Chapter 7

Adult Hepatic Progenitor Cells

Peter Siig Vestentoft

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/58814

1. Introduction

The liver is the largest internal organ of the body only second in size compared to the skin. The liver not only functions as an endocrine and exocrine organ, but it also performs a multitude of vital functions including glycogen storage, detoxification and plasma protein synthesis [1–4]. The liver receives nutrients and environmental toxins from the digestive tract through the portal vein. This direct transport of potentially harmful agents is hypothesized to have exerted an evolutionary pressure on the liver to possess multiple pathways for regeneration [4,5]. In fact, the regenerative capacity of the liver is so enormous that this was renowned in ancient times and described in Mediterranean folklore. According to Greek mythology the Titan Prometheus stole fire from the Gods of Olympia and gave it to the mortals. As a consequence, Zeus, the king of Gods, chained Prometheus to a rock. An eagle would then appear each day and pecked out part of Prometheus' liver only to let it regenerate overnight [1]. This punishment was to be repeated for eternity, but according to one version of the story, Heracles (Hercules) eventually killed the eagle and freed Prometheus.

Despite of the famed renewal capacity of the liver, hepatic diseases constitute a worldwide problem. Hepatic diseases can broadly be divided into two major groups: acute and chronic liver diseases. Acute liver failure is characterized by the manifestation of sudden severe hepatic injury that can have several etiologies [6,7]. Frequent causes included viral hepatitis or drug intoxication, commonly paracetamol, leading to hepatic encephalopathy, coagulopathy and often progressive multiorgan failure [6,8–11]. In developed countries, acute liver failure is relatively rare with an incidence estimated between 1-6 cases per million people each year [6, 12]. In contrast, chronic liver diseases are caused by prolonged insults. Common causes include sustained alcohol consumption, non-alcoholic fatty liver disease and hepatitis B or C virus infection [2]. These insults can lead to hepatic fibrosis, a form of wound healing characterized by the presence of collagen-rich septae connecting the so-called portal areas. If untreated, this potentially reversible manifestation, can progress to end stage cirrhosis, where hepatic

architecture is greatly disturbed and scar tissue encircles nodules of remaining hepatocytes [7,13–17]. Chronic liver diseases are estimated to affect 170 million patients worldwide. Those cases eventually progress to fibrosis and possibly cirrhosis in 25-30 % of these patients [2]. Where acute hepatic failure involves sudden massive cell death, chronic liver diseases are conversely characterized by continuous cell death [18–20].

When hepatic regeneration is hindered orthotopic liver transplantation is the only treatment that radically improves the outcome of hepatic failure [2,21]. However, the worldwide shortage of liver donors result in death of many patients waiting for transplantation [22]. Research into alternative methods of therapeutic treatment is therefore highly needed. The possibility of culturing hepatic stem cells holds the promise to treat certain liver diseases, even with autologous stem cells. This include correcting metabolic diseases characterized by inherited defects of hepatic enzymes or treating fulminant hepatic failure characterized by rapid onset of liver failure and death, when donor organs are unavailable [23]. The use of autologous stem cells would additionally prevent the lifespan administration of immunosuppressive agents currently employed to prevent allograft rejection. Therefore, there has been an increasing interest into using hepatic stem cell-based therapies as novel alternatives to traditional liver treatments. However, the stem cell biology of the liver is not well understood. In particular, the lack of specific markers for hepatic stem cell identification has hindered their characterization and isolation [24–28].

The present chapter will provide an overview of current knowledge of the rodent and human hepatic stem cell niche.

In particular, the chapter will go through the development of the hepatic stem cell niche, the associated extracellular matrix molecules and support cells. Attention will also be given to the various modes of hepatic regeneration and the involvement of hepatic stem cells in cancerous disease states.

2. Stem cells

Even though stem cells have been identified and characterized in several organs, no universally accepted definition of what constitutes a stem cell has been defined [29]. However, a broadly accepted view is that stem cells are cells that hold a capacity for unlimited or prolonged self-renewal and can also give rise to at least one type of highly differentiated progeny [30]. However, many classes of stem cell exist with different potentials. These range from the totipotent fertilized egg from which entire organisms develop over pluripotent embryonic stem cells that can give rise to the three germ layers to the unipotent tissue stem cells.

Typically, tissue or intra-organ stem cells are less differentiated cells that exist in a mitotically quiescent form [31]. This class of stem cells are so-called “determined”, meaning that they lack markers of final differentiation, but are able to divide and differentiate into highly specialized effector cells [32,33]. When needed tissue stem cells are activated to divide and clonally regenerate the tissue in which they are located [32,34]. Upon activation, tissue stem cells would

perform either symmetric or asymmetric cell division [30]. Symmetric stem cell division give rise to two daughter cells that themselves are stem cells, thereby maintaining the stem cell pool. Alternatively, this form for division may result in two daughter cells committed for differentiation. Asymmetric stem cell division, on the other hand, produces one stem cell and one differentiated daughter cell. Differentiated daughter cells are also known as progenitor cells or transit amplifying cells. They divide rapidly in order to generate a pool of continually more differentiated cells en route to replace senescent or damaged tissue cells [34]. Early progenitor cells hold multi-lineage potential and have characteristics similar to the parent stem cell whereas late progenitor cells are more differentiated and produce single-lineage progeny [32]. Therefore, even though stem cells have a high self-renewal capacity, they may divide relatively infrequently, whereas the transit amplifying cells greatly increase in number and differentiate into given tissue cells.

3. Stem cell niche

The potency of stem cells requires tight regulation of their behavior. Stem cell quiescence and activation must be regulated according to the needs of the organism. A critical actor in mediating the balanced response of stem cells to the needs of the organism is the stem cell niche.

The stem cell niche concept was first proposed by Schofield who conducted bone marrow studies [35]. It was suggested that stem cells reside in compartments that promote and maintain their characteristics [35]. It is believed that once postnatal tissues are formed, intra-organ stem cells reside in these special tissue microenvironments or niches. However, upon activation, the niche must change the composition of its microenvironment from favoring stem cell quiescence to induce stem cell activation and proliferation. Studies on *Drosophila spp*. gonads have helped understanding the factors constituting the stem cell niche and greatly expanded knowledge of stem cell activation and the generation of transit amplifying cells [36]. These studies have revealed a basal theme to reoccur. Structurally, the typical stem cell niche consists of stem cells resting on a scaffold of extracellular matrix components, having cell-cell interactions with differentiated neighboring cells [36–38]. In *Drosophila spp.* gonads, the extracellular matrix forms a repressing environment to stem cell differentiation, while promoting cellular adhesion [36]. Immediately outside this repressive zone, stem cell adherence is reduced while cellular differentiation is stimulated [36]. More specifically, integrins have been identified as key elements in this adhesion process. These transmembrane proteins that mediate adhesion to the extracellular matrix, are often highly expressed in stem cells and can suppress terminal differentiation in epidermal stem cells, for instance [39,40]. Conversely, the loss of integrins is associated with the epidermal stem cell niche disappearance, characterized by cellular differentiation [30].

The key factor to identify stem cell niches is the stem cell localization itself. For this purpose,label-retention assays may be applied, two common labels being ^{3}H-thymidine and the thymidine analog BrdU. Upon asymmetric cellular division, stem cells may incorporate either

of these labels into their DNA thereby retaining 50% of the label with the resulting daughter stem cell and 50% with the transit amplifying cell. As transit amplifying cells are fast cycling the label is gradually diluted in the following chase period while the slow cycling daughter stem cell retain the marker. Use of these and similar label-retaining assays have been employed to identify the stem cell niche of the skin, hair follicle and peripheral cornea [41–45]. Though being an intriguing method for locating stem cells, label-retention techniques has certain disadvantages. Stem cells that did not enter the cell cycle during the labelling period will for instance remain unmarked, while progenitor cells that terminally differentiate and stop cell division can retain markers for longer periods of time [46]. Stem cells and their niches have, never the less, been identified in several organs. In vertebrates these include the bulge region of the hair follicle, the bone marrow and the lover region of the crypts in the small intestine [41,47–49]. The common denominator of these organs, however, is that they are characterized by a continuous supply of cells descending from the stem cells. Stem cells in tissues characterized by a lower cellular turnover are, on the contrary, more difficult to identify. One such organ is the liver, where mitotically quiescent hepatocytes have relatively long life spans and high proliferative capacities [50,51].

4. Hepatic anatomy

Although many cell types are present, the liver is characterized by two epithelial tissue components; cholangiocytes and hepatic cords containing hepatocytes, respectively. The hepatocytes secrete serum proteins, including albumin, and express monooxygenases from the cytochrome P450 family, the major enzymes involved in oxidative metabolism of xenobiotics [52]. Cholangiocytes, on the other hand, form biliary channels transporting bile from the liver towards the bile bladder.

Examination of hepatic tissue sections reveal an unvarying landscape of cords of hepatocytes with scattered central veins and so-called portal triads or portal tracts. The latter contain bile ducts and branches from the portal vein and portal artery, thereby forming a triad [53]. However, from a three-dimensional perspective, this dull landscape masks a highly complex organ [53,54]. Accurately defining the livers functional entities have historically been difficult, as multiple functions could be applied based on either enzymatic expression patterns or histological observations. A frequently used definition is the simple histological unit “lobule” (figure 1). The classic lobule is envisioned as a two-dimensional hexagonal structure centered around a central vein [55]. Each hexagonal corner contain a portal tract and cords of hepatocytes extend from the hepatocytic limiting plate at the periportal space, towards the central vein [55]. The terminal segments of the biliary system in the portal tracts connect directly with the hepatic cords through a specialized structure known as the Canal of Hering – thought to constitute the hepatic progenitor cell niche [56]. Canals of Hering are formed partly by biliary cells and partly by hepatocytes near the limiting plate [56]. Hepatic cords are separated from each other by a special form of blood vessels called sinusoids [55]. The sinusoids are lined by endothelial cells with open pores, or fenestrae, lacking a diaphragm and a basal lamina [57]. The resulting high endothelial permeability facilitate the exchange of macromolecules, solutes

http://dx.doi.org/10.5772/58814

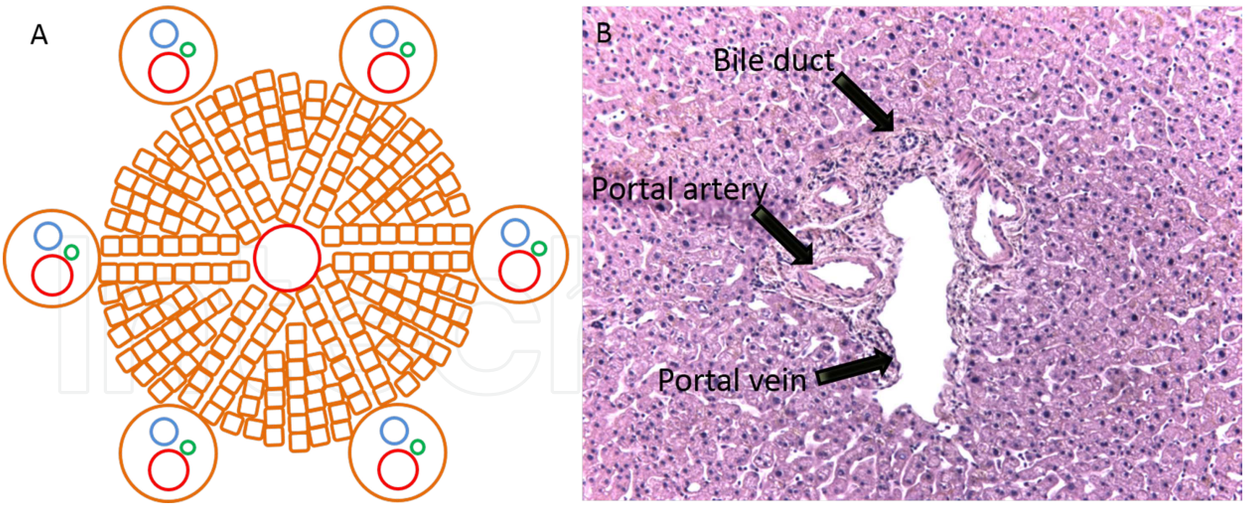

Figure 1. A. Cartoon of a stylized hepatic lobule. Each hexagonal corner of the hepatic lobule is marked by a portal area containing a portal vein, a portal artery and a bile duct. A central vein mark the center of the lobule. B. Hematoxylin and eosin staining of a tissue section from adult normal human liver. A portal area containing a portal vein, portal artery and bile duct is discernible. Magnification x100.

and water between sinusoidal blood and hepatocytes [57]. The sinusoidal wall is additionally separated from the hepatocytes by a lumen termed the space of Disse. The predominant view is that blood drain from the portal vein and portal artery branches and blends in the sinusoids from where it drains into the central vein [54,55]. Lymph, on the other hand, is thought to be generated by filtration of sinusoidal blood into the space of Disse from where it flows towards lymphatic vessels located in the portal tracts [54]. Bile canaliculi are narrow spaces formed from the apical membranes of adjacent hepatocytes in the hepatic cords [54]. Bile originating from the bile canaliculi is transported towards terminal bile ducts in the portal tracts through the canal of Hering [54,56].

Non-parenchymal cell types also present in the liver include stellate cells and Kupffer cells. Hepatic stellate cells, also known as Ito cells, are starshaped and contain lipid droplets with vast amounts of vitamin A [58]. In normal liver they are located to the space of Disse which is suggested to constitute the hepatic stellate cell niche [59]. Kupffer cells, on the other hand, are hepatic macrophages involved in the phagocytosis of cellular debris, extracellular matrix components and release of inflammatory factors [60].

