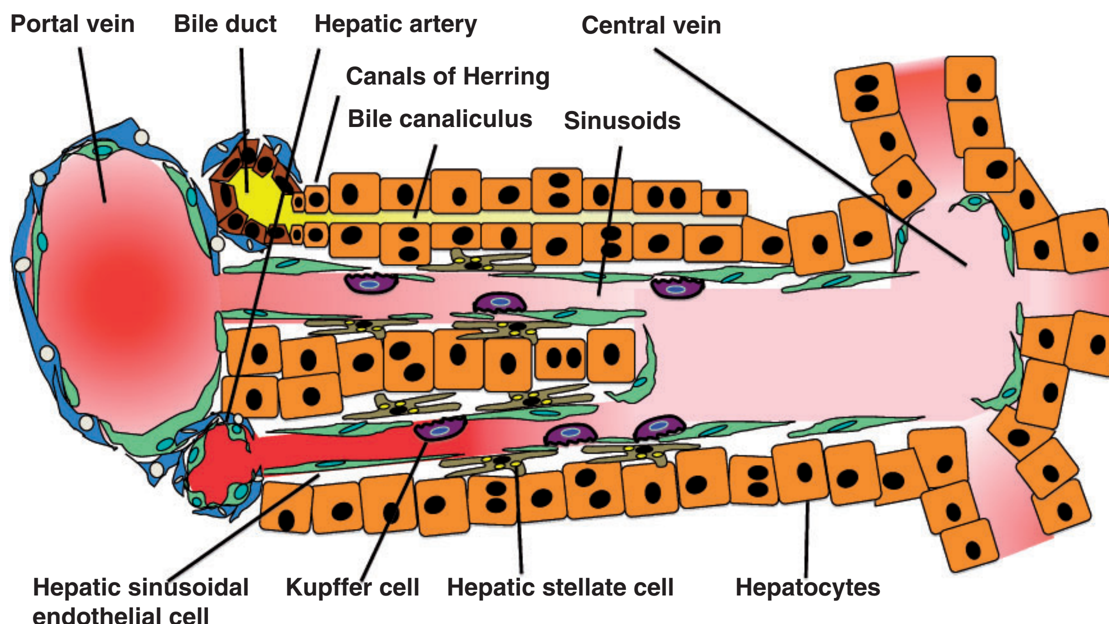

Fig. 1 Liver architecture. In the liver, blood flows from portal blood vessels through sinusoids to central efferent veins. Sinusoids are liver-specific capillaries with fenestrated endothelial cells, hepatic stellate cells (Ito cells) and blood cells such as liver-resident macrophages (Kupffer cells). Hepatocytes are highly polarized epithelial cells forming cords, and plates of hepatocytes are lined by sinusoidal capillaries that radiate towards a central efferent vein. Tight junctions formed between hepatocytes create a canaliculus that surrounds each hepatocyte. Bile salts produced in hepatocytes are excreted into canaliculi that are linked to bile ducts. The region that connects the bile canaliculus and the biliary tree is called 'canals of Hering'.

form cords. Their basolateral surfaces face fenestrated sinusoid endothelial cells, facilitating the transfer of materials between hepatocytes and blood flows. Tight junctions formed between hepatocytes create a canaliculus that surrounds each hepatocyte. Bile salts produced in hepatocytes are excreted into canaliculi that are linked to bile ducts at the portal triad. Bile ducts are formed by a specialized type of epithelial cell called a biliary epithelial cell or a cholangiocyte.

In general, stem cells are characterized by their ability to self-renew and differentiate to multiple lineages. As hepatocytes and cholangiocytes, the two types of liver epithelial cells, are derived from a common origin during organogenesis, those cells with the potential to proliferate and give rise to both types of liver epithelial cells are considered to be liver stem cells. Although there are many reports describing liver stem cells, the definitions of stem cells are rather vague in many of them. As it is not an easy task to distinguish stem cells from progenitors because of the difficulty of proving the unlimited self-renewal activity of stem cells in many situations, we use the term stem/progenitor cells to describe such cells in this review article.

