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Hepatic Progenitors of the Liver and Extra-Hepatic Tissues

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1. Introduction

The liver has a tremendous capacity to regenerate at all developmental stages (for reviews, see (1-3)). Liver cell mass can be restored even after repeated partial hepatectomies as well as after toxic injury. The contribution of stem cells to these processes is still under debate. Adult liver cells have been shown to regenerate liver tissue repeatedly when transplanted serially (4). However, hepatocytes cannot be considered stem cells because they are unipotent (for a glossary of terms, see **Table 1**). This chapter describes various liver progenitors that have been found by different researchers in humans and other mammalian species. Intra and extra-hepatic progenitors are discussed that can give rise to liver lineages. Intra-hepatic progenitors of non-hepatic lineages, such as endothelial or hematopoietic restricted progenitors, are not discussed. Although the focus of this chapter is on progenitors that have been characterized in normal, non-pathological conditions of the liver, oval cells will be described briefly.

Term	Description
Totipotent	Capable to give rise to cells of all three embryonic germ layers (i.e. endoderm, mesoderm and ectoderm) as well as extra-embryonic tissue of the placenta.
Pluripotent	Capable to give rise to cells of all three embryonic germ layers (i.e. endoderm, mesoderm and ectoderm) but not to extra-embryonic tissue. Most commonly used e.g. for embryonic stem cells, which derive from the inner cell mass of the blastocyst.
Multipotent	Capable to give rise to multiple but not all lineages. For example, bone marrow mesenchymal stem cells are considered multipotent.
Bipotent	Able to give rise to two fates. In liver, hepatoblasts are considered bipotential as they can develop into biliary and hepatic lineages.
Unipotent	Able to give rise to only one cell type. Hepatocytes are considered unipotential.
Progenitor	Broad term to describe various types of precursors with different potential.

Term	Description
Stem cell	Cell, which is capable to differentiate into multiple lineages and is also able of self-renewal.
Hepatoblast	Hepatic parenchymal cell of the fetal liver. Defined by its expression of immature protein alpha-fetoprotein and absence of several mature hepatic functions and proteins.
Hepatocyte	Hepatic parenchymal cell of the adult liver. In non-pathological conditions defined by its expression of mature functions and proteins, such as albumin and cytochrome P450 enzymes, and the absence of immature proteins such as alpha-fetoprotein.
Oval cell	Small cells with oval-shaped nuclei that emerge in livers, which have been treated with certain toxins.

Table 1. Common terminology relevant to liver progenitor biology. Further details can be found also in (5, 6).

2. Embryonic liver development

During embryonic development, the liver arises from the definitive endoderm (for reviews on liver development, see (7-9)). The definitive endoderm is an embryonic layer, whereas visceral endoderm is a non-embryonic derived layer, also called extra-embryonic endoderm. The definitive endoderm is one of the three germ layers, which include also ectoderm and mesoderm. The definitive endoderm is initially located beneath the ectoderm and mesoderm. In the mouse, the definitive endoderm layer forms a liver bud between E8.5 and E9.5. This layer will also form the pancreas, lung, stomach, intestine, and thyroid. The cardiac mesoderm and septum transversum mesenchyme release signals, such as fibroblast growth factors (FGF) and bone morphogenetic proteins (BMP), which are necessary to induce liver specification. The septum transversum mesenchyme has been implicated to give rise to stellate cells (also called Ito cells), which are fat and vitamin A-storing and extracellular matrix producing liver cells (10); cells positive for the Lim-homeobox gene (Lhx2) migrate from the septum transversum into the forming liver bud and become desmin and Lhx2 positive stellate cells. Cells in the developing liver bud are termed hepatoblasts and express alpha-fetoprotein (AFP). Hepatoblasts have been described as bipotential progenitors, developing into mature hepatocytes as well as bile duct epithelial cells (cholangiocytes), based on findings from *ex vivo* and *in vitro* studies (11-15). Suppression of transcription factor CCAAT-enhancer-binding protein alpha (CEBPα) has been suggested to induce their specification towards biliary differentiation (16, 17).

3. Human hepatic progenitors in fetal and adult livers

Different hepatic progenitors in human livers have been described. Based on early findings in developmental biology, hepatic stem cells were originally defined as AFP positive hepatoblasts. More recent research, however, reveals that hepatic stem cells are AFP negative and are the precursors to hepatoblasts (12, 18). Furthermore, stem cells of assumed mesendodermal origin capable of multilineage differentiation towards liver- and mesenchymal lineages have been discovered (19). An overview about human hepatic progenitors that have been isolated and characterized is given in **Table 2**.