5. Liver development

The liver in an endodermal derived organ with hepatocytes and cholangiocytes originating from a common progenitor termed "hepatoblast" or "primitive hepatocyte" [61]. Development of the liver goes through sequential stages including induction, specification, proliferation and maturation steps. The endoderm is important for inducing development of the neighboring cardiogenic mesoderm followed by maturation of the heart. Embryonic development of the liver is initiated in the ventral part of the anterior endoderm, whereas pancreas coordinately

develops from the dorsal part. Within a short period of time, in a so-called "window of opportunity" around embryonic day (E) 8.5-11.5 in mouse endoderm, the anterior endoderm is competent for activation of a hepatic development gene program [62]. At the time of hepatic induction, the adjacent mesenchymal tissue, comprising the cardiogenic mesoderm and septum transversum, produces subtypes of growth factors: fibroblast growth factor (FGF) and bone morphogenetic protein (BMP), respectively [63]. Growth factors acid FGF, basic FGF, FGF4, BMP 2, and BMP4 initiate a hepatic gene expression program, while FGF or cardiogenic mesoderm suppresses the pancreatic gene expression program [62]. In the absence of BMPs or FGFs, the pancreatic gene expression program is initiated while the hepatic gene program is suppressed [62].

Following induction of hepatic gene expression, the endodermal cells adopt a columnar appearance at E8.5 and express albumin. At E9.5 a thickening of the endoderm is observed, interceded by primitive endothelial cells from the septum transversum [62,64]. This prospective liver, termed the hepatic diverticulum or "liver bud", is visible in the human embryo at the 17 somite stage, corresponding to 3 weeks and 5 days post conception [53,64]. Signaling molecules, including BMPs, hepatocyte growth factor (Hgf) and vascular endothelial growth factor receptor 2 (Vegfr-2) from the septum transversum and endothelial cells induce proliferation and migration of hepatoblast positive for cytokeratin (CK19), Hepatocyte Paraffin 1 (HepPar1), α-fetoprotein (AFP) and albumin, into the adjacent septum transversum [62,65–69]. At E11 the hepatoblasts addionationally stain for the intermediate filament proteins CK8, CK14 and CK18 [70–72]. Concurrent with the hepatoblast invasion the endothelial cells coalesce around spaces in the septum transversum thereby forming anastomosing primitive blood vessels around which hepatoblast are situated. The endodermal invasion displaces the septum transversum that eventually form the liver capsule, mesenchyme and possibly the hepatic stellate cells [62,68,69,73,74].

At E14 (in mouse) hematopoietic cells colonize the liver, making it a prenatal site for hematopoiesis. Concomitantly, hepatoblasts express markers of both the hepatocytic and cholangiocytic lineages and are capable of differentiating into either of the two epithelial cell types. The hepatoblasts, however, gradually commit to either the hepatocytic or cholangiocytic lineages. Three transcriptions factors, Hepatocyte Nuclear Factor (HNF)-4α, HNF-6 and HNF-1β, are found to be particularly important in this process. Microarray data have demonstrated that HNF-4α bind approximately half of the active genes in liver and is essential for determination toward a hepatocytic fate [75,76]. On the other hand, HNF-6 and HNF-1β are essential for development of the biliary lineage. Knockout mice for HNF6 and its downstream target HNF1β, develop no gallbladder and display abnormal development of the intrahepatic and extrahepatic bile ducts [77,78]. Around E16 (mouse) the hepatoblast are commited to either the hepatocytic or cholangiocytic lineages and are thereby no longer bipotential [62,79,80].

During development of the liver, morphogenesis of the biliary tree is also said to proceed through a series of developmental stages. These are categorized as the ductal plate, remodeling bile duct and remodeled bile duct stages [79]. The earliest indicator of biliary development comes from studies of the transcription factor SRY-related HMG box transcription factor 9 (SOX9). SOX9 is essential for the formation of certain stem cell niches, such as the hair follicle

stem cell compartment [81]. SOX9 is expressed in the hepatic diverticulum but disappears during the endodermal invasion of the septum transversum. At E11.5, however, SOX9 is reexpressed in hepatoblasts located near the developing portal veins [82]. These prospective cholangiocytes lining the mesenchyme surrounding developing portal veins form a single-layered ring at E14.5 termed the "ductal plate". Studies on cells isolated from the ductal plate and from adult livers have shed important information on this structure. Cells from adult livers and the ductal plate, positive for epithelial cell adhesion molecule (EpCAM) and CK19 and negative for AFP can give rise to both the hepatic and biliary lineages, when injected into immunodeficient NOD/SCID mice [83,84]. The ductal plate is therefore not only suggested to constitute the pre-and perinatal hepatic progenitor cell niche, but also to be directly antecedent to the canal of Hering, the presumed adult hepatic progenitor cell niche [83–85].

The ductal plate, which can be envisioned as a biliary sleeve, increase expression of CK8, 18 and 19 relative to the remaining parenchymal cells [86,87]. Through a unique mode of tubulogenesis the cholangiocytes induce neighboring hepatoblast to differentiate into cholangiocytes themselves thereby developing a two-layered transiently asymmetric ductal plate around E16.5 [79,82]. Focal lumina appear between the mesenchymal and parenchymal ductal plate facing layers, thereby giving rise to early bile ducts at E16.5 [79]. In the following remodeling phase, these primitive bile ducts migrate into the portal mesenchyme in a complex process timely coordinated with the formation of hepatic portal arteries [65]. The parts of the ductal plate, which are not involved in bile duct formation, possibly regress as a result of apoptosis [88]. As of a result, the intrahepatic bile ducts loose contact with the ductal plate and become fully embedded in the portal mesenchyme in the remodeled stage. However, the intrahepatic bile duct system is still immature until several weeks after birth and remnants of the ductal plate can be identified, in particular, at the smaller vein branches [86,89]. As a final step in the maturation process, developing cholangiocytes initiate expression of CK7, a marker of adult bile duct cells [53,86]. The outlined development of the intrahepatic bile duct system is initiated at the hepatic hilum from where it gradually progresses towards the periphery of the liver, where the smaller portal branches reside [89].

6. Hepatic tissue homeostasis

The wide range of important metabolic functions performed by the liver and its proximity to ingested environmental toxins are hypothesized to have imparted the livers tremendous capacity for adaptation and regeneration [3,90].

Hepatocytes are the main component of liver and therefore, the most vulnerable to damage. The generation of adult hepatocytes, under non-pathogenic conditions, has been widely disputed. In normal liver, parenchymal turnover is slow with hepatocyte lifespans estimated 150 to 450 days in rat [50,51,91,92]. With a turnover rate of normal liver cells of approximately 1 in 20,000-40,000 at any given time the entire liver is estimated to be replaced by normal tissue at least once a year [93]. As hepatocytes supposedly are terminally differentiated cells, they were once hypothesized only to possess the capacity for one or two cell divisions. A number

of studies of label-retaining markers of cells based on the incorporation of markers such as tritiated thymidine into DNA in rats or lack of markers such as cytochrome c in humans have located proliferative hepatocytes in the periportal region [94,95]. Cell tracking has illustrated a gradual invasion of these recognizable cells from the portal tract towards the terminal central vein. Based on these and similar experiments the "streaming liver" hypothesis was suggested in which mitotically active hepatocytes at the limiting plate in the periportal region continuously provided hepatocytic offspring. In a unidirectional fashion, these hepatocytes are hypothesized to stream along the sinusoids as they gradually change enzymatic expression and eventually replace dead hepatocytes in the perivenous region [94]. However, this model is still quite controversal. Long-term labelling of hepatocytes with beta-galactosidase, an enzyme capable of converting X-gal into an insoluble blue compound, found positive clusters of hepatocytes, ergo cells that had divided, throughout the liver lobule thereby contradicting the streaming liver hypothesis [51].

The relative mitotic quiescence of hepatocytes and cholangiocytes mask their huge proliferative potential. Resecting two-thirds of the liver in accordance with the partial hepatectomy protocol (PHx) leads to complete regrowth in approximately 10 days [1,3]. This regrowth is, however, not a true regeneration, given that it does not recreate original hepatic morphology but is compensatory hyperplasia in the residual liver lobes [55]. Even with this relatively harsh treatment of the liver, only 1-2 proliferative events of hepatic epithelial cells are needed to lead to complete compensatory regrowth with no or very little hepatic stem cell contribution [1,96]. Impressively, this procedure can be repeated at least 12 times in rats without regenerative failure or endangering liver function as hepatocytes maintain a fully differentiated state [97]. In an experimental animal model, mice deficient for the tyrosine catabolic enzyme, fumarylacetoacetate hydrolase (FAH), suffer from hepatocyte damage due to accumulation of fumarylacetoacetate and its precursor maleylacetoacetate [98]. However, wild-type hepatocytes are capable of rescuing this phenotype. In an elegant study serial transplantations of wild-type hepatocytes into FAH deficient mice repopulated 6 generations of livers corresponding to 69 cell doublings [98]. Therefore, during normal tissue homeostasis, hepatocytes could be regarded as the functional unipotent hepatic stem cell, capable of giving rise to more than 50 livers [1]. Furthermore, in some chronic biliary diseases such as primary biliary cirrhosis and primary sclerosing cholangitis, hepatocytes have even been observed differentiating into biliary cells [99,100]. Nonetheless, the replicative activity of even hepatocytes can apparently decrease in chronic hepatic injury in mice and advance cirrhosis in humans, possibly due to telomere shortening [101]. Regeneration through replication of hepatocytes and cholangiocytes is also known as the "first tier of defense" or a "level 1 response" [4]. This form of response was responsible was regenerating Prometheus' liver during night as the eagle essentially conducted partial hepatectomy during the day.

7. Localizing the hepatic progenitor cell niche

Locating stem cells is the first step into characterizing their niche. Stem cells and their niches have been defined in several tissues, including the hair follicle and skin, the hematopoietic

system and in the intestinal crypts [42,44,49,102–105]. These organs are, unlike the liver, generally under constant renewal and require frequent stem cell division for tissue replenishment. Stem cells in these organs are therefore fulltime committed to perform stem cell function. However, stem cells in tissues with low turnover have been notoriously difficult to detect. As with arrangements in other stem cell niches the hepatic progenitor cell niche is thought to be structurally composed of a stem or progenitor cell population situated on a basal lamina and in contact with surrounding support cells [36,38]. As cellular turnover in the liver is already low and hepatic homeostasis and regeneration to a large extent is completed by differentiated parenchymal and non-parenchymal cells stem cells in this organ have been difficult to characterize.

While hepatocytes can conceptually be considered as the livers functional stem cells, the contribution of hepatic stem or progenitor cells to liver regeneration has been debated. Clues to a possible existence of stem cells in the liver came from early studies conducted by Farber, Wilson and Leduc [106,107]. Following dietary administration of DL-ethionine or carcinogenic 2-acetylaminofluorene (2-AAF) to rats, Farber observed the presence of pseudoductular structures consisting of small cells near the hepatic portal areas in rat liver (figure 2). These small cells are termed "oval cells" due to their oval shaped nucleus and scant cytoplasm [106]. In a following study Wilson and Leduc examined murine livers following dietary administration of ethionine and bentonite [107]. The presence of small cholangioles apparently giving rise to both bile-duct cells and parenchymal cells suggested the presence or a population of reserve cells or stem cells [107]. In acute liver failure and chronic liver diseases similar so-called "ductular reactions" may be noted at the portal triad interface [7,108–110]. The ductular response is thought to result from proliferating progenitor cells and represent the livers "second tier of defense" or "level 2 response" [4]. These cells termed oval cells in rodents are named progenitor cells in humans as rodent hepatic injury models and human diseases may not be directly comparable. However, we will collectively refer to them as hepatic progenitor cells (HPCs). The resulting arborizing network of ductular structures sprouting from the portal area is classified as an atypical ductular reaction due to a poorly defined lumen.