The onset of liver development

Liver organogenesis begins at embryonic day (E) 8.5 in the mouse from the foregut endoderm. The ventral wall of the foregut endoderm faces the developing heart by approximately E8 and receives inductive signals for hepatic fate, such as fibroblast growth factor (FGF) from the heart (*1–3*) and bone morphogenetic protein from the septum transversum mesenchyme (STM) (*4*). Wnt2b is expressed in the lateral plate mesoderm adjacent to the endoderm destined to be the liver and is essential for the onset of liver development in zebrafish (*5*). By these signals, hepatoblasts emerge from the foregut endoderm and migrate as cords into the surrounding STM (*6*, *7*). Analysis of Flk1-deficient mouse embryos revealed that Flk1^{+} endothelial cells are required for proliferation of hepatoblasts (*8*). Because hepatoblasts proliferate and give rise to both hepatocytes and cholangiocytes as described below, they are considered to be embryonic liver stem/progenitor cells.

Identification and characterization of hepatoblasts

As cell sorting using antibodies is a powerful means to isolate and characterize a specific cell type, efforts have been made to search for specific cell surface antigens on hepatoblasts (Fig. 2). Kubota and Reid (*9*) showed that the RT1A1^{-} OX18^{low} ICAM-1^{+} fraction of E13 rat foetal liver contained hepatoblasts. Suzuki *et al*. (*10*) developed a single cell-based assay designated the hepatic colony-forming unit in culture (H-CFU-C) and showed that the CD45^{-} TER119^{-} c-Kit^{-} CD29^{+} CD49f^{+} and CD45^{-} TER119^{-} c-Kit^{-} c-Met^{+} CD49f$^{+/low}$ fraction of E13.5 mouse liver contained hepatic progenitor/stem cells. They also showed that CD45^{-} TER119^{-} c-Kit^{-} c-Met^{+} CD49f$^{+/low}$ cells of E11.5 mouse liver had high H-CFU-C potential and that clonally expanding cells reconstituted the liver, pancreas and intestine *in vivo*. On the other hand, Minguet *et al*. (*11*) reported that CD45^{-} TER119^{-} c-Kit^{low} cells in E11 mouse liver contained the earliest hepatic progenitors, also displaying features of

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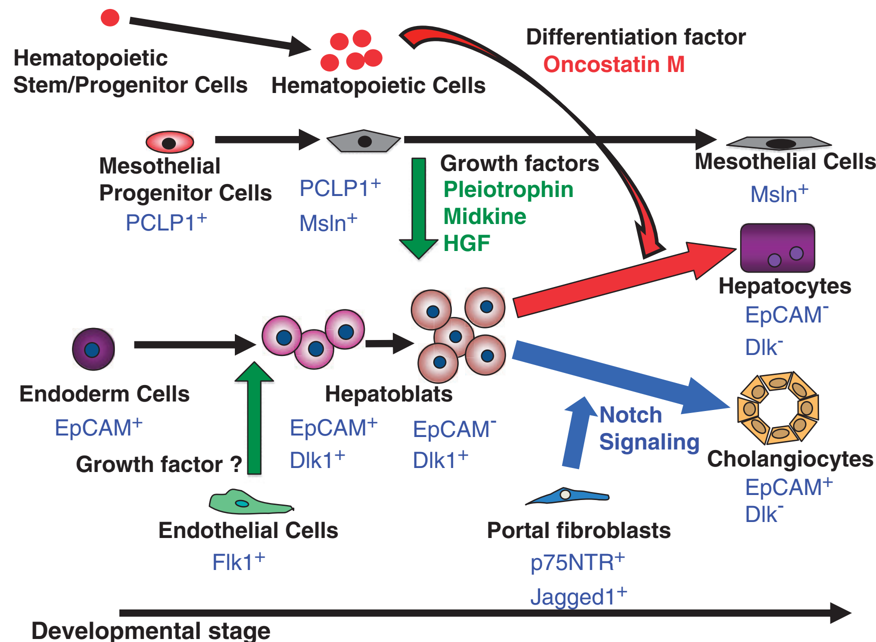

Fig. 2 Development of liver cells and expression of cell surface markers. The EpCAM$^+$DLK1$^+$ hepatoblasts emerge from EpCAM$^+$ foregut endoderm cells and form liver primordium. Then, the hepatoblasts dramatically reduce the expression of EpCAM. EpCAM is upregulated again in biliary epithelial cell precursor cells around the portal vein, where p75NTR$^+$ Jagged1$^+$ portal fibroblasts interacted with hepatoblasts. PCLP1$^+$ mesothelial progenitor cells produce growth factors for hepatoblasts to proliferate. OSM secreted by haematopoietic cells induces hepatocytic differentiation of hepatoblasts.