Publication	Develop-mental stage of liver tissue	Presumable lineage	Term used by authors	Isolation method	Phenotype	*In vivo* model for repopula-tion	*In vitro* characteristics
Najimi *et al*. 2007 (20), Khuu *et al*. 2011 (21)	Adult	Mesenchymal	Adult derived human liver stem/ progenitor cell (ADHLSCs)	Culture	Positive: CD90, CD73, CD29, CD44, CD13, HLA-class I. Weak: CD49e, CD49b, CD49f. Negative: CD105, CD133, CD117, CD45, CD34, HLA-DR	uPA$^{+/+}$-SCID with and without 70% hepatectomy	Hepatic functions after induced differentiation
Dan *et al*. 2006 (19)	Fetal	Mesendodermal	Human fetal liver multipotent progenitor cells (hFLMPC)	Culture	Positive: CD34, CD90, CD117, CD326, c-met, SSEA4, CK18, CK19, CD44h, vimentin. Negative: CD133, CD45, AFP, albumin	Rag2$^{-/-}$ $\gamma^{-/-}$ retrorsine/ CCl_4	Long-term culture, ~46h PDT, multipotent
Herrera *et al*. 2006 (22)	Adult		Human liver stem cells (HLSCs)	Culture	Positive: Albumin, AFP, CD29, CD73, CD44, CD90, vimentin, nestin. Weak: CK8, CK18. Negative: CD34, CD45, CD117, CD133, CK19.	SCID, N-acetyl-p-aminophen	Multipotent, high expansion potential, ~36h PDT
Schmelzer *et al*. 2006, 2007 (12, 18)	Fetal (16-20 weeks of gestation), neonatal, pediatric, adult	Endodermal	Human hepatic stem cells (hHpSC)	MACS, culture	Positive: CD326, CD133, CD56, E-cadherin, CD29, CD44h, claudin3, CK19. Weak: albumin. Negative: AFP.	NOD/SCID	Long-term culture, >150 population doublings, precursors of hepatoblasts
Malhi *et al*. 2002 (23)	Fetal		Human fetal liver progenitor/ stem cells	Culture	Positive: AFP, GGT, CK8, CK19, CD34	SCID CCl_4	Long-term culture

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Schmelzer *et al*. 2006, 2007 (12, 18)	Fetal (16-20 weeks of gestation)		Human hepatoblasts	MACS, culture	Positive: AFP. Variable: CD326	NOD/SCID	Can arise from hHpSC colonies in culture

Abbreviations: AFP: alpha-fetoprotein; CCl_4: Carbon tetrachloride; CD: cluster of differentiation; CK: cytokeratin; GGT: γ-glutamyl transpeptidase; HLA: human leukocyte antigen; NOD: non-obese diabetic; SCID: severe-combined immunodeficient; MACS: magnetic activated cells sorting; PDT: population doubling time; uPA: urokinase-type plasminogen activator.

Table 2. Progenitors with hepatic potential isolated from human livers. Details are given in the respective sections.

3.1 Human liver multipotent progenitors

Dan *et al*. isolated liver stem cells co-expressing endodermal and mesenchymal phenotypes from human fetal liver by culture selection on feeder cells (19). These cells could differentiate not only into hepatocytes and bile duct cells, but also into fat, bone, cartilage, and endothelial cells. Because of their multilineage differentiation potential, these cells were termed human fetal liver multipotent progenitor cells (hFLMPC). The *in vivo* percentage of this progenitor was not given, as these cells were isolated by culture selection. Cell surface and intracellular markers included: CD34, CD90, CD117, CD326 (also called epithelial cell adhesion molecule (EpCAM)), c-met, SSEA4, CK18, CK19, CD44h, and vimentin. Cells were negative for albumin, CD133, CD45, and AFP. They could be cultured monoclonal and long-term for up to 100 population doublings. Cells had population doubling times of 46h. Early and late passages demonstrated identical morphology, differentiation potential, and telomere length. Cultured cells formed typical clusters with cells having a high nuclear to cytoplasm ratio. The morphology of these clusters resembled hepatic stem cells colonies described by Schmelzer *et al.* (12). When transplanted into immunotolerant $Rag2^{-/-}\gamma^{-/-}$ mice (using a modified retrorsine/carbon tetra-chloride model), human-specific albumin in mouse serum and human-specific albumin in sections of the liver could be detected. Liver sections of transplanted mice demonstrated clusters of human hepatocytes. A repopulation of 0.8–1.7% was estimated. The multipotential differentiation potential and resemblance to hepatic stem cell colonies suggests that hFLMPC represent mesendodermal precursors of hepatic stem cells.