A number of rodent hepatic injury models have been developed to investigate various modes of regeneration and to mimic human hepatic diseases. Particularly notable models include partial hepatectomy, which induces proliferation of differentiated hepatocytes and cholangiocytes and thereby represent the first tier of defense [18,111]. Ligation of the common bile duct (Bile Duct Ligation) obstructs bile flow from the liver (figure 2). This surgical technique mimics cholestasis and induces proliferation of hepatocytes and the larger bile ducts without signs of differentiation towards the hepatocytic lineage in the latter [112,113]. Several injury models specifically induce HPC responses and thereby the second tier of defense. For example administration of the choline-deficient ethionine-supplemented (CDE) diet or carcinogenic agents such as 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) to rodents induces ductular reactions while carbon tetrachloride (CCl_4) administration additionally result in advanced hepatic fibrosis [114–116]. In the 2-AAF/PHx model administration of 2-acetylaminofluorene to rats is followed by two-thirds partial hepatectomy. This procedure block hepatocyte differentiation, ergo the first tier of defense, while at the same time providing a strong stimulus

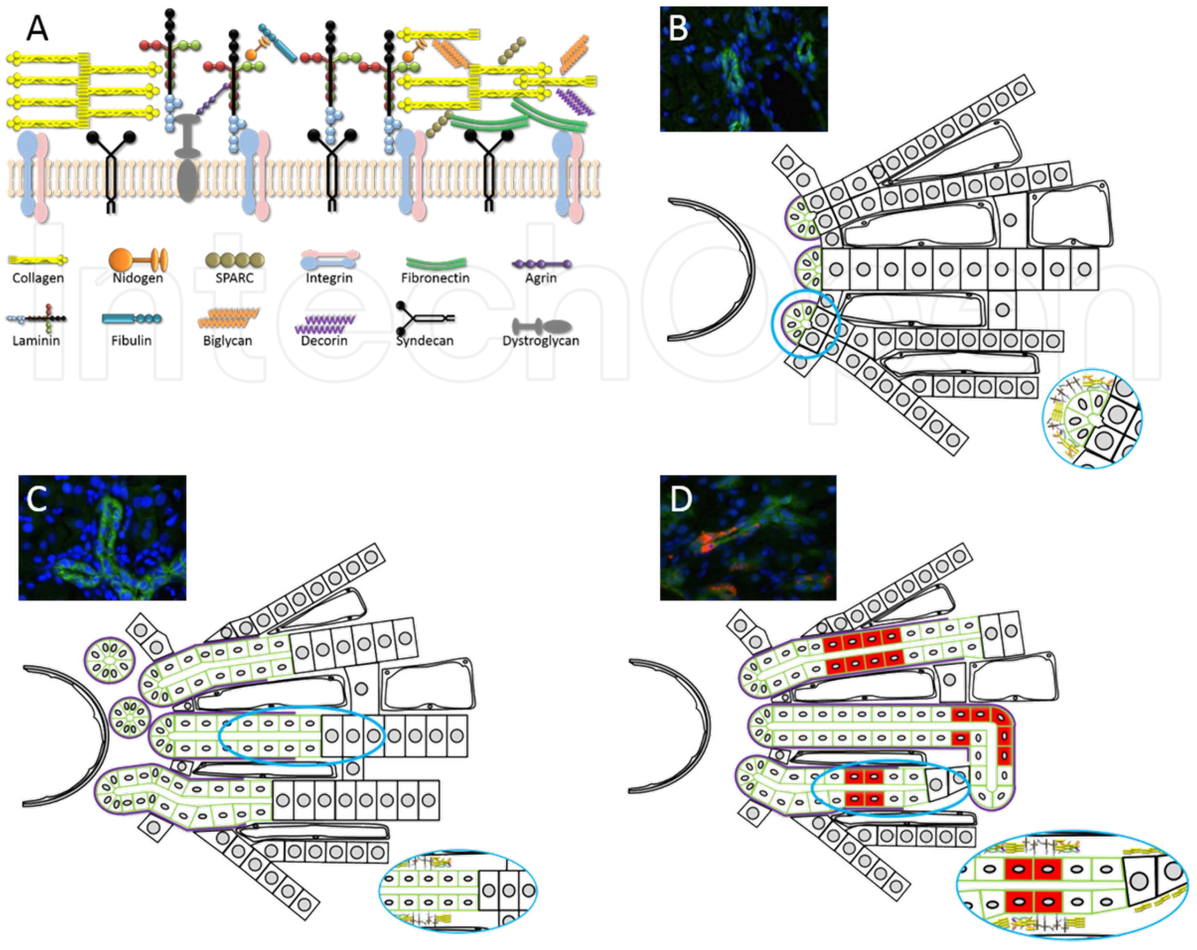

Figure 2. A. Cartoon of typical constituents of the extracellular matrix. B, C, D. Microphotographic images and cartoons of livers from rats subjected to B) sham operation, C) bile duct ligation and D) the 2-AAF-PHx model. Cholangiocytes and progenitor cells are stained for HAI-1 (green) and DLK1 (red). Cartoons in B, C and D portray part of portal areas with bile ducts (green) and their extracellular matrix in the portal mesenchyme bordering the limiting plate. B) In sham operated rat liver cholangiocytes are marked by HAI-1. C) In the bile duct ligation model in rats the larger bile ducts proliferate. D) In the 2-AAF-PHx model in rat liver a ductular reaction contain a subpopulation of hepatic progenitor cells positive for DLK1. Regardless of injury model extracellular matrix components escort the cholangiocytes. Upon exiting the hepatic progenitor cell niche the progenitor cells differentiate into hepatocytes. Microphotograph magnification x100. Adapted from Vestentoft et al. 2013 [129].

for growth. As a result, a ductular response is mounted (figure 2). Although these proliferating epithelial cells are collectively referred to as oval cells, it remains unclear if the oval cells resulting from different hepatic insults across different species have common characteristics as mice and rat respond differently to the same insults [117].

The ductular response is thought to represent proliferating progenitor cells. However, the origin of these progenitor cells is debated. Ductular reactions initiated, for example in the 2-AAF/PHx protocol, display both biliary and hepatocytic markers [26,118,119]. Moreover, destruction of the biliary tree through administration of 4,4'-methylenedianiline (MDA) inhibits progenitor cell proliferation, suggesting that progenitor cells originate from the biliary lineage [120]. However, administration of dexamethasone diminishes progeni-

tor cell induction, but has no consequence on proliferation of larger bile ducts [113]. The anatomical location of the Canal of Hering, at the portal triad interface, makes this structure a prime candidate for the adult HPC compartment or "niche" [56,120–122]. Based on these experiments, the Canal of Hering represents, therefore, the HPC niche and the ductular reaction represents the activated HPC niche, respectively. However, the assumed stem cells located in the Canal of Hering may in fact not be "true" stem cells, but rather subpopulations of biliary or hepatocytic cells with increased stemness compared to other cells of their respective lineage [3]. Although progenitor cells morphologically resemble biliary cells, ductular reactions are phenotypically heterogenous [123]. In the ductular end connected to the biliary tree, the cells display cholangiocytic markers such as CK19, whereas the ductular end facing the parenchyma display hepatocytic markers including HepPar1 and the transcription factor HNF4 [119,123]. Between these extremes, hepatobiliary cells expressing cholangiocytic and hepatocytic markers to various degrees are found [123,124]. It is now clear that the ductular response can be divided into several distinct phases that are evident in the 2-AAF/PHx protocol [117]. In the activation phase, on day 1, few proliferating HPCs expressing CK19 are detectable in the biliary ductules. In the early proliferation and migration phase on day 5 multiple CK19-positive HPCs can be observed whereas progenitor cell expression of delta-like 1 homolog (DLK1/Pref1) and AFP is rare. In the late proliferation and migration phase on day 9, arborizing ductular structures expand from the portal area with HPCs expressing CK19, Dlk1 and AFP proteins.

Even though a number of HPC markers have been reported, none are specific for a pure population of hepatic stem cells [24,123,125,126]. What is more, only few of the reported HPC markers are expressed on the cellular surface and are therefore able to be employed for cellular isolation studies. CK19, OV-6 (an antibody recognizing a shared epitope between CK14 and CK19), EpCAM, CD24, hepatocyte growth factor activator inhibitor type 1 (HAI-1) and suppressor of tumorigenicity 14 (ST14) decorate both the intrahepatic bile ducts and the ductular reactions but only EpCAM, CD24, HAI-1 and ST14 are expressed on the surface [126–129]. AFP and DLK1, however, mark a subpopulation of HPCs suggesting the presence of an established hierarchy amongst the HPCs [129–131]. AFP and DLK1 are normally not expressed in the liver. However, both proteins are observed in hepatoblasts, the embryonic precursors to the cholangiocytic and hepatocytic lineages, suggesting that oval cells recapitulate a fetal phenotype when activated in hepatic injuries [72,132]. DLK1 is a transmembrane protein often described as an inhibitor of cellular differentiation and is expressed in less differentiated cells [133,134]. For example, forced expression of Dlk1 inhibits adipogenesis, whereas suppression promotes this process [133]. It is therefore conceivable that Dlk1 inhibits HPC differentiation thereby allowing transit amplifying cells to increase in numbers similar to the one observed in other stem cell niches [36]. With regard to AFP, elevated serum levels of this protein are associated with a favorable prognosis for patients with fulminant hepatic failure [135,136]. This observation supports the assumption that AFP marks cells capable of, at least, differentiating towards the hepatocytic lineage.

8. Support cells and the hepatic progenitor cell response

Proliferation and morphogenesis of cholangiocytes and HPCs is a complex interplay between the biliary cells, surrounding support cells and the extracellular matrix. All of these components contribute to the HPC niche. Cell-cell interactions and cell-matrix interplays are likely to be important for regulating stem cell behavior within niches [37].

Hepatic stellate cells possibly originate from the septum transversum-derived mesothelium lining the liver [137]. They are recognizable in their quiescent state by the expression of desmin and glial fibrillary acidic protein (GFAP), whereas they express alpha smooth muscle actin (α-SMA) when activated, often as a result of hepatic injury [138–141]. In the quiescent state they reside in the space of Disse, which constitute a laminin coated hepatic stellate cell niche, but when activated they give rise to contractile myofibroblast [59]. Both cell types are major producers of extracellular matrix components and activated hepatic stellate cells are the main source of matrix metalloproteinases and their inhibitors. However, so-called portal fibroblasts and vascular myofibroblasts can also transform into myofibroblasts thus giving rise to much confusion about the origins of the latter [142,143]. Additional confusion has been caused by misinterpretation of cellular markers. In particular, Thy-1, a cell surface protein initially suggested to mark oval cells, was later reclassified as a marker for hepatic myofibroblasts [144]

Hepatic stellate cells and myofibroblasts are greatly involved in the HPC response. In both the CDE model of HPC induction in mice, and the 2-AAF/PHx model in rat, hepatic stellate cell and myofibroblast response are invoked [121,145,146]. Hepatic stellate cells and myofibroblasts not only intimately escort the HPC invasion into the parenchyma, but cellular processes from the hepatic stellate cells disrupt the HPC basal lamina and form direct cellular contact [121]. Such direct cell-cell interactions between hepatic stellate cells and liver epithelial cells has been shown to induce differentiation of the latter into a hepatocytic fate *in vitro* [147]. The HPC response is a regulated process undergoing several stages. Both initiation and termination is under tight regulation and hepatic stellate cells may be involved in these processes. HGF is a potent mitogen for hepatocytes whereas TGF-β is a strong inhibitor of their proliferation [148]. TGF-β is additionally identified as a partaker in maintaining quiescence of stem cells in other niches, such as the melanocyte stem cells located to the hair follicle bulge region [149]. Not only are hepatic stellate cells activated and induced to transform into myofibroblasts by TGF-β, but hepatic stellate cells themselves are major producers of this cytokine [148]. Conditioned media harvested from hepatic stellate cells in the early HPC response is rich in hepatocyte growth factor (HGF). This media promote HPC proliferation, possibly due to an override of the antiproliferative effect of TGF-β [150]. In the terminal phases of liver regeneration hepatic stellate cells change cytokine expression profile and produce high levels of TGF-β which inhibits proliferation of hepatocytes [150]. Thus, hepatic stellate cells may be involved in both initiation and termination of the HPC response.

Other cells types involved in the HPC response are macrophages and Kupffer cells. Macrophages can remodel the extracellular matrix, partly through the production of matrix metalloproteinases [151]. As for hepatic stellate cells and myofibroblasts also bone marrow derived macrophages intimately associate with ductular reactions in rats [145]. Kupffer cells, on the

other hand, are resident hepatic macrophages. Kupffer cells are greatly activated in the CDE model of HPC response in mice. Before onset of HPC proliferation and parenchymal invasion activated Kupffer cells gradually shift from a more periportal location towards a more centrilobular location. Depletion of Kupffer cells through clodronate injections result in greatly reduced invasion of HPCs into the hepatic parenchyma. However, HPC proliferation is unaltered. In conclusion these data suggest that hepatic stellate cells are involved in the initiation, proliferation and termination of the HPC response, whereas Kupffer cells are needed for HPC invasion into the hepatic parenchyma.

9. Extracellular matrix components and the hepatic progenitor cell response

Extracellular matrix can be defined as the complex molecular material surrounding cells and encompass both the basement membrane and the interstitial matrix [152]. Major components include the respective protein families of collagens, laminins, elastins, proteoglycans and glycosaminoglycans (figure 2) [152–155].

The extracellular matrix is a dynamic scaffold known to affect aspects of stem cell behavior such as morphology, growth and survival. A proportion of these responses are due to interactions between the extracellular matrix components and integrins, a family of dimeric extracellular matrix receptors that are linked to and transmit signals to the cytoskeleton [156,157]. The extracellular matrix may also contain growth factors which provide growth and morphogenic signals to nearby cells. Even physical features of the matrix, such as rigidity and geometry may influence cellular phenotype and behavior and has been shown to direct stem cell lineage specification [152,158–160]. Studies of *Drosophila spp.* stem cell niches have clarified that the microenvironment, as expected, may promote adherence to the niche and repress stem cell differentiation [36]. What is more, with age the molecular composition of the extracellular matrix change in an unfavorable direction for stem cell function and proliferation. This has been illustrated in experiments where stem cells transplanted from older mice, where stem cell self-renewal and differentiation has deteriorated, to extracellular matrix from younger mice rejuvenate stem cell function to levels comparable to that observed in younger mice [161,162].

Upon induction of the HPC response, the molecular composition of the HPC niche is thought to change in favor of promoting progenitor cell proliferation. Therefore, a key to understanding HPC biology and to characterize the HPC niche lies within unravelling the extracellular matrix composition of the niche. It is of particular interest to clarify which extracellular matrix molecules regulate the hepatic progenitor cell responses. A number of extracellular matrix molecules taking part in development of the intrahepatic bile ducts or in modulating the HPC response have been identified. Particularly, laminin and collagen I and IV are associated with these processes, but also other extracellular matrix components including tenascin, nidogen 1, agrin and fibronectin contribute.

The family of collagen fibrils comprises 28 members, all with at least one triple helical domain and arranged in a rope-like fashion [163,164]. Collagens are deposited in the extracellular space

and particularly collagen I and collagen IV are implicated in hepatic development and regeneration [165]. However, their roles seem quite different. Collagen I is the main component of hepatic fibrosis, where it is laid down by the non-parenchymal hepatic stellate cells and myofibroblasts and contribute to the formation of scaring tissue [165,166]. Collagen IV, on the other hand, is part of the basement membrane of adult biliary cells and contributes to the ductal plate, the prenatal hepatic progenitor cell niche [167,168]. Collagen I and IV delineate expanding biliary cells not only in the HPC response, but also in the bile duct ligation model [129,146].