liver-repopulating stem cells. Delta-like protein 1 (Dlk1), also known as Pref-1, was strongly expressed in liver buds as early as E10.5 in mice. Dlk1$^+$ cells isolated from E14.5 livers expressed albumin (ALB) and formed colonies composed of the hepatocyte and cholangiocyte lineages in the presence of hepatocyte growth factor and epidermal growth factor, indicating that liver stem cell activity is present in this population (*12*). As in mouse foetal liver, Dlk1 is also expressed strongly in human foetal liver (*13*, *14*). Nierhoff *et al.* showed that murine foetal liver alpha-fetoprotein (AFP)$^+$/ALB$^+$ cells were positive for Dlk1 and E-cadherin and that purified E-cadherin$^+$ epithelial cells formed clusters in cell culture and differentiated along the hepatocytic lineage. Interestingly, AFP$^+$/E-cadherin$^+$ epithelial cells were Sca-1$^+$, but showed no expression of c-Kit. In order to examine their *in vivo* capacity, wild-type E12.5 mouse liver epithelial cells were transplanted into adult dipeptidyl peptidase IV (DPPIV) knockout mice, and DPPIV expression was used as a marker to discriminate the donor from recipient cells. This resulted in incorporation of the DPPIV$^+$ donor-derived cells into the hepatic parenchymal cords of the recipient liver, showing a repopulation and differentiation capacity of the E12.5 E-cadherin$^+$ cells (*15*).

Epithelial cell adhesion molecule (EpCAM) is expressed in HNF4α$^+$ hepatoblasts of liver buds as early as E9.5 in mice (Fig. 2). Colony-forming assays using sorted E11.5 liver cells revealed that the EpCAM$^+$ Dlk1$^+$ cell population contained *in vitro* colony-forming cells, indicating that liver stem cell activity is present in this population. EpCAM expression declined by E13.5 in mouse liver, while Dlk1 expression was sustained by E16.5 (*16*). In humans, Dan *et al.* (*17*) reported that multipotent progenitor cells derived from human foetal liver expressed EpCAM,and Schmelzer *et al.* (*18*) reported that pluripotent precursors of hepatoblasts expressed EpCAM and were located in ductal plates in human foetal liver. CD13 (aminopeptidase N) was detected on the cells of the Dlk1$^+$ hepatic stem/progenitor fraction. Colony formation assays revealed that hepatic stem/progenitor cells were enriched in the CD13$^+$ fraction, compared with the Dlk1$^+$ fraction, of non-haematopoietic cells in foetal liver (*19*).

Characteristics of foetal liver stem/progenitor cells

Dlk1$^+$ cells contain some clonogenic cells named hepatic progenitor proliferating on laminin that continuously proliferate on laminin-coated plates and differentiate to both hepatocytes and cholangiocytes depending on culture conditions, suggesting that they are liver stem cells (*20*). Bipotential cell lines, referred to as bipotential mouse embryonic liver cell (BMEL), were also obtained after a long latency in culture of foetal liver cells and they were shown to give rise to both hepatocytes and cholangiocytes in recipient mice, although the origin of BMEL was unknown (*21*). These cell lines are used to study the mechanisms of hepatocytic and/or cholangiocytic differentiation from liver stem cells.

In the past decade, a number of cell surface markers for foetal liver cells have been found and used to prospectively isolate and to localize them in the liver. While some studies used transplantation assays to

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investigate the repopulation capacity, the ability to form a colony and differentiate to both lineages *in vitro* is a practical criterion to evaluate hepatoblasts in most of these studies. In the case of haematopoietic stem cells, a single purified stem cell can be shown to propagate and give rise to all kinds of haematopoietic cells for the long term in an irradiated recipient mouse, providing clear evidence for stemness *in vivo*, that is self-renewal ability and multi-lineage differentiation. By contrast, as liver repopulation assays require a large number of cells to be transplanted to demonstrate engraftment capacity, rigorous proof of stemness *in vivo* is difficult. Nonetheless, there is little doubt that hepatoblasts possess capacities of liver stem cells on the basis of numerous previous works as described above (*9–12*, *15–19*).