Herrera *et al*. isolated a similar population from human adult livers (22) using culture selection. These cells also expressed hepatic and mesenchymal markers. Cell surface and intracellular markers included albumin, AFP, CD29, CD73, CD44, CD90, vimentin and nestin; however, there was a negative expression of CD34, CD45, CD117, CD133, and CK19, and a weakly positive expression of CK8 and CK18. The cells were different from those described by Dan *et al*., as albumin and AFP expression could be observed and hematopoietic markers CD34 and CD117 were absent. *In vitro*, progenitors differentiated not only into hepatocytes, but also into osteogenic, endothelial, and islet-like, insulin-producing

structures. Adipogenic differentiation could not be induced. As these cells were culture-selected, percentages of their *in vivo* occurrence were not established. Cells *in vitro* demonstrated exponential growth rates. When transplanted, human cells could be localized *in vivo* within the liver parenchyma of severe-combined immunodeficient (SCID) mice treated with N-acetyl-p-aminophen.

Mesenchymal progenitors isolated from adult human livers were investigated for their potential to differentiate into hepatocytes (20, 21). Mesenchymal-like cells were obtained by selective culture (not sorting) of total liver cells. FACS analyses of cultured cells revealed a phenotype similar to mesenchymal stem cells with positive expression for CD90, CD73, CD29, CD44, CD13, and HLA-class I, but negative expression for CD105, CD133, CD117, CD45, CD34, and HLA-DR; cells were weakly positive for CD49e and CD49b, and only a minor fraction expressed CD49f. When cells were intrasplenically transplanted into uPA$^{+/+}$-SCID mice, human albumin and AFP positive cells could be observed and human albumin secretion was detected. When transplanted into SCID mice with and without 70% hepatectomy, human albumin gene expression could be measured in mice livers that had undergone hepatectomy, and human albumin positive cells could be detected in mouse liver sections in both models. Potential fusion events were not analyzed. When cells were induced to hepatic lineages *in vitro* (21), hepatic functions were increased compared to non-induced controls, but lower than those of freshly isolated adult liver cells.

3.2 Hepatic stem cells in the human liver

Hepatic stem cells can be isolated from fetal, neonatal, pediatric, and adult human livers with identical characteristics (12, 18), as described by Schmelzer *et al*. Cell surface and intracellular markers include CD326, CD133, CD56, E-cadherin, CD29, Patched (24), claudin 3 (18), CK19, and show weak positivity for albumin. Cells are negative for AFP, CD45, CD34, CD38, CD14, CD90, CD235a, VEGFr, vWF, CD31, CD146, desmin, ASMA, transferrin, connexins, PEPCK, DPP4, CYP450; CD117 is variably expressed. Sonic and Indian Hedgehog signaling pathway components are expressed (24). Stem cells could be selected by MACS sorting as well as under selective culture conditions, which included serum-free medium and culture on plastic. Under these culture conditions, hepatic stem cell colonies formed. These colonies (**Figure 1**) exhibit a typical epithelial morphology of densely packed, small cells with high nucleus-to-cytoplasm ratio. Stem cell colonies are positive for CD326 (**Figure 2**), CD44h, CD56, and weakly express albumin, but are negative for AFP.