Members of the laminin family are trimeric proteins that, as for collagen IV, are part of the basal lamina [169]. In the HPC response laminin expression can be detected in hepatic stellate cells, myofibroblasts, endothelial cells and the progenitor cells themselves [170–172]. As for collagen IV, laminin contribute to the ductal plate during development and form the basal lamina escorting the HPC response in close apposition to stellate cells [121,168]. Several studies have highlighted the importance of remodeling the extracellular matrix in connection with the HPC response. In the CDE-induced murine model of HPC activation α-SMA positive cells and an extracellular matrix rich in collagen I are deposited in the periportal area prior to oval cell proliferation [146]. The ECM is laid down in a porto-veinous direction, thereby preforming a niche for the HPCs to invade. However, this invasion process is tightly correlated with ECM remodeling. Hepatic macrophages and stellate cells are sources of a variety of extracellular matrix degrading enzymes, such as matrix metalloproteinases (MMP) 2, 9, 12 and 13, and their inhibitor, tissue inhibitor of metalloproteinase type 1 (TIMP-1) [151,173,174]. Where the CCl_4 or CDE-models of HPC activation initiate a florid HPC response in wild-type mice this response is markedly attenuated in mice expressing a degradation resistant form of collagen I [175]. These mice also display a distinct paucity of laminin deposition suggesting that degradation of collagen is a prerequisite for HPC proliferation and parenchymal invasion.

Where ECM remodeling and collagen I degradation is necessary for the HPC response only laminin is important for the biliary phenotype. Primary murine HPCs cultured on laminin up-regulate expression of HPC and biliary associated genes, such as DLK1 and aquaporin 1, respectively, while hepatocytic gene expression, exemplified by C/EBPα is inhibited [145]. Collagen I and IV, on the other hand, inhibit or do not influence these biliary genes, whereas fibronectin promote C/EBPα expression. In support of these results, culturing HPCs with laminin support proliferation and expansion *in vitro* whereas culturing HPCs with collagen I result in growth arrest and differentiation [176,177]. The importance of the laminin-rich activated progenitor cell niche for maintaining the biliary/progenitor phenotype *in vivo* is also evident in the HPC response, as disappearance of the basement membrane induces differentiation [129,178]. Assuming that the canal of Hering truly constitutes the hepatic progenitor cell niche, this niche therefore appear to be sharply limited by the deposition of collagen I, collagen IV, laminin, nidogen 1 and agrin [129]. The niche support maintaining the biliary phenotype and proliferation of HPCs that will differentiate to a hepatocytic phenotype upon exit from the HPC niche, not unlike the scenario of other stem cell niches [36].

Stem cell niche component		Comment
Progenitor cell niche position	The Canal of Hering	Most distal part of the bile duct system. Composed of cholangiocytes and hepatocytes. Link bile ducts with canaliculi between hepatocytes.
Progenitor cell origin	Possibly the biliary lineage	Administration of dexamethasone selective diminishes the progenitor cell response.
Progenitor cell composition	Phenotypically heterogenous	Progenitor cells display biliary (CK19) and hepatocytic markers (HepPar1, HNF4α) to various degrees.
Progenitor cell markers	AFP, NCAM, DLK1/Pref1	NCAM and DLK1/Pref1 are cell surface markers. AFP and DLK1/Pref1 are expressed during hepatic development.
Associated cell types	Kupffer cells	Necessary for invasion of ductular reactions into the hepatic parenchyma.
	Hepatic stellate cells	Intimately associate with ductular reactions. Are necessary for initiation, proliferation and termination of ductular reactions.
	Macrophages	Intimately associate with ductular reactions.
Associated extracellular matrix components	Laminin Collagen I + IV Nidogen 1 Agrin	Laminin is essential for maintaining the biliary phenotype.

Table 1. Summary of components associated with the activated hepatic progenitor cell niche.

10. Activation and aberrant hepatic stem cell activation.

Animal studies have clarified that when regeneration through hepatocytic division fail HPCs from the canal of Hering contribute to liver regeneration. Despite several protein markers, such as Dlk1, EpCAM, CK19 and AFP, are associated with HPCs a pure population of hepatic stem cells or their niche have not been defined [83,179–182]. However, as elevated levels of AFP are associated with increased survival of patients suffering from acetaminophen-induced liver injury, hepatic stem cells may be activated in acute hepatic diseases [136]. OV6 and CK7 mark ductular reactions and intermediate hepatocytes, i.e. progenitor cells on route to a hepatocytic fate, suggesting stem cell involvement in a variety of human diseases and syndromes. These include hepatitis C virus infection, fatty liver disease and acute processes such as submassive liver cell necrosis, [183]. Generally, HPC activation seems correlated with the severity of inflammation and fibrosis [184]. In addition, the more aggressive the hepatocellular injury is, the larger a proportion of intermediate hepatocytes are observed [184]. Interestingly, the protein deleted in malignant brain tumor 1 (dbmt1) is specifically associated with ductular cell

populations emerging after acetaminophen intoxication or infection with hepatitis B virus but not in primary biliary cirrhosis or large bile duct obstruction. Dbmt1 therefore may have a role in cellular fate decision [185].

Liver cancer is the second most frequent cause of cancer death in men and the sixth leading cause of cancer death in women [186]. Hepatocellular carcinoma (HCC) represent the major histological subtype accounting for 70-85 % of primary livers cancers, followed by an increase in incidents of intrahepatic cholangiocarcinomas (ICC) [186,187]. Given that cancer cells and stem cells share certain characteristics cancer is proposed to represent an abnormal stem cell disease [188,189]. Both categories of cells can self-renew, divide unlimited and give rise to heterogeneous progeny. Indeed, certain gliomas, intestinal adenomas and squamous skin tumours are now attributed to cancer stem cells (CSCs) [190–193]. Liver cancer most frequently arise in chronic liver diseases, such as chronic hepatitis, cirrhosis or both, where hepatocytic regeneration and continuous inflammation occur [194,195]. Hepatocarcinogenesis is considered as a slow process in which genomic changes progressively alter the hepatocellular phenotype [194]. In chronic liver diseases, the hepatic microenvironment is substantially altered in a fashion promoting cellular damage. Stellate cells are activated and infiltrating lymphocytes may cause inflammation through the release of free radicals and cytokines, resulting in DNA damage and cell proliferation, factors that may promote aberrant HPC activation [196,197]. However, as the hepatic stem cells are not fully defined, their involvements in these liver cancers have not been conclusively established. In addition to accumulation of genomic and epigenetic changes of genes and regulatory pathways the hepatic microenvironment is also involved in promoting liver cancer. For instance, activated hepatic stellate cells locate in the space between endothelial cells and trabeculae of cancer cells in HCC patients [198]. Conditioned media from such activated hepatic stellate cells both increase proliferation and migration of human HCC cells [199]. Thus, activated hepatic stellate cells may both drive fibrosis and proliferation of HCC cells.

A third form of primary liver cancer is the rare HCC-cholangiocarcinoma (HCC-CCA). In addition to the heterogenous cellular morphology also displayed by HCCs and ICCs, HCC-CCA's show signs of both hepatocellular and biliary epithelial differentiation [200]. Indeed, analysis of the expression pattern of hepatocytic marker HepPar1 and cholangiocytic markers CK7, CK19, EpCAM and CD133 in HCC-CCA's reveal subpopulations of cancer cells coexpressing both categories of markers [182,200–203]. These results seemingly confirm the hypothesis that HCC-CCA are of HPC origin and human hepatocarcinogenesis may originate from the transformation of HPCs [200]. The identification of bipotent CSCs possibly originating from HPCs is interesting, as stem cell like expression patterns in liver cancers reflect a particularly malignant nature and poor prognostic outcome [182,204–206]. However, the identification of bipotent cancer stem cells also opens for new therapeutic applications. Identification and elimination of CSCs could provide more effective treatment of certain tumors and prevent reoccurrence. Unfortunately, the niche controlling self-renewal, proliferation and differentiation of HPCs and CSCs is still not well described and the putative hepatic stem cells remain unidentified.

11. Conclusion and perspectives

The present chapter has attempted to provide a simplified overview of current knowledge of hepatic stem cells and their niches. Unfortunately, the putative hepatic stem cell has not been identified and therefore not been characterized. Knowledge of the hepatic stem cell therefore mainly originates from analysis of its progeny, the hepatic progenitor cells, in animal models where regeneration through hepatocyte division is impaired. As a result, the location of the HPC niche is unknown. However, evidences point to the canal of Hering as the HPC niche. Assuming that the canal of Hering truly represent the hepatic stem cell niche, and that HPCs are descendants of hepatic stem cell, animal studies and their corresponding human diseases has provided us with some knowledge of the constituents in the activated HPC niche:

- Activated hepatic progenitor cells are phenotypically heterogeneous and to various degrees display markers of the biliary and hepatocytic lineages.
- A cellular hierarchy is present with AFP, DLK1 and NCAM marking subpopulations of HPCs [123].
- Hepatic stellate cells and macrophages intimately associate with the ductular reactions.
- Hepatic stellate cells are necessary for initiation, proliferation and termination of ductular reactions.
- Kupffer cells are necessary for invasion of ductular reactions into the hepatic parenchyma.
- The extracellular matrix in the HPC micromillieu contain laminin, collagen I and IV, nidogen 1 and agrin.
- Laminin is necessary for maintaining the biliary phenotype of the HPCs.
- HPCs differentiate into hepatocytes upon exit from the activated HPC niche.

The establishment of HPC subpopulations suggests the presence of progenitor cells at different stages of differentiation. Identifying additional proteins expressed on the HPC surface could facilitate isolation and characterization of these subpopulations and evaluation of their potential for differentiation. Furthermore, as hepatic stem cells may be implicated in development of primary liver cancers, better characterization of the hepatic progenitor cells could provide new targets for treatment of cancerous diseases. Aberrant progenitor cell activation and proliferation is also dependent on the hepatic microenvironment. As stellate cells and Kupffer cells are involved in the proliferation and invasion of HPCs these cell types could also provide targets for alleviating HPC derived cancers. In addition, targeting stellate cells has the potential to reduce hepatic fibrosis. Therefore, more research is needed into characterizing the hepatic progenitor cell niche and obtaining a better understanding of the activation and differentiation of the hepatic progenitor cells.

12. Nomenclature

2-AAF: 2-Acetylaminofluorene.

AFP: α-Fetoprotein.

ASMA: alpha smooth muscle actin.

BMP: Bone morphogenetic protein.

BrdU: 5-Bromo-2′-Deoxyuridine.

CCl4: Carbon tetrachloride.

CDE: Choline-deficient, ethionine supplemented.

CK: Cytokeratin.

CSC: Cancer Stem Cell.

DDC: 3,5-diethoxycarbonyl-1,4-dihydrocollidine.

DLK1: Delta-Like 1 Homolog.

Dmbt1: Deleted in malignant brain tumors 1

FGF: Fibroblast growth factor.

GFAP: Glial fibrillary acidic protein.

HAI-1: Hepatocyte Growth Factor Activator Inhibitor Type 1.

HCC: Hepatocellular carcinoma.

HCC-CCA: HCC-cholangiocarcinoma.

HepPar1: Hepatocyte Paraffin 1.

HGF: Hepatocyte growth factor.

HNF: Hepatocyte nuclear factor.

HPC: Hepatic progenitor cell.

ICC: Intrahepatic cholangiocarcinoma.

MDA: 4,4'-Methylenedianiline.

MMP: Matrix metalloproteinase.

NCAM: Neural Cell Adhesion Molecule.

OV6: Oval Cell Marker Antibody 6.

PHx: Partial hepatectomy.

ST14: Suppressor of tumorigenicity 1.

SOX9: SRY-related HMG box transcription factor 9.

TIMP-1: tissue inhibitor of metalloproteinase 1.

Vegfr2: Vascular endothelial growth factor receptor-2.

Author details

Peter Siig Vestentoft*

Address all correspondence to: sqp608@alumni.ku.dk

Dako, Glostrup, Denmark

References

[1] Michalopoulos GK, DeFrances MC (1997) Liver regeneration. Science 276: 60-66.

[2] Russo FP, Parola M (2011) Stem and progenitor cells in liver regeneration and repair. Cytotherapy 13: 135-144. 10.3109/14653249.2010.545386 [doi].

[3] Michalopoulos GK (2007) Liver regeneration. J Cell Physiol 213: 286-300. 10.1002/jcp.21172 [doi].

[4] Riehle KJ, Dan YY, Campbell JS, Fausto N (2011) New concepts in liver regeneration. J Gastroenterol Hepatol 26 Suppl 1: 203-212. 10.1111/j.1440-1746.2010.06539.x [doi].

[5] Michalopoulos GK (2010) Liver regeneration after partial hepatectomy: critical analysis of mechanistic dilemmas. Am J Pathol 176: 2-13. S0002-9440(10)60317-3 [pii]; 10.2353/ajpath.2010.090675 [doi].

[6] Bernal W, Auzinger G, Dhawan A, Wendon J (2010) Acute liver failure. Lancet 376: 190-201. S0140-6736(10)60274-7 [pii];10.1016/S0140-6736(10)60274-7 [doi].