Differentiation of hepatoblasts to cholangiocytes

Bile ducts are formed only around the portal vein, suggesting that regionally specific signals induce cholangiocytes from hepatoblasts. Indeed, two signalling pathways, TGFβ/Activin and Notch, are specifically activated in hepatoblasts near the portal vein. TGFβ2 and TGFβ3 are predominantly expressed in the portal region (*22*), and the Onecut family of transcription factors, HNF6 (OC-1) and OC-2, promote expression of α2-macroglobulin and follistatin, inhibitors of the TGFβ/Activin pathway, in the parenchymal region (*23*). Dlk1$^+$ hepatoblasts express Notch2, whereas p75NTR$^+$ periportal fibroblasts express Jagged-1 (*24*). Forced expression of Notch intracellular domain in Dlk1$^+$ hepatoblasts resulted in differentiation to cholangiocytes (*25*). These results strongly suggest that cholangiocyte differentiation is induced by Notch signalling in the periportal region. Although differentiation of hepatoblasts to cholangiocytes by TGFβ and Notch signalling occurs in mid-gestation, surprisingly, hepatocytes turned to cholangiocytes and formed ectopic duct structures in the parenchyma by Notch activation after birth (*26*). These results indicate that not only hepatoblasts but also hepatocytes are competent to differentiate to cholangiocytes at least by the neonatal period (Fig. 2).

Immature cholangiocytes form a ductal plate, a single cell layer, around the portal vein. Tubular morphogenesis of bile ducts proceeds through the rearrangement of a single layer of the ductal plate. Recent studies on mice lacking Sox9, a transcription factor, or Notch 2 in the liver indicated the second wave of cholangiocyte differentiation adjacent to the initial single layer of the ductal plate, which was regulated by TGFβ and Notch pathways and involved in tubular morphogenesis. In a model proposed on the basis of those studies, after the initial induction of cholangiocytes near the portal vein, cholangiocyte differentiation and tubular morphogenesis progress in parallel (*22*, *26–30*). However, the precise mechanisms of bile duct morphogenesis have not been completely understood.

In addition, studies using mutant mice have implicated transcription factors including HES1, HNF6, HNF1β, Tbx3, FoxA2 and A3, FoxM1b, Hex and Sall4 in bile duct differentiation and/or morphogenesis (*31–38*) (Fig. 3). Although a network of these transcription factors and a link between transcription factors and Notch/TGFβ pathways are being uncovered (*39*), studies on gene expression and histology of mutant mice are insufficient to understand how these factors regulate complicated processes of tubulogenesis. As an alternative approach, *in vitro* culture systems allowing hepatoblasts to form bile duct structures are helpful to understand the lineage commitment of hepatoblasts and tubular morphogenesis (*22*, *25*, *40*, *41*).

Proliferation and differentiation of hepatocytes in foetal liver

At an early stage of hepatogenesis, endothelial cells contribute to the proliferation of hepatoblasts (*8*) and the vast majority of hepatoblasts become parenchymal hepatocytes at a later stage. The liver parenchyma is covered with the mesothelium consisting of the surface mesothelial cell (MC) layer, ALCAM$^+$ submesothelial cells and fibroblasts (*42*). At a later stage of hepatogenesis, MCs seem to contribute to the expansion of hepatoblasts (*43*). Foetal liver MCs are characterized by the expression of a sialomucin, PCLP1, and become adult liver MCs expressing mesothelin. Comparison of the gene expression profiles between foetal and adult MCs revealed that foetal PCLP1$^+$ MCs express various growth factors for hepatocytes such as Midkine and Pleiotrophin, and co-culture of Dlk1$^+$ foetal hepatocytes with PCLP1$^+$ foetal MCs in a transwell enhanced hepatocyte proliferation. Wilms' tumour 1 (Wt1) knockout mice were embryonic lethal, exhibiting impaired liver development. Cytokine production by Wt1 knockout MCs was reduced, while proliferation of Dlk1$^+$ cells from Wt1 knockout embryos was normal in a co-culture with wild-type MCs, indicating that defects in liver development of Wt1

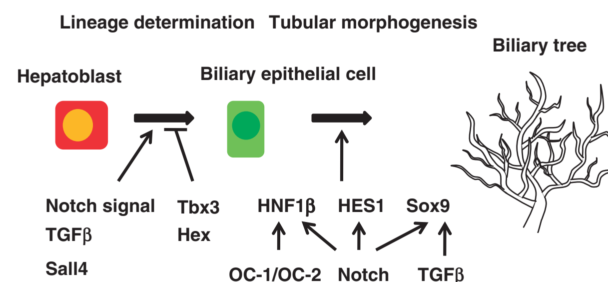

Fig. 3 Bile duct development. There are two steps in bile duct development. First, hepatoblasts are induced to differentiate to biliary epithelial cells around the portal vein. This step is promoted by TGFβ and Notch signals as well as a transcription factor, Sall4, whereas it is inhibited by two transcription factors, Tbx3 and Hex. Biliary epithelial cells then undergo tubular morphogenesis and form the biliary tree. Three transcription factors, HNF1β, HES-1 and Sox9, are involved in tubular morphogenesis. The Notch signal is upstream of all the three transcription factors, whereas OC-1 and OC-2, and the TGFβ signal are upstream of HNF1β and Sox9, respectively.