Cells were capable of self-renewal, as shown by clonogenic expansion for more than 150 population doublings. 0.5 – 2.5% of all liver cells from all ages were positive for CD326 expression. Hepatic stem cells have a small diameter of about 9 µm. *In vivo*, they are located in the ductal plates in fetal and neonatal livers and in the Canals of Hering in pediatric and adult livers. The Canal of Hering has been previously described as the reservoir of stem cells in postnatal livers (25, 26). Carpentier *et al*. recently studied lineage tracing by using a Cre recombinase Sox9 mouse model and confirmed that ductal plate cells give rise to cholangiocytes, periportal hepatocytes, and adult liver progenitor cells (27). Furuyama *et al*. (28) demonstrated that adult intestinal cells, hepatocytes and pancreatic acinar cells are physiologically supplied from Sox9-expressing progenitors using Cre-based lineage tracing in mice. In CCl_4 mediated liver injury, Sox9-positive progenitors contributed to liver

regeneration. Hepatic stem cells have been shown to differentiate into biliary and hepatocytic lineages *in vivo* and *in vitro* (12). Freshly isolated cells or stem cells expanded in culture developed into mature liver tissue expressing human-specific proteins when transplanted into NOD/SCID mice, and lost their expression of stem cell marker CD326, CD133, and CK19. Whether those cells also possess multilineage differentiation potential beyond endodermal fates, i.e. mesodermal or ectodermal, has not yet been investigated. Khan *et al*. transplanted human fetal liver derived $CD326^+$ sorted progenitors into patients with liver fibrosis (29). Patients demonstrated improvements in clinical and biochemical parameters and a decrease in mean MELD (model for end-stage liver disease) score at six-month follow-up.

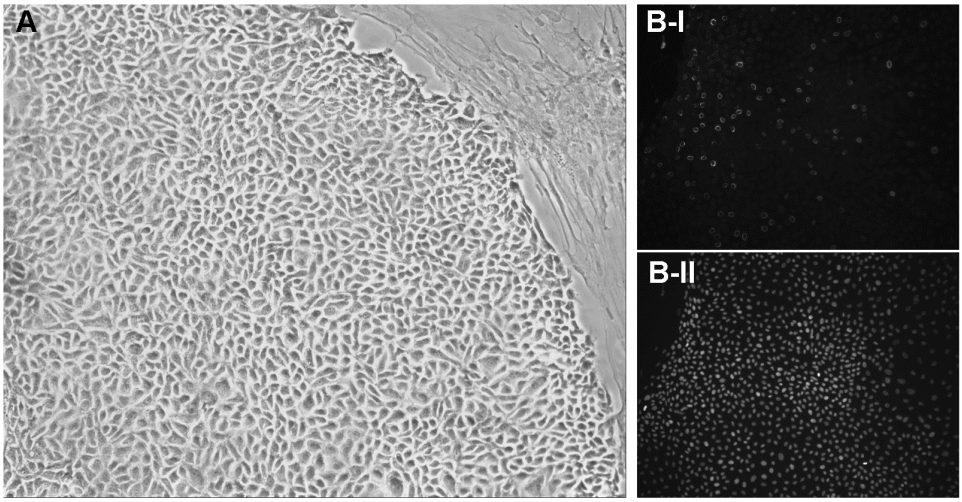

Fig. 1. Human hepatic stem cell colony in culture, established from fetal liver cell suspensions as described in (12); phase contrast microscopy (**A**), and fluorescence microscopy (**B**) of proliferating cells with positive nuclei for incorporated thymidine analog bromodeoxyuridine (**B-I**) and corresponding total nuclei stained with 4',6-diamidino-2-phenylindole (**B-II**).

3.3 Hepatoblasts in human liver

Hepatoblasts are the main parenchymal cell type of the fetal liver and are defined by their expression of AFP. AFP positive cells are rare in normal adult livers, except in livers with severe injury or disease (30-32) (for review, see (33)). Hepatoblasts can give rise to hepatocytes and cholangiocytes, and are therefore also named bipotential progenitors (15). AFP-negative hepatic stem cells are the precursors to hepatoblasts that can mature into AFP-positive hepatoblasts (12). Human fetal hepatoblasts could be cultured long-term and clonally, and contributed to liver parenchyma when transplanted into SCID mice (23). Hepatoblasts express biliary and hepatocyte markers such as CK19, CK14, gamma glutamyl transpeptidase, glucose-6-phosphatase, glycogen, albumin, AFP, E-cadherin (34), α-1 microglobulin, HepPar1, glutamate dehydrogenase, and dipeptidyl peptidase IV (15, 18).