[7] Dechene A, Sowa JP, Gieseler RK, Jochum C, Bechmann LP, El FA, Schlattjan M, Saner F, Baba HA, Paul A, Dries V, Odenthal M, Gerken G, Friedman SL, Canbay A (2010) Acute liver failure is associated with elevated liver stiffness and hepatic stellate cell activation. Hepatology 52: 1008-1016. 10.1002/hep.23754 [doi].

[8] O'Grady JG (2005) Acute liver failure. Postgraduate Medical Journal 81: 148-154.

[9] Larsen FS, Kirkegaard P, Rasmussen A, Hansen BA (1995) The Danish liver transplantation program and patients with serious acetaminophen intoxication. Transplant Proc 27: 3519-3520.

[10] Ostapowicz G, Fontana RJ, Schiodt FV, Larson A, Davern TJ, Han SH, McCashland TM, Shakil AO, Hay JE, Hynan L, Crippin JS, Blei AT, Samuel G, Reisch J, Lee WM

(2002) Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. Ann Intern Med 137: 947-954. 200212170-00007 [pii].

[11] Rolando N, Wade J, Davalos M, Wendon J, Philpott-Howard J, Williams R (2000) The systemic inflammatory response syndrome in acute liver failure. Hepatology 32: 734-739. S0270913900079660 [pii];10.1053/jhep.2000.17687 [doi].

[12] Larson AM (2010) Diagnosis and management of acute liver failure. Curr Opin Gastroenterol 26: 214-221. 10.1097/MOG.0b013e32833847c5 [doi].

[13] Kisseleva T, Cong M, Paik Y, Scholten D, Jiang C, Benner C, Iwaisako K, Moore-Morris T, Scott B, Tsukamoto H, Evans SM, Dillmann W, Glass CK, Brenner DA (2012) Myofibroblasts revert to an inactive phenotype during regression of liver fibrosis. Proc Natl Acad Sci U S A 109: 9448-9453. 1201840109 [pii];10.1073/pnas.1201840109 [doi].

[14] Troeger JS, Mederacke I, Gwak GY, Dapito DH, Mu X, Hsu CC, Pradere JP, Friedman RA, Schwabe RF (2012) Deactivation of hepatic stellate cells during liver fibrosis resolution in mice. Gastroenterology 143: 1073-1083. S0016-5085(12)00925-0 [pii];10.1053/j.gastro.2012.06.036 [doi].

[15] Ellis EL, Mann DA (2012) Clinical evidence for the regression of liver fibrosis. J Hepatol 56: 1171-1180. S0168-8278(12)00044-X [pii];10.1016/j.jhep.2011.09.024 [doi].

[16] Guo J, Friedman SL (2007) Hepatic fibrogenesis. Semin Liver Dis 27: 413-426. 10.1055/s-2007-991517 [doi].

[17] Rojkind M, Giambrone MA, Biempica L (1979) Collagen types in normal and cirrhotic liver. Gastroenterology 76: 710-719.

[18] Rutherford A, Chung RT (2008) Acute liver failure: mechanisms of hepatocyte injury and regeneration. Semin Liver Dis 28: 167-174. 10.1055/s-2008-1073116 [doi].

[19] Riordan SM, Williams R (2003) Mechanisms of hepatocyte injury, multiorgan failure, and prognostic criteria in acute liver failure. Semin Liver Dis 23: 203-215. 10.1055/s-2003-42639 [doi].

[20] Canbay A, Friedman S, Gores GJ (2004) Apoptosis: the nexus of liver injury and fibrosis. Hepatology 39: 273-278. 10.1002/hep.20051 [doi].

[21] Riordan SM, Williams R (2000) Use and validation of selection criteria for liver transplantation in acute liver failure. Liver Transpl 6: 170-173. S1527646500459411 [pii]; 10.1002/lt.500060221 [doi].

[22] Lee WM (1993) Acute liver failure. N Engl J Med 329: 1862-1872. 10.1056/NEJM199312163292508 [doi].

[23] Azuma H, Paulk N, Ranade A, Dorrell C, Al-Dhalimy M, Ellis E, Strom S, Kay MA, Finegold M, Grompe M (2007) Robust expansion of human hepatocytes in Fah-/-/Rag2-/-/Il2rg-/-mice. Nat Biotechnol 25: 903-910. nbt1326 [pii];10.1038/nbt1326 [doi].

[24] Hixson DC, Allison JP (1985) Monoclonal antibodies recognizing oval cells induced in the liver of rats by N-2-fluorenylacetamide or ethionine in a choline-deficient diet. Cancer Res 45: 3750-3760.

[25] Evarts RP, Nagy P, Marsden E, Thorgeirsson SS (1987) A precursor-product relationship exists between oval cells and hepatocytes in rat liver. Carcinogenesis 8: 1737-1740.

[26] Evarts RP, Nagy P, Nakatsukasa H, Marsden E, Thorgeirsson SS (1989) In Vivo Differentiation of Rat Liver Oval Cells into Hepatocytes. Cancer Res 49: 1541-1547.

[27] Van Den Heuvel MC, Slooff MJ, Visser L, Muller M, de Jong KP, Poppema S, Gouw AS (2001) Expression of anti-OV6 antibody and anti-N-CAM antibody along the biliary line of normal and diseased human livers. Hepatology 33: 1387-1393. S0270913901415254 [pii];10.1053/jhep.2001.24453 [doi].

[28] Jensen CH, Jauho EI, Santoni-Rugiu E, Holmskov U, Teisner B, Tygstrup N, Bisgaard HC (2004) Transit-amplifying ductular (oval) cells and their hepatocytic progeny are characterized by a novel and distinctive expression of delta-like protein/preadipocyte factor 1/fetal antigen 1. Am J Pathol 164: 1347-1359.

[29] Jones DL, Wagers AJ (2008) No place like home: anatomy and function of the stem cell niche. Nat Rev Mol Cell Biol 9: 11-21. nrm2319 [pii];10.1038/nrm2319 [doi].

[30] Watt FM, Hogan BL (2000) Out of Eden: stem cells and their niches. Science 287: 1427-1430. 8287 [pii].

[31] Morrison SJ, Shah NM, Anderson DJ (1997) Regulatory mechanisms in stem cell biology. Cell 88: 287-298. S0092-8674(00)81867-X [pii].

[32] Potten CS, Loeffler M (1990) Stem cells: attributes, cycles, spirals, pitfalls and uncertainties. Lessons for and from the crypt. Development 110: 1001-1020.

[33] Weissman IL (2000) Stem cells: units of development, units of regeneration, and units in evolution. Cell 100: 157-168. S0092-8674(00)81692-X [pii].

[34] Lechler T, Fuchs E (2005) Asymmetric cell divisions promote stratification and differentiation of mammalian skin. Nature 437: 275-280. nature03922 [pii];10.1038/nature03922 [doi].

[35] Schofield R (1978) The relationship between the spleen colony-forming cell and the haemopoietic stem cell. Blood Cells 4: 7-25.

[36] Losick VP, Morris LX, Fox DT, Spradling A (2011) Drosophila stem cell niches: a decade of discovery suggests a unified view of stem cell regulation. Dev Cell 21: 159-171. S1534-5807(11)00252-8 [pii];10.1016/j.devcel.2011.06.018 [doi].

[37] Spradling A, Drummond-Barbosa D, Kai T (2001) Stem cells find their niche. Nature 414: 98-104. 10.1038/35102160 [doi];35102160 [pii].

[38] Fuchs E, Tumbar T, Guasch G (2004) Socializing with the neighbors: stem cells and their niche. Cell 116: 769-778. S0092867404002557 [pii].

[39] Jensen UB, Lowell S, Watt FM (1999) The spatial relationship between stem cells and their progeny in the basal layer of human epidermis: a new view based on whole-mount labelling and lineage analysis. Development 126: 2409-2418.

[40] Zhu AJ, Haase I, Watt FM (1999) Signaling via beta1 integrins and mitogen-activated protein kinase determines human epidermal stem cell fate in vitro. Proc Natl Acad Sci U S A 96: 6728-6733.

[41] Taylor G, Lehrer MS, Jensen PJ, Sun TT, Lavker RM (2000) Involvement of follicular stem cells in forming not only the follicle but also the epidermis. Cell 102: 451-461. S0092-8674(00)00050-7 [pii].

[42] Tumbar T, Guasch G, Greco V, Blanpain C, Lowry WE, Rendl M, Fuchs E (2004) Defining the epithelial stem cell niche in skin. Science 303: 359-363. 10.1126/science.1092436 [doi];1092436 [pii].

[43] Ohyama M, Terunuma A, Tock CL, Radonovich MF, Pise-Masison CA, Hopping SB, Brady JN, Udey MC, Vogel JC (2006) Characterization and isolation of stem cell-enriched human hair follicle bulge cells. J Clin Invest 116: 249-260.

[44] Cotsarelis G, Sun TT, Lavker RM (1990) Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. Cell 61: 1329-1337. 0092-8674(90)90696-C [pii].

[45] Cotsarelis G, Cheng SZ, Dong G, Sun TT, Lavker RM (1989) Existence of slow-cycling limbal epithelial basal cells that can be preferentially stimulated to proliferate: implications on epithelial stem cells. Cell 57: 201-209. 0092-8674(89)90958-6 [pii].

[46] Goldman B (2008) Magic marker myths. Nature Reports Stem Cells.

[47] Oshima H, Rochat A, Kedzia C, Kobayashi K, Barrandon Y (2001) Morphogenesis and renewal of hair follicles from adult multipotent stem cells. Cell 104: 233-245. S0092-8674(01)00208-2 [pii].

[48] Ehninger A, Trumpp A (2011) The bone marrow stem cell niche grows up: mesenchymal stem cells and macrophages move in. J Exp Med 208: 421-428. jem.20110132 [pii];10.1084/jem.20110132 [doi].

[49] Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M, Haegebarth A, Korving J, Begthel H, Peters PJ, Clevers H (2007) Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature 449: 1003-1007. nature06196 [pii];10.1038/nature06196 [doi].

[50] MACDONALD RA (1961) "Lifespan" of liver cells. Autoradio-graphic study using tritiated thymidine in normal, cirrhotic, and partially hepatectomized rats. Arch Intern Med 107: 335-343.

[51] Bralet MP, Branchereau S, Brechot C, Ferry N (1994) Cell lineage study in the liver using retroviral mediated gene transfer. Evidence against the streaming of hepatocytes in normal liver. Am J Pathol 144: 896-905.

[52] Bertz RJ, Granneman GR (1997) Use of in vitro and in vivo data to estimate the likelihood of metabolic pharmacokinetic interactions. Clin Pharmacokinet 32: 210-258. 10.2165/00003088-199732030-00004 [doi].

[53] Vestentoft PS, Jelnes P, Hopkinson BM, Vainer B, Mollgard K, Quistorff B, Bisgaard HC (2011) Three-dimensional reconstructions of intrahepatic bile duct tubulogenesis in human liver. BMC Dev Biol 11: 56. 1471-213X-11-56 [pii];10.1186/1471-213X-11-56 [doi].

[54] Saxena R, Theise ND, Crawford JM (1999) Microanatomy of the human liver-exploring the hidden interfaces. Hepatology 30: 1339-1346. S0270913999004991 [pii];10.1002/hep.510300607 [doi].

[55] Fausto N, Campbell JS (2003) The role of hepatocytes and oval cells in liver regeneration and repopulation. Mech Dev 120: 117-130.

[56] Roskams TA, Theise ND, Balabaud C, Bhagat G, Bhathal PS, Bioulac-Sage P, Brunt EM, Crawford JM, Crosby HA, Desmet V, Finegold MJ, Geller SA, Gouw AS, Hytiroglou P, Knisely AS, Kojiro M, Lefkowitch JH, Nakanuma Y, Olynyk JK, Park YN, Portmann B, Saxena R, Scheuer PJ, Strain AJ, Thung SN, Wanless IR, West AB (2004) Nomenclature of the finer branches of the biliary tree: canals, ductules, and ductular reactions in human livers. Hepatology 39: 1739-1745.

[57] Braet F, Wisse E (2002) Structural and functional aspects of liver sinusoidal endothelial cell fenestrae: a review. Comp Hepatol 1: 1.

[58] Blaner WS, O'Byrne SM, Wongsiriroj N, Kluwe J, D'Ambrosio DM, Jiang H, Schwabe RF, Hillman EM, Piantedosi R, Libien J (2009) Hepatic stellate cell lipid droplets: a specialized lipid droplet for retinoid storage. Biochim Biophys Acta 1791: 467-473. S1388-1981(08)00207-2 [pii];10.1016/j.bbalip.2008.11.001 [doi].

[59] Sawitza I, Kordes C, Reister S, Haussinger D (2009) The niche of stellate cells within rat liver. Hepatology 50: 1617-1624. 10.1002/hep.23184 [doi].

[60] Van HN, Lanthier N, Espanol SR, Abarca QJ, van RN, Leclercq I (2011) Kupffer cells influence parenchymal invasion and phenotypic orientation, but not the proliferation, of liver progenitor cells in a murine model of liver injury. Am J Pathol 179: 1839-1850. S0002-9440(11)00665-1 [pii];10.1016/j.ajpath.2011.06.042 [doi].

[61] Wilson JW, Groat CS, Leduc EH (1963) Histogenesis of the liver. Annals of the New York Academy of Sciences 111: 8-24. 10.1111/j.1749-6632.1963.tb36945.x.

[62] Zaret KS (2002) Regulatory phases of early liver development: paradigms of organogenesis. Nat Rev Genet 3: 499-512. 10.1038/nrg837 [doi];nrg837 [pii].

[63] Cascio S, Zaret KS (1991) Hepatocyte differentiation initiates during endodermal-mesenchymal interactions prior to liver formation. Development 113: 217-225.