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knockout mouse are due to MCs. MCs were also shown to delaminate and give rise to mesenchymal cells in the liver (*44*). These results indicate that the mesothelium is not only a protective sheet covering the liver parenchyma but also actively involved in liver organogenesis (Fig. 2).

Foetal liver is a major tissue for haematopoiesis, and hepatocytes acquire various metabolic functions at perinatal and postnatal stages. Mice lacking gp130, the common receptor subunit of the IL-6 family cytokines, develop liver with impaired functions, indicating that some of the IL-6 family cytokines are required for functional maturation of the liver (*45*). Oncostatin M (OSM), a member of the IL-6 family, strongly enhanced differentiation of foetal hepatocytes, while liver development is normal in OSM-deficient mice, suggesting that another member of the family may play a similar role. In the foetal liver, immigrating haematopoietic stem cells proliferate and produce numerous blood cells with the help of liver cells including hepatocytes and endothelial cells. Haematopoietic activity in foetal liver declines with hepatocyte differentiation (Fig. 2). As OSM is secreted from haematopoietic cells proliferating in the foetal liver and induces differentiation of hepatocytes, it is likely that OSM plays a role for coordination of liver development and haematopoiesis (*46*).

Adult liver stem/progenitor cells

Adult liver has a potential to regenerate under conditions of severe parenchymal loss, although hepatocytes and cholangiocytes are mitotically dormant under normal conditions. Hepatocytes themselves have a remarkable ability to self-replicate to restore liver mass (*47*) and are capable of at least 80 doublings by serial transplantation (*48*), allowing the liver to regenerate. Thus, the contribution of liver stem cells to regeneration after partial hepatectomy (PH) seems to be minimal if any. However, in liver injury that limits this pathway there is an accompanying expansion of a potential stem cell compartment in the periportal area, which is known as ductular reaction (*49–51*) (Fig. 4). These proliferating epithelial cells are often referred to as oval cells in rodents because of their oval nucleus (*52*). Upon activation of oval cells, they expand into liver parenchyma from the portal area, and selective damage of the periportal zone reduces oval cell proliferation, supporting the notion that oval cells are derived from the periportal region, in particular canals of Hering that connect the bile canaliculus and the biliary tree (*53*). In addition, an extrahepatic origin of oval cells such as bone marrow was also suggested (*54*); however, the exact origin of oval cells still remains to be established. While oval cells have been most extensively studied in rodents, similar cells have been found in various human liver diseases, such as chronic viral hepatitis, alcoholic liver disease, nonalcoholic fatty liver disease and fulminant hepatitis, and also implicated in tumourigenesis (*55*, *56*). Oval cells express both ALB and cytokeratin 19, which are hepatocytic and cholangiocytic markers, respectively, and are believed to differentiate to hepatocytic and biliary lineages, similar to hepatoblasts in the embryonic liver.