Human hepatoblasts do not express the mesenchymal or hematopoietic markers CD90, vimentin, and CD34 (34). In mice, hepatoblasts express the surface marker Dlk-1 (35-37), which was subsequently demonstrated to be expressed by human fetal hepatoblasts as well (34). Mouse fetal liver cells sorted for Dlk-1 can be cultured long-term; transplantation of Dlk-1 positive cells into the spleen gives rise to hepatocytes in the liver. Several signaling pathways and transcription factors contribute towards differentiation into either cell type. In mice, Notch signaling controls differentiation towards biliary epithelium by upregulation of HNF1β but downregulation of HNF1α, HNF4, and C/EBPα (38), and, in turn, suppression of C/EBPα expression in periportal hepatoblasts is suggested to induce biliary epithelial differentiation by increasing HNF6 and HNF1β expression (17).

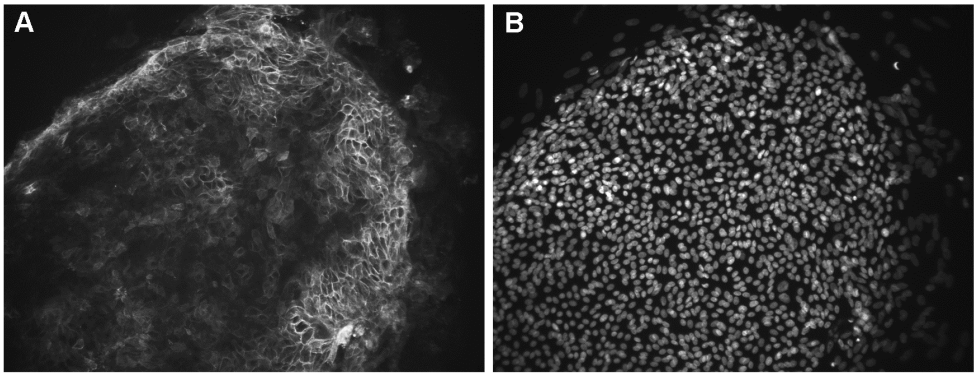

Fig. 2. Human hepatic stem cell colonies established as described in (12) are positive for CD326 (**A**). Fluorescence microscopy for the transmembrane glycoprotein CD326 (also named epithelial cell adhesion molecule (EpCAM)) in (**A**), and corresponding nuclei stained with 4',6-diamidino-2-phenylindole in (**B**).

4. Murine and rat hepatic progenitors in the fetal and adult liver

Various surface markers have been applied to identify hepatic stem or progenitor populations in rodents.

Germain *et al*. described the bipotential capacity of fetal rat liver cells to differentiate into hepatic and biliary cells *in vitro* (39), as did Kubota *et al*. using clonal cultures (40). Small hepatocytes were detected in non-parenchymal fractions of adult rat liver cells (41-44). These small hepatocytes produced colonies that expressed hepatic and biliary markers. A similar type of colony could be obtained when adult liver cell clusters were placed into culture (45). Suzuki *et al*. sorted progenitor populations from fetal mice and rat livers with a phenotype of c-met$^+$, CD49f$^+$, CD117$^-$, CD45$^-$, and TER119$^-$ (46-50). Sorted cells developed into albumin and glycogen positive cells when transplanted into retrorsine-treated adult rats that had undergone two-third partial hepatectomy. Cells negative for c-met or positive for CD45 could not repopulate recipient livers. These progenitors could be also cultured clonally. Feng *et al*. (51) demonstrated that these cells could also undergo pancreatic differentiation in culture as well as *in vivo* when transplanted into alloxan-induced diabetic

mice. Similarly, Nierhoff *et al*. (35) demonstrated that fetal mouse liver epithelial cells positive for AFP or E-cadherin did not express hematopoietic stem cell markers CD34, CD117, Ter119, or CD45, but were positive for progenitor markers Sca-1 and Pan-cytokeratin. Both E-cadherin positive sorted as well as unsorted fetal liver cell fractions from wild type mice gave rise to liver parenchyma when transplanted into retrorsine treated DPPIV$^{-/-}$ mice.

As described for human hepatoblasts above, mouse fetal liver hepatoblasts have been shown to express the surface marker Dlk-1 (35-37). Dlk-1 positive sorted mouse fetal liver cells can be cultured long-term and, when transplanted into the spleen, give rise to hepatocytes in the liver. Dabeva *et al*. (52) described the re-population potential of wild type fetal rat liver cells when transplanted into DPPIV-/- rat models. These models included knockouts that had undergone two-third partial hepatectomy and were either treated with retrorsine or not. In rats treated with retrorsine, which blocked proliferation of endogenous hepatocytes, mainly bipotential, transplanted progenitors were observed expressing AFP, albumin, and CK19. In non-treated rats, transplanted cells expressed mainly either hepatocytic or biliary markers.