[64] Severn CB (1971) A morphological study of the development of the human liver. I. Development of the hepatic diverticulum. Am J Anat 131: 133-158. 10.1002/aja.1001310202 [doi].

[65] Fabris L, Cadamuro M, Libbrecht L, Raynaud P, Spirli C, Fiorotto R, Okolicsanyi L, Lemaigre F, Strazzabosco M, Roskams T (2008) Epithelial expression of angiogenic growth factors modulate arterial vasculogenesis in human liver development. Hepatology 47: 719-728. 10.1002/hep.22015 [doi].

[66] Haruna Y, Saito K, Spaulding S, Nalesnik MA, Gerber MA (1996) Identification of bipotential progenitor cells in human liver development. Hepatology 23: 476-481. S0270913996000766 [pii];10.1002/hep.510230312 [doi].

[67] Terrace JD, Currie IS, Hay DC, Masson NM, Anderson RA, Forbes SJ, Parks RW, Ross JA (2007) Progenitor cell characterization and location in the developing human liver. Stem Cells Dev 16: 771-778. 10.1089/scd.2007.0016 [doi].

[68] Lemaigre FP (2009) Mechanisms of liver development: concepts for understanding liver disorders and design of novel therapies. Gastroenterology 137: 62-79. S0016-5085(09)00463-6 [pii];10.1053/j.gastro.2009.03.035 [doi].

[69] Zhao R, Duncan SA (2005) Embryonic development of the liver. Hepatology 41: 956-967. 10.1002/hep.20691 [doi].

[70] Brill S, Zvibel I, Reid LM (1995) Maturation-dependent changes in the regulation of liver-specific gene expression in embryonal versus adult primary liver cultures. Differentiation 59: 95-102. S0301-4681(09)60795-7 [pii];10.1046/j.1432-0436.1995.5920095.x [doi].

[71] Tee LB, Kirilak Y, Huang WH, Smith PG, Morgan RH, Yeoh GC (1996) Dual phenotypic expression of hepatocytes and bile ductular markers in developing and preneoplastic rat liver. Carcinogenesis 17: 251-259.

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

[73] Severn CB (1972) A morphological study of the development of the human liver. II. Establishment of liver parenchyma, extrahepatic ducts and associated venous channels. Am J Anat 133: 85-107. 10.1002/aja.1001330106 [doi].

[74] Lemaigre F, Zaret KS (2004) Liver development update: new embryo models, cell lineage control, and morphogenesis. Curr Opin Genet Dev 14: 582-590. 10.1016/j.gde.2004.08.004 [doi];S0959-437X(04)00128-5 [pii].

[75] Odom DT, Zizlsperger N, Gordon DB, Bell GW, Rinaldi NJ, Murray HL, Volkert TL, Schreiber J, Rolfe PA, Gifford DK, Fraenkel E, Bell GI, Young RA (2004) Control of

pancreas and liver gene expression by HNF transcription factors. Science 303: 1378-1381. 10.1126/science.1089769 [doi];303/5662/1378 [pii].

[76] Li J, Ning G, Duncan SA (2000) Mammalian hepatocyte differentiation requires the transcription factor HNF-4alpha. Genes Dev 14: 464-474.

[77] Clotman F, Lannoy VJ, Reber M, Cereghini S, Cassiman D, Jacquemin P, Roskams T, Rousseau GG, Lemaigre FP (2002) The onecut transcription factor HNF6 is required for normal development of the biliary tract. Development 129: 1819-1828.

[78] Coffinier C, Gresh L, Fiette L, Tronche F, Schutz G, Babinet C, Pontoglio M, Yaniv M, Barra J (2002) Bile system morphogenesis defects and liver dysfunction upon targeted deletion of HNF1beta. Development 129: 1829-1838.

[79] Lemaigre FP (2003) Development of the biliary tract. Mech Dev 120: 81-87. S0925477302003349 [pii].

[80] Costa RH, Kalinichenko VV, Holterman AX, Wang X (2003) Transcription factors in liver development, differentiation, and regeneration. Hepatology 38: 1331-1347. 10.1016/j.hep.2003.09.034 [doi];S0270913903010048 [pii].

[81] Vidal VP, Chaboissier MC, Lutzkendorf S, Cotsarelis G, Mill P, Hui CC, Ortonne N, Ortonne JP, Schedl A (2005) Sox9 is essential for outer root sheath differentiation and the formation of the hair stem cell compartment. Curr Biol 15: 1340-1351. S0960-9822(05)00747-5 [pii];10.1016/j.cub.2005.06.064 [doi].

[82] Antoniou A, Raynaud P, Cordi S, Zong Y, Tronche F, Stanger BZ, Jacquemin P, Pierreux CE, Clotman F, Lemaigre FP (2009) Intrahepatic bile ducts develop according to a new mode of tubulogenesis regulated by the transcription factor SOX9. Gastroenterology 136: 2325-2333. S0016-5085(09)00300-X [pii];10.1053/j.gastro.2009.02.051 [doi].

[83] Schmelzer E, Zhang L, Bruce A, Wauthier E, Ludlow J, Yao HL, Moss N, Melhem A, McClelland R, Turner W, Kulik M, Sherwood S, Tallheden T, Cheng N, Furth ME, Reid LM (2007) Human hepatic stem cells from fetal and postnatal donors. J Exp Med 204: 1973-1987. jem.20061603 [pii];10.1084/jem.20061603 [doi].

[84] Schmelzer E, Wauthier E, Reid LM (2006) The phenotypes of pluripotent human hepatic progenitors. Stem Cells 24: 1852-1858. 2006-0036 [pii];10.1634/stemcells.2006-0036 [doi].

[85] Zhang L, Theise N, Chua M, Reid LM (2008) The stem cell niche of human livers: symmetry between development and regeneration. Hepatology 48: 1598-1607. 10.1002/hep.22516 [doi].

[86] Van EP, Sciot R, Callea F, van der Steen K, Moerman P, Desmet VJ (1988) The development of the intrahepatic bile ducts in man: a keratin-immunohistochemical study. Hepatology 8: 1586-1595. S0270913988001661 [pii].

[87] Shah KD, Gerber MA (1989) Development of intrahepatic bile ducts in humans. Immunohistochemical study using monoclonal cytokeratin antibodies. Arch Pathol Lab Med 113: 1135-1138.

[88] Terada T, Nakanuma Y (1995) Detection of apoptosis and expression of apoptosis-related proteins during human intrahepatic bile duct development. Am J Pathol 146: 67-74.

[89] Van EP, Sciot R, Desmet V (1988) Intrahepatic bile duct development in the rat: a cytokeratin-immunohistochemical study. Lab Invest 59: 52-59.

[90] Starzl TE, Fung J, Tzakis A, Todo S, Demetris AJ, Marino IR, Doyle H, Zeevi A, Warty V, Michaels M,. (1993) Baboon-to-human liver transplantation. Lancet 341: 65-71.

[91] Wood RL (1971) Regeneration of Liver and Kidney. JAMA: The Journal of the American Medical Association 218: 1306-1307.

[92] Zajicek G, Oren R, Weinreb M, Jr. (1985) The streaming liver. Liver 5: 293-300.

[93] Steiner JW, Perz ZM, Taichman LB (1966) Cell population dynamics in the liver. A review of quantitative morphological techniques applied to the study of physiological and pathological growth. Exp Mol Pathol 5: 146-181. 0014-4800(66)90012-8 [pii].

[94] Fellous TG, Islam S, Tadrous PJ, Elia G, Kocher HM, Bhattacharya S, Mears L, Turnbull DM, Taylor RW, Greaves LC, Chinnery PF, Taylor G, McDonald SA, Wright NA, Alison MR (2009) Locating the stem cell niche and tracing hepatocyte lineages in human liver. Hepatology 49: 1655-1663. 10.1002/hep.22791 [doi].

[95] Oren R, Zajicek G, Maaravi Y, Kenet G, Karmely F, Hubert A, Raanani P, Arber N (1997) Methimazole slows hepatocyte streaming in rats. Dig Dis Sci 42: 1433-1437.

[96] Grisham JW (1962) A morphologic study of deoxyribonucleic acid synthesis and cell proliferation in regenerating rat liver; autoradiography with thymidine-H3. Cancer Res 22: 842-849.

[97] Stocker E, Wullstein HK, Brau G (1973) [Capacity of regeneration in liver epithelia of juvenile, repeated partially hepatectomized rats. Autoradiographic studies after continous infusion of 3H-thymidine (author's transl)]. Virchows Arch B Cell Pathol 14: 93-103.

[98] Overturf K, Al-Dhalimy M, Ou CN, Finegold M, Grompe M (1997) Serial transplantation reveals the stem-cell-like regenerative potential of adult mouse hepatocytes. Am J Pathol 151: 1273-1280.

[99] Crosby HA, Hubscher S, Fabris L, Joplin R, Sell S, Kelly D, Strain AJ (1998) Immunolocalization of putative human liver progenitor cells in livers from patients with end-stage primary biliary cirrhosis and sclerosing cholangitis using the monoclonal antibody OV-6. Am J Pathol 152: 771-779.

[100] Laconi E, Oren R, Mukhopadhyay DK, Hurston E, Laconi S, Pani P, Dabeva MD, Shafritz DA (1998) Long-term, near-total liver replacement by transplantation of iso-

lated hepatocytes in rats treated with retrorsine. Am J Pathol 153: 319-329. S0002-9440(10)65574-5 [pii];10.1016/S0002-9440(10)65574-5 [doi].

[101] Rudolph KL, Chang S, Millard M, Schreiber-Agus N, DePinho RA (2000) Inhibition of experimental liver cirrhosis in mice by telomerase gene delivery. Science 287: 1253-1258. 8254 [pii].

[102] Morris RJ, Potten CS (1999) Highly persistent label-retaining cells in the hair follicles of mice and their fate following induction of anagen. J Invest Dermatol 112: 470-475. 10.1046/j.1523-1747.1999.00537.x [doi].

[103] Foudi A, Hochedlinger K, Van BD, Schindler JW, Jaenisch R, Carey V, Hock H (2009) Analysis of histone 2B-GFP retention reveals slowly cycling hematopoietic stem cells. Nat Biotechnol 27: 84-90. nbt.1517 [pii];10.1038/nbt.1517 [doi].

[104] Wilson A, Laurenti E, Oser G, van der Wath RC, Blanco-Bose W, Jaworski M, Offner S, Dunant CF, Eshkind L, Bockamp E, Lio P, Macdonald HR, Trumpp A (2008) Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair. Cell 135: 1118-1129. S0092-8674(08)01386-X [pii];10.1016/j.cell.2008.10.048 [doi].

[105] Sato T, Vries RG, Snippert HJ, van de Wetering M, Barker N, Stange DE, van Es JH, Abo A, Kujala P, Peters PJ, Clevers H (2009) Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature 459: 262-265. nature07935 [pii];10.1038/nature07935 [doi].

[106] 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.

[107] Wilson JW, Leduc EH (1958) Role of cholangioles in restoration of the liver of the mouse after dietary injury. J Pathol 76: 441-449. 10.1002/path.1700760213.

[108] Xiao JC, Jin XL, Ruck P, Adam A, Kaiserling E (2004) Hepatic progenitor cells in human liver cirrhosis: immunohistochemical, electron microscopic and immunofluorencence confocal microscopic findings. World J Gastroenterol 10: 1208-1211.

[109] Richardson MM, Jonsson JR, Powell EE, Brunt EM, Neuschwander-Tetri BA, Bhathal PS, Dixon JB, Weltman MD, Tilg H, Moschen AR, Purdie DM, Demetris AJ, Clouston AD (2007) Progressive fibrosis in nonalcoholic steatohepatitis: association with altered regeneration and a ductular reaction. Gastroenterology 133: 80-90. S0016-5085(07)00994-8 [pii];10.1053/j.gastro.2007.05.012 [doi].

[110] Chen YK, Zhao XX, Li JG, Lang S, Wang YM (2006) Ductular proliferation in liver tissues with severe chronic hepatitis B: an immunohistochemical study. World J Gastroenterol 12: 1443-1446.

[111] Curado S, Stainier DY (2010) deLiver'in regeneration: injury response and development. Semin Liver Dis 30: 288-295. 10.1055/s-0030-1255357 [doi].

[112] Alatas FS, Masumoto K, Matsuura T, Hayashida M, Saeki I, Kohashi K, Oda Y, Taguchi T (2011) Synchronized expressions of hepatic stellate cells and their transactivation and liver regeneration during liver injury in an animal model of cholestasis. J Pediatr Surg 46: 2284-2290. S0022-3468(11)00755-X [pii];10.1016/j.jpedsurg.2011.09.016 [doi].

[113] Nagy P, Kiss A, Schnur J, Thorgeirsson SS (1998) Dexamethasone inhibits the proliferation of hepatocytes and oval cells but not bile duct cells in rat liver. Hepatology 28: 423-429. S0270913998003218 [pii];10.1002/hep.510280220 [doi].

[114] Preisegger KH, Factor VM, Fuchsbichler A, Stumptner C, Denk H, Thorgeirsson SS (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.

[115] Pritchard MT, Nagy LE (2010) Hepatic fibrosis is enhanced and accompanied by robust oval cell activation after chronic carbon tetrachloride administration to Egr-1-deficient mice. Am J Pathol 176: 2743-2752. S0002-9440(10)60796-1 [pii];10.2353/ajpath.2010.091186 [doi].

[116] Akhurst B, Croager EJ, Farley-Roche CA, Ong JK, Dumble ML, Knight B, Yeoh GC (2001) A modified choline-deficient, ethionine-supplemented diet protocol effectively induces oval cells in mouse liver. Hepatology 34: 519-522. S0270-9139(01)11774-X [pii];10.1053/jhep.2001.26751 [doi].