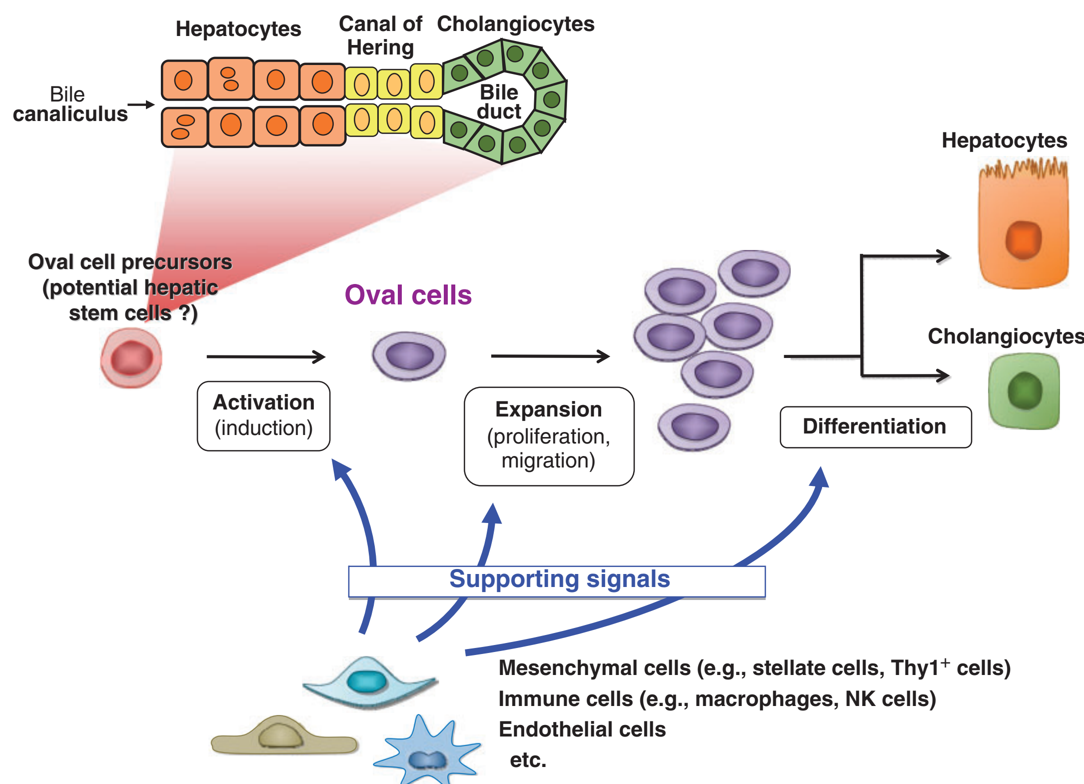

Fig. 4 Induction of oval cells. Oval cells and Thy1^{+} cells are simultaneously induced in severe liver damage conditions. The origin of oval cells is still under debate. FGF7 is produced by Thy1^{+} cells in response to liver damage conditions, and oval cells receive its signal.

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Thus they are thought to be facultative stem/progenitor cells in the adult liver (Fig. 4).

The nature of oval cells as liver stem cells was debated in numerous reports of studies using various rodent models. The 2-acetylaminofluoren (2-AAF)/PH model, *i.e.* blocking hepatocyte proliferation by 2-AAF prior to PH, has been extensively used to characterize oval cells in rat (*57*, *58*). However, the same procedure does not induce oval cells in mice, and alternative protocols such as a choline-deficient, ethionine-supplemented (CDE) diet and 3,5-diethoxycarbonyl-1,4-dihydro-collidine (DDC) diet have been developed to induce oval cells in mice (*59–61*). Although the proliferating epithelial cells in the periportal region upon injury by various insults are collectively referred to as oval cells, it remains unclear whether or not the oval cells in different species by different protocols have common characteristics. A major problem in characterizing oval cells was the lack of appropriate cell surface markers to identify and isolate the oval cell compartment.

In the rat 2-AAF/PH model, Dabeva *et al.* reported that $EpCAM^+$ oval cells are bipotential adult hepatic progenitors (*62*, *63*). Suzuki *et al.* (*64*) reported that $CD133^+$ cells isolated from DDC-treated mouse liver could form large colonies in culture. These large colony-forming cells gave rise to both hepatocytes and cholangiocytes, while maintaining undifferentiated cells by self-renewing cell divisions. In order to isolate and characterize mouse oval cells, Okabe *et al.* (*65*) searched for cell surface molecules expressed on oval cells in mouse fed DDC diet. EpCAM was expressed in both mouse normal cholangiocytes and oval cells, and its related protein TROP2 was expressed exclusively in oval cells, establishing TROP2 as a novel marker to distinguish oval cells from normal cholangiocytes (*65*). Some of the $EpCAM^+$ cells isolated from injured liver proliferate to form colonies *in vitro*, and the clonally expanded cells differentiate into hepatocytes and cholangiocytes, suggesting that the oval cell fraction contains potential liver stem cells.

Interestingly, such cells with liver stem cell characteristics are also found in $EpCAM^+$ cells of the normal liver. Intriguingly, comparison of the colony formation of $EpCAM^+$ cells between normal and injured livers revealed little difference in the frequency of potential liver stem cell activity between them, strongly suggesting that most of the proliferating mouse oval cells represent transit-amplifying cells rather than stem cells. Bipotential clonal cell lines can be obtained from the healthy liver of adult mice and participate in liver regeneration in severe combined immunedeficient mice expressing urokinase-type plasminogen by the ALB promoter, where they differentiate in clusters of hepatocytes and occasionally bile ducts (*66*). Kamiya *et al.* (*67*) found progenitor cells in the $CD13^+CD49f^+CD133^+$ subpopulation of non-haematopoietic cells derived from postnatal livers. These results demonstrate the existence, in normal adult mouse liver, of a pool of clonogenic cells that are (or can become) bipotential.

As mentioned above, oval cells are induced in liver with severe or chronic damage. Chronic injury conditions in the liver are usually associated with inflammation, and the roles of lymphocytes and inflammatory responses in oval cell regulation have also been suggested (*68*, *69*). In accordance with this notion, several kinds of inflammatory cytokines, such as tumour necrosis factor (TNF)-α, lymphotoxin-β, interferon-γ and IL-6, have been shown to modulate oval cell response (*70*, *71*). Perhaps the best established inflammatory cytokine to be involved in oval cell response is a TNF family member ligand, TNF-like weak inducer of apoptosis (Tweak). Thus, transgenic mice overexpressing this cytokine in the liver exhibit periportal oval cell hyperplasia, while administration of a blocking anti-Tweak monoclonal antibody significantly reduced oval cell response in mice fed DDC diet (*72*). Furthermore, in mice lacking Fn14, the cognate receptor for Tweak, induction of oval cells was attenuated in both DDC diet and CDE diet models (*72*, *73*). These inflammatory cytokines are considered to function as part of the innate immune system sensing damage to the tissue and serve as the earliest signals for triggering the process of liver regeneration (Fig. 4).

Mesenchymal cells such as stellate cells have long been suggested to physically interact with oval cells and thus considered to induce some signals in them (*53*). Recent studies using several rat and mouse models have demonstrated that a population of mesenchymal cells expressing thymus cell antigen-1 (Thy-1; also known as CD90) resides in close proximity to and expands in parallel with oval cells (*74*) (H. Takase, T. Itoh and A. Miyajima, unpublished observation). Furthermore, these $Thy1^+$ cells were found to express FGF7, and its cognate receptor FGFR2b was detected in oval cells. FGF7 knockout mice showed a defect in oval cell response, while overexpression of FGF7 *in vivo* in normal mouse liver led to induction and proliferation of cells with markers of oval cells in the periportal area. Together, these results strongly suggest that FGF7 plays a key role in adult liver stem/progenitor cell response as well as that the $Thy1^+$ cells may serve as the niche for oval cells by providing this cytokine (Takase, H., Itoh, T. and Miyajima A., unpublished observations). As a signal related to oval cell response, several recent studies have implicated the canonical Wnt/β-catenin pathway in oval cell regulation (*75–78*) (Fig. 4). The Wnt/β-catenin pathway is well known to play important roles in stem cell regulation including self-renewal in various other organs and tissues, and also in carcinogenesis including liver tumours. In both rat and mouse models, expression of some Wnt ligands in damaged liver and concomitant activation of the β-catenin pathway in oval cells were observed. In conditional knockout mice lacking β-catenin in both hepatocytes and cholangiocytes, DDC diet-induced oval cell response in the liver was significantly reduced, although not completely abrogated. While several factors have been shown to be involved in oval cell response, the precise modes of their actions and their relationship are currently unclear and should be determined.

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Concluding remarks

Traditionally, research on liver biology mostly relied on relatively crude cell separation methods based on cell density and centrifugation. In the last decade, identification of specific cell surface markers for each of the liver cell types, production of corresponding monoclonal antibodies and cell sorting techniques have together revolutionized the field and enabled us to perform much more detailed characterization of liver cells, particularly non-parenchymal cells including the stem/progenitor cells. It has also become possible to analyse the modes of interaction among different types of these cells *in vivo* by means of combinatorial use of specific markers/antibodies as well as *in vitro* with co-culture systems using the isolated viable cell populations. Elucidation of the molecular basis for the signals that regulate development, proliferation and differentiation of liver stem/progenitor cells should not only advance our understanding of the basic pathophysiology of the liver but also help to establish better protocols to generate mature hepatocytes and other liver cells *in vitro* for cell-based therapy, transplantation and drug discovery.

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Conflict of interest
None declared.

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