[117] Jelnes P, Santoni-Rugiu E, Rasmussen M, Friis SL, Nielsen JH, Tygstrup N, Bisgaard HC (2007) Remarkable heterogeneity displayed by oval cells in rat and mouse models of stem cell-mediated liver regeneration. Hepatology 45: 1462-1470. 10.1002/hep.21569 [doi].

[118] Evarts RP, Hu Z, Omori N, Omori M, Marsden ER, Thorgeirsson SS (1996) Precursor-product relationship between oval cells and hepatocytes: comparison between tritiated thymidine and bromodeoxyuridine as tracers. Carcinogenesis 17: 2143-2151.

[119] Nagy P, Bisgaard HC, Thorgeirsson SS (1994) Expression of hepatic transcription factors during liver development and oval cell differentiation. J Cell Biol 126: 223-233.

[120] Petersen BE, Zajac VF, Michalopoulos GK (1997) Bile ductular damage induced by methylene dianiline inhibits oval cell activation. Am J Pathol 151: 905-909.

[121] Paku S, Schnur J, Nagy P, Thorgeirsson SS (2001) Origin and structural evolution of the early proliferating oval cells in rat liver. Am J Pathol 158: 1313-1323.

[122] Theise ND, Saxena R, Portmann BC, Thung SN, Yee H, Chiriboga L, Kumar A, Crawford JM (1999) The canals of Hering and hepatic stem cells in humans. Hepatology 30: 1425-1433. S027091399900511X [pii];10.1002/hep.510300614 [doi].

[123] Zhou H, Rogler LE, Teperman L, Morgan G, Rogler CE (2007) Identification of hepatocytic and bile ductular cell lineages and candidate stem cells in bipolar ductular reactions in cirrhotic human liver. Hepatology 45: 716-724. 10.1002/hep.21557 [doi].

[124] Koenig S, Probst I, Becker H, Krause P (2006) Zonal hierarchy of differentiation markers and nestin expression during oval cell mediated rat liver regeneration. Histochem Cell Biol 126: 723-734. 10.1007/s00418-006-0204-3 [doi].

[125] Yovchev MI, Grozdanov PN, Zhou H, Racherla H, Guha C, Dabeva MD (2008) Identification of adult hepatic progenitor cells capable of repopulating injured rat liver. Hepatology 47: 636-647. 10.1002/hep.22047 [doi].

[126] Yovchev MI, Grozdanov PN, Joseph B, Gupta S, Dabeva MD (2007) Novel hepatic progenitor cell surface markers in the adult rat liver. Hepatology 45: 139-149. 10.1002/hep.21448 [doi].

[127] Okabe M, Tsukahara Y, Tanaka M, Suzuki K, Saito S, Kamiya Y, Tsujimura T, Nakamura K, Miyajima A (2009) Potential hepatic stem cells reside in EpCAM+cells of normal and injured mouse liver. Development 136: 1951-1960. 136/11/1951 [pii]; 10.1242/dev.031369 [doi].

[128] Bisgaard HC, Parmelee DC, Dunsford HA, Sechi S, Thorgeirsson SS (1993) Keratin 14 protein in cultured nonparenchymal rat hepatic epithelial cells: characterization of keratin 14 and keratin 19 as antigens for the commonly used mouse monoclonal antibody OV-6. Mol Carcinog 7: 60-66.

[129] Vestentoft PS, Jelnes P, Andersen JB, Tran TA, Jorgensen T, Rasmussen M, Bornholdt J, Grovdal LM, Jensen CH, Vogel LK, Thorgeirsson SS, Bisgaard HC (2013) Molecular constituents of the extracellular matrix in rat liver mounting a hepatic progenitor cell response for tissue repair. Fibrogenesis Tissue Repair 6: 21. 1755-1536-6-21 [pii]; 10.1186/1755-1536-6-21 [doi].

[130] Tanimizu N, Tsujimura T, Takahide K, Kodama T, Nakamura K, Miyajima A (2004) Expression of Dlk/Pref-1 defines a subpopulation in the oval cell compartment of rat liver. Gene Expr Patterns 5: 209-218. S1567-133X(04)00118-8 [pii];10.1016/j.modgep.2004.08.003 [doi].

[131] Bisgaard HC, Nagy P, Ton PT, Hu Z, Thorgeirsson SS (1994) Modulation of keratin 14 and alpha-fetoprotein expression during hepatic oval cell proliferation and liver regeneration. J Cell Physiol 159: 475-484. 10.1002/jcp.1041590312 [doi].

[132] Floridon C, Jensen CH, Thorsen P, Nielsen O, Sunde L, Westergaard JG, Thomsen SG, Teisner B (2000) Does fetal antigen 1 (FA1) identify cells with regenerative, endocrine and neuroendocrine potentials? A study of FA1 in embryonic, fetal, and placental tissue and in maternal circulation. Differentiation 66: 49-59. S0301-4681(09)60574-0 [pii];10.1046/j.1432-0436.2000.066001049.x [doi].

[133] Smas CM, Sul HS (1993) Pref-1, a protein containing EGF-like repeats, inhibits adipocyte differentiation. Cell 73: 725-734. 0092-8674(93)90252-L [pii].

[134] Moon YS, Smas CM, Lee K, Villena JA, Kim KH, Yun EJ, Sul HS (2002) Mice lacking paternally expressed Pref-1/Dlk1 display growth retardation and accelerated adiposity. Mol Cell Biol 22: 5585-5592.

[135] Taketa K (1990) Alpha-fetoprotein: reevaluation in hepatology. Hepatology 12: 1420-1432.

[136] Schmidt LE, Dalhoff K (2005) Alpha-fetoprotein is a predictor of outcome in acetaminophen-induced liver injury. Hepatology 41: 26-31. 10.1002/hep.20511 [doi].

[137] Asahina K, Zhou B, Pu WT, Tsukamoto H (2011) Septum transversum-derived mesothelium gives rise to hepatic stellate cells and perivascular mesenchymal cells in developing mouse liver. Hepatology 53: 983-995. 10.1002/hep.24119 [doi].

[138] Yokoi Y, Namihisa T, Kuroda H, Komatsu I, Miyazaki A, Watanabe S, Usui K (1984) Immunocytochemical detection of desmin in fat-storing cells (Ito cells). Hepatology 4: 709-714. S0270913984001051 [pii].

[139] Gard AL, White FP, Dutton GR (1985) Extra-neural glial fibrillary acidic protein (GFAP) immunoreactivity in perisinusoidal stellate cells of rat liver. J Neuroimmunol 8: 359-375.

[140] Ramadori G, Veit T, Schwogler S, Dienes HP, Knittel T, Rieder H, Meyer zum Buschenfelde KH (1990) Expression of the gene of the alpha-smooth muscle-actin isoform in rat liver and in rat fat-storing (ITO) cells. Virchows Arch B Cell Pathol Incl Mol Pathol 59: 349-357.

[141] Friedman SL (2008) Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver. Physiol Rev 88: 125-172. 88/1/125 [pii];10.1152/physrev.00013.2007 [doi].

[142] Knittel T, Kobold D, Piscaglia F, Saile B, Neubauer K, Mehde M, Timpl R, Ramadori G (1999) Localization of liver myofibroblasts and hepatic stellate cells in normal and diseased rat livers: distinct roles of (myo-)fibroblast subpopulations in hepatic tissue repair. Histochem Cell Biol 112: 387-401. 91120387.418 [pii].

[143] Knittel T, Kobold D, Saile B, Grundmann A, Neubauer K, Piscaglia F, Ramadori G (1999) Rat liver myofibroblasts and hepatic stellate cells: different cell populations of the fibroblast lineage with fibrogenic potential. Gastroenterology 117: 1205-1221. S0016508599004655 [pii].

[144] Dezso K, Jelnes P, Laszlo V, Baghy K, Bodor C, Paku S, Tygstrup N, Bisgaard HC, Nagy P (2007) Thy-1 is expressed in hepatic myofibroblasts and not oval cells in stem cell-mediated liver regeneration. Am J Pathol 171: 1529-1537. ajpath.2007.070273 [pii]; 10.2353/ajpath.2007.070273 [doi].

[145] Lorenzini S, Bird TG, Boulter L, Bellamy C, Samuel K, Aucott R, Clayton E, Andreone P, Bernardi M, Golding M, Alison MR, Iredale JP, Forbes SJ (2010) Characterisation of a stereotypical cellular and extracellular adult liver progenitor cell niche in rodents and diseased human liver. Gut 59: 645-654. 59/5/645 [pii];10.1136/gut.2009.182345 [doi].

[146] Van Hul NK, Abarca-Quinones J, Sempoux C, Horsmans Y, Leclercq IA (2009) Relation between liver progenitor cell expansion and extracellular matrix deposition in a CDE-induced murine model of chronic liver injury. Hepatology 49: 1625-1635. 10.1002/hep.22820 [doi].

[147] Nagai H, Terada K, Watanabe G, Ueno Y, Aiba N, Shibuya T, Kawagoe M, Kameda T, Sato M, Senoo H, Sugiyama T (2002) Differentiation of liver epithelial (stem-like) cells into hepatocytes induced by coculture with hepatic stellate cells. Biochem Biophys Res Commun 293: 1420-1425. 10.1016/S0006-291X(02)00406-0 [doi];S0006-291X(02)00406-0 [pii].

[148] Karkampouna S, ten DP, Dooley S, Julio MK (2012) TGFbeta signaling in liver regeneration. Curr Pharm Des 18: 4103-4113. CPD-EPUB-20120522-28 [pii].

[149] Nishimura EK, Suzuki M, Igras V, Du J, Lonning S, Miyachi Y, Roes J, Beermann F, Fisher DE (2010) Key roles for transforming growth factor beta in melanocyte stem cell maintenance. Cell Stem Cell 6: 130-140. S1934-5909(09)00633-X [pii];10.1016/j.stem.2009.12.010 [doi].

[150] Chen L, Zhang W, Zhou QD, Yang HQ, Liang HF, Zhang BX, Long X, Chen XP (2012) HSCs play a distinct role in different phases of oval cell-mediated liver regeneration. Cell Biochem Funct 30: 588-596. 10.1002/cbf.2838 [doi].

[151] Fallowfield JA, Mizuno M, Kendall TJ, Constandinou CM, Benyon RC, Duffield JS, Iredale JP (2007) Scar-associated macrophages are a major source of hepatic matrix metalloproteinase-13 and facilitate the resolution of murine hepatic fibrosis. J Immunol 178: 5288-5295. 178/8/5288 [pii].

[152] Geiger B, Spatz JP, Bershadsky AD (2009) Environmental sensing through focal adhesions. Nat Rev Mol Cell Biol 10: 21-33. nrm2593 [pii];10.1038/nrm2593 [doi].

[153] Guilak F, Cohen DM, Estes BT, Gimble JM, Liedtke W, Chen CS (2009) Control of stem cell fate by physical interactions with the extracellular matrix. Cell Stem Cell 5: 17-26. S1934-5909(09)00293-8 [pii];10.1016/j.stem.2009.06.016 [doi].

[154] Hynes RO (2009) The extracellular matrix: not just pretty fibrils. Science 326: 1216-1219. 326/5957/1216 [pii];10.1126/science.1176009 [doi].

[155] Badylak SF, Freytes DO, Gilbert TW (2009) Extracellular matrix as a biological scaffold material: Structure and function. Acta Biomater 5: 1-13. S1742-7061(08)00282-1 [pii];10.1016/j.actbio.2008.09.013 [doi].

[156] van der Flier A, Sonnenberg A (2001) Function and interactions of integrins. Cell Tissue Res 305: 285-298.

[157] Daley WP, Peters SB, Larsen M (2008) Extracellular matrix dynamics in development and regenerative medicine. J Cell Sci 121: 255-264. 121/3/255 [pii];10.1242/jcs.006064 [doi].

[158] Brown BN, Barnes CA, Kasick RT, Michel R, Gilbert TW, Beer-Stolz D, Castner DG, Ratner BD, Badylak SF (2010) Surface characterization of extracellular matrix scaffolds. Biomaterials 31: 428-437. S0142-9612(09)00993-4 [pii];10.1016/j.biomaterials.2009.09.061 [doi].

[159] Discher DE, Mooney DJ, Zandstra PW (2009) Growth factors, matrices, and forces combine and control stem cells. Science 324: 1673-1677. 324/5935/1673 [pii];10.1126/science.1171643 [doi].

[160] Engler AJ, Sen S, Sweeney HL, Discher DE (2006) Matrix elasticity directs stem cell lineage specification. Cell 126: 677-689. S0092-8674(06)00961-5 [pii];10.1016/j.cell.2006.06.044 [doi].

[161] Sun Y, Li W, Lu Z, Chen R, Ling J, Ran Q, Jilka RL, Chen XD (2011) Rescuing replication and osteogenesis of aged mesenchymal stem cells by exposure to a young extracellular matrix. FASEB J 25: 1474-1485. fj.10-161497 [pii];10.1096/fj.10-161497 [doi].

[162] Ryu BY, Orwig KE, Oatley JM, Avarbock MR, Brinster RL (2006) Effects of aging and niche microenvironment on spermatogonial stem cell self-renewal. Stem Cells 24: 1505-1511. 2005-0580 [pii];10.1634/stemcells.2005-0580 [doi].

[163] Ricard-Blum S (2011) The collagen family. Cold Spring Harb Perspect Biol 3: a004978. cshperspect.a004978 [pii];10.1101/cshperspect.a004978 [doi].

[164] Bozec L, van der Heijden G, Horton M (2007) Collagen fibrils: nanoscale ropes. Biophys J 92: 70-75. S0006-3495(07)70805-6 [pii];10.1529/biophysj.106.085704 [doi].

[165] Duffield JS, Forbes SJ, Constandinou CM, Clay S, Partolina M, Vuthoori S, Wu S, Lang R, Iredale JP (2005) Selective depletion of macrophages reveals distinct, opposing roles during liver injury and repair. J Clin Invest 115: 56-65. 10.1172/JCI22675 [doi].

[166] Ramadori G, Saile B (2002) Mesenchymal cells in the liver--one cell type or two? Liver 22: 283-294. 1726 [pii].

[167] Yasoshima M, Tsuneyama K, Harada K, Sasaki M, Gershwin ME, Nakanuma Y (2000) Immunohistochemical analysis of cell-matrix adhesion molecules and their ligands in the portal tracts of primary biliary cirrhosis. J Pathol 190: 93-99. 10.1002/(SICI)1096-9896(200001)190:1<93::AID-PATH507>3.0.CO;2-A [pii];10.1002/(SICI)1096-9896(200001)190:1<93::AID-PATH507>3.0.CO;2-A [doi].

[168] Terada T, Nakanuma Y (1994) Expression of tenascin, type IV collagen and laminin during human intrahepatic bile duct development and in intrahepatic cholangiocarcinoma. Histopathology 25: 143-150.

[169] Timpl R, Rohde H, Robey PG, Rennard SI, Foidart JM, Martin GR (1979) Laminin--a glycoprotein from basement membranes. J Biol Chem 254: 9933-9937.

[170] Knittel T, Janneck T, Muller L, Fellmer P, Ramadori G (1996) Transforming growth factor beta 1-regulated gene expression of Ito cells. Hepatology 24: 352-360. S0270-9139(96)00330-8 [pii];10.1053/jhep.1996.v24.pm0008690404 [doi].

[171] Kikkawa Y, Mochizuki Y, Miner JH, Mitaka T (2005) Transient expression of laminin alpha1 chain in regenerating murine liver: restricted localization of laminin chains and nidogen-1. Exp Cell Res 305: 99-109. S0014-4827(05)00009-1 [pii];10.1016/j.yexcr.2005.01.005 [doi].

[172] Nikolova G, Strilic B, Lammert E (2007) The vascular niche and its basement membrane. Trends Cell Biol 17: 19-25. S0962-8924(06)00327-8 [pii];10.1016/j.tcb.2006.11.005 [doi].

[173] Pham VT, Couchie D, Martin-Garcia N, Laperche Y, Zafrani ES, Mavier P (2008) Expression of matrix metalloproteinase-2 and-9 and of tissue inhibitor of matrix metalloproteinase-1 in liver regeneration from oval cells in rat. Matrix Biol 27: 674-681. S0945-053X(08)00103-0 [pii];10.1016/j.matbio.2008.07.002 [doi].

[174] Pellicoro A, Aucott RL, Ramachandran P, Robson AJ, Fallowfield JA, Snowdon VK, Hartland SN, Vernon M, Duffield JS, Benyon RC, Forbes SJ, Iredale JP (2012) Elastin accumulation is regulated at the level of degradation by macrophage metalloelastase (MMP-12) during experimental liver fibrosis. Hepatology 55: 1965-1975. 10.1002/hep.25567 [doi].

[175] Kallis YN, Robson AJ, Fallowfield JA, Thomas HC, Alison MR, Wright NA, Goldin RD, Iredale JP, Forbes SJ (2011) Remodelling of extracellular matrix is a requirement for the hepatic progenitor cell response. Gut 60: 525-533. gut.2010.224436 [pii];10.1136/gut.2010.224436 [doi].

[176] Clayton E, Forbes SJ (2009) The isolation and in vitro expansion of hepatic Sca-1 progenitor cells. Biochem Biophys Res Commun 381: 549-553. S0006-291X(09)00350-7 [pii];10.1016/j.bbrc.2009.02.079 [doi].

[177] McClelland R, Wauthier E, Uronis J, Reid L (2008) Gradients in the liver's extracellular matrix chemistry from periportal to pericentral zones: influence on human hepatic progenitors. Tissue Eng Part A 14: 59-70. 10.1089/ten.a.2007.0058 [doi].

[178] Paku S, Nagy P, Kopper L, Thorgeirsson SS (2004) 2-acetylaminofluorene dose-dependent differentiation of rat oval cells into hepatocytes: confocal and electron microscopic studies. Hepatology 39: 1353-1361. 10.1002/hep.20178 [doi].

[179] Oertel M, Shafritz DA (2008) Stem cells, cell transplantation and liver repopulation. Biochim Biophys Acta 1782: 61-74. S0925-4439(07)00235-9 [pii];10.1016/j.bbadis.2007.12.004 [doi].

[180] Xu X, Liu RF, Zhang X, Huang LY, Chen F, Fei QL, Han ZG (2012) DLK1 as a potential target against cancer stem/progenitor cells of hepatocellular carcinoma. Mol Cancer Ther 11: 629-638. 1535-7163.MCT-11-0531 [pii];10.1158/1535-7163.MCT-11-0531 [doi].

[181] Yamashita T, Forgues M, Wang W, Kim JW, Ye Q, Jia H, Budhu A, Zanetti KA, Chen Y, Qin LX, Tang ZY, Wang XW (2008) EpCAM and alpha-fetoprotein expression defines novel prognostic subtypes of hepatocellular carcinoma. Cancer Res 68: 1451-1461. 68/5/1451 [pii];10.1158/0008-5472.CAN-07-6013 [doi].

[182] Yamashita T, Ji J, Budhu A, Forgues M, Yang W, Wang HY, Jia H, Ye Q, Qin LX, Wauthier E, Reid LM, Minato H, Honda M, Kaneko S, Tang ZY, Wang XW (2009) EpCAM-positive hepatocellular carcinoma cells are tumor-initiating cells with stem/progenitor cell features. Gastroenterology 136: 1012-1024. S0016-5085(08)02179-3 [pii];10.1053/j.gastro.2008.12.004 [doi].

[183] Roskams TA, Libbrecht L, Desmet VJ (2003) Progenitor cells in diseased human liver. Semin Liver Dis 23: 385-396. 10.1055/s-2004-815564 [doi].

[184] Bird TG, Lorenzini S, Forbes SJ (2008) Activation of stem cells in hepatic diseases. Cell Tissue Res 331: 283-300. 10.1007/s00441-007-0542-z [doi].

[185] Bisgaard HC, Holmskov U, Santoni-Rugiu E, Nagy P, Nielsen O, Ott P, Hage E, Dalhoff K, Rasmussen LJ, Tygstrup N (2002) Heterogeneity of Ductular Reactions in Adult Rat and Human Liver Revealed by Novel Expression of Deleted in Malignant Brain Tumor 1. Am J Pathol 161: 1187-1198.

[186] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D (2011) Global cancer statistics. CA Cancer J Clin 61: 69-90. caac.20107 [pii];10.3322/caac.20107 [doi].

[187] de Jong MC, Nathan H, Sotiropoulos GC, Paul A, Alexandrescu S, Marques H, Pulitano C, Barroso E, Clary BM, Aldrighetti L, Ferrone CR, Zhu AX, Bauer TW, Walters DM, Gamblin TC, Nguyen KT, Turley R, Popescu I, Hubert C, Meyer S, Schulick RD, Choti MA, Gigot JF, Mentha G, Pawlik TM (2011) Intrahepatic cholangiocarcinoma: an international multi-institutional analysis of prognostic factors and lymph node assessment. J Clin Oncol 29: 3140-3145. JCO.2011.35.6519 [pii];10.1200/JCO.2011.35.6519 [doi].

[188] Cohnheim J (1875) Congenitales, quergestreiftes Muskelsarkom der Nieren. Archiv f pathol Anat 65: 64-69. 10.1007/BF01978936.

[189] Wicha MS, Liu S, Dontu G (2006) Cancer stem cells: an old idea--a paradigm shift. Cancer Res 66: 1883-1890. 66/4/1883 [pii];10.1158/0008-5472.CAN-05-3153 [doi].

[190] Driessens G, Beck B, Caauwe A, Simons BD, Blanpain C (2012) Defining the mode of tumour growth by clonal analysis. Nature. nature11344 [pii];10.1038/nature11344 [doi].

[191] Chen J, Li Y, Yu TS, McKay RM, Burns DK, Kernie SG, Parada LF (2012) A restricted cell population propagates glioblastoma growth after chemotherapy. Nature. nature11287 [pii];10.1038/nature11287 [doi].

[192] Schepers AG, Snippert HJ, Stange DE, van den Born M, van Es JH, van de Wetering M, Clevers H (2012) Lineage tracing reveals Lgr5+stem cell activity in mouse intestinal adenomas. Science 337: 730-735. science.1224676 [pii];10.1126/science.1224676 [doi].

[193] Modrek AS, Bayin NS, Placantonakis DG (2014) Brain stem cells as the cell of origin in glioma. World J Stem Cells 6: 43-52. 10.4252/wjsc.v6.i1.43 [doi].

[194] Thorgeirsson SS, Grisham JW (2002) Molecular pathogenesis of human hepatocellular carcinoma. Nat Genet 31: 339-346. 10.1038/ng0802-339 [doi];ng0802-339 [pii].

[195] Seitz HK, Stickel F (2006) Risk factors and mechanisms of hepatocarcinogenesis with special emphasis on alcohol and oxidative stress. Biol Chem 387: 349-360. 10.1515/BC.2006.047 [doi].

[196] Hussain SP, Schwank J, Staib F, Wang XW, Harris CC (2007) TP53 mutations and hepatocellular carcinoma: insights into the etiology and pathogenesis of liver cancer. Oncogene 26: 2166-2176. 1210279 [pii];10.1038/sj.onc.1210279 [doi].

[197] Budhu A, Forgues M, Ye QH, Jia HL, He P, Zanetti KA, Kammula US, Chen Y, Qin LX, Tang ZY, Wang XW (2006) Prediction of venous metastases, recurrence, and prognosis in hepatocellular carcinoma based on a unique immune response signature of the liver microenvironment. Cancer Cell 10: 99-111. S1535-6108(06)00214-5 [pii];10.1016/j.ccr.2006.06.016 [doi].

[198] Enzan H, Himeno H, Iwamura S, Onishi S, Saibara T, Yamamoto Y, Hara H (1994) Alpha-smooth muscle actin-positive perisinusoidal stromal cells in human hepatocellular carcinoma. Hepatology 19: 895-903. S0270913994001187 [pii].

[199] Amann T, Bataille F, Spruss T, Muhlbauer M, Gabele E, Scholmerich J, Kiefer P, Bosserhoff AK, Hellerbrand C (2009) Activated hepatic stellate cells promote tumorigenicity of hepatocellular carcinoma. Cancer Sci 100: 646-653. CAS1087 [pii];10.1111/j.1349-7006.2009.01087.x [doi].

[200] Zhang F, Chen XP, Zhang W, Dong HH, Xiang S, Zhang WG, Zhang BX (2008) Combined hepatocellular cholangiocarcinoma originating from hepatic progenitor cells: immunohistochemical and double-fluorescence immunostaining evidence. Histopathology 52: 224-232. HIS2929 [pii];10.1111/j.1365-2559.2007.02929.x [doi].

[201] Komuta M, Spee B, Vander BS, De VR, Verslype C, Aerts R, Yano H, Suzuki T, Matsuda M, Fujii H, Desmet VJ, Kojiro M, Roskams T (2008) Clinicopathological study

on cholangiolocellular carcinoma suggesting hepatic progenitor cell origin. Hepatology 47: 1544-1556. 10.1002/hep.22238 [doi].

[202] Ma S, Chan KW, Hu L, Lee TK, Wo JY, Ng IO, Zheng BJ, Guan XY (2007) Identification and characterization of tumorigenic liver cancer stem/progenitor cells. Gastroenterology 132: 2542-2556. S0016-5085(07)00786-X [pii];10.1053/j.gastro.2007.04.025 [doi].

[203] Qiang L, Yang Y, Ma YJ, Chen FH, Zhang LB, Liu W, Qi Q, Lu N, Tao L, Wang XT, You QD, Guo QL (2009) Isolation and characterization of cancer stem like cells in human glioblastoma cell lines. Cancer Lett 279: 13-21. S0304-3835(09)00034-2 [pii]; 10.1016/j.canlet.2009.01.016 [doi].

[204] Lee JS, Heo J, Libbrecht L, Chu IS, Kaposi-Novak P, Calvisi DF, Mikaelyan A, Roberts LR, Demetris AJ, Sun Z, Nevens F, Roskams T, Thorgeirsson SS (2006) A novel prognostic subtype of human hepatocellular carcinoma derived from hepatic progenitor cells. Nat Med 12: 410-416. nm1377 [pii];10.1038/nm1377 [doi].

[205] Woo HG, Wang XW, Budhu A, Kim YH, Kwon SM, Tang ZY, Sun Z, Harris CC, Thorgeirsson SS (2011) Association of TP53 mutations with stem cell-like gene expression and survival of patients with hepatocellular carcinoma. Gastroenterology 140: 1063-1070. S0016-5085(10)01695-1 [pii];10.1053/j.gastro.2010.11.034 [doi].

[206] Cairo S, Wang Y, de RA, Duroure K, Dahan J, Redon MJ, Fabre M, McClelland M, Wang XW, Croce CM, Buendia MA (2010) Stem cell-like micro-RNA signature driven by Myc in aggressive liver cancer. Proc Natl Acad Sci U S A 107: 20471-20476. 1009009107 [pii];10.1073/pnas.1009009107 [doi].