The positive expression of aldehyde dehydrogenase (ALDH) has been used as a feature to select progenitors from adult mouse liver (53). ALDH^{+} cells were shown to have stem cell characteristics and to express markers of human hepatic stem cells such as CD326, CK19, CD133, and Sox9.

Various hepatic progenitor cell lines have been developed from normal, genetically modified, or toxin treated rodents (54-62). Several of these lines were described as bipotential *in vitro* or when transplanted *in vivo*.

5. Oval cells

Oval cells were first described in rodents, emerging when the liver is exposed to certain toxins (for review, see (63)) (64). Termed "oval cells" because of their oval shaped nucleus, these small cells have a diameter of less than 10 µm. They are located near the portal triads and expand in the livers of animals exposed to oncogenic insults. The term "oval cells" frequently refers to liver stem cells or progenitors. However, oval cells can be distinguished from normal hepatic progenitors phenotypically and in their growth regulatory requirements (65). Several protocols have been shown to lead to the emergence of oval cells: administration of 2-acetylamino fluorine or dipin in combination with partial hepatectomy; administration of carbon tetrachloride, 3-methyl-diaminobenzidine, galactosamine, furane, or 3,5-diethoxycarbonyl-1,4-dihydrocollidine; etluonine addition to a choline-deficient diet; or transgenic albumin-urokinase-type plasminogen-activator mice.

Oval cells were described as positive for several surface and intracellular markers (including hematopoietic and mesenchymal markers not found on normal epithelial hepatic stem cells) such as CD34 (66), CD117 (67), AFP, CK14, CK19 (68), GGT, OC.2, OV-6, and CD90 (69). CD90, however, was subsequently demonstrated to be expressed not by oval cells but by myofibroblasts (70).

Some primary liver tumors are suggested to emerge from oval cells (71).

6. Hepatic progenitors found in various mammalian species

Few data have been published on hepatic progenitors from species other than human or rodent. In general, pigs are used as an animal model closely resembling human physiology and metabolic functions. This makes the pig model more favorable than the rodent model. However, this model is scarcely used due to obvious constraints in keeping animals. Kano *et al*. (72, 73) investigated hepatic progenitors isolated by culture selection from non-parenchymal liver cell suspensions of six-seven months old pigs. Cell clusters in culture were positive for the hepatic markers AFP, albumin, transferrin, CK18, CK7, and c-met, but did they not express biliary markers such as gamma-glutamyltransferase, CK19, and CK14, although they were positive for oval cell marker OV6. Duct-like structures emerged from clusters expressing biliary epithelial markers. Clonal cell growth could be established (74). Comparable cells could be obtained (75) by isolating small liver cells from pigs that had undergone partial hepatectomy. In addition to the hepatic markers albumin and AFP, these cells also expressed biliary marker CK19 and were positive for OV6. In culture, cells were positive for stem-cell factor, CD117, CD90, AFP, CK19, and OV6. Fetal porcine liver cells were used to establish colonies of pluripotent progenitors (76, 77).

7. Extra-hepatic sources of potential liver progenitors

Several extra-hepatic sources have been described to harbor progenitors able to differentiate into hepatic lineages *in vitro* and *in vivo*. It is widely debated whether cells of extra-hepatic origin are able to differentiate into hepatic cell types or if they fuse with the recipient's liver cells when transplanted. Tissue sources include bone marrow, adipose tissue, umbilical cord, and peripheral blood. Hepatic differentiation potentials of embryonic stem cells (ESC), placenta derived stem cells, or induced pluripotent stem cells (iPS cells) are not discussed here; further literature can be found in reviews (78-82).

Bone marrow cells or bone marrow derived hematopoietic stem cells have been suggested to be able to trans-differentiate into hepatic lineages. Petersen *et al*. performed initial experiments with cross-strain and cross-sex bone marrow and liver transplantations in rats (83). When male bone marrow was transplanted into female recipients and liver damage was induced, Y-chromosome positive cells could be detected in the female livers. Also, when male dipeptidyl peptidase (DPPIV) positive bone marrow was transplanted into female DPPIV negative recipients and liver damage was induced, DPPIV positive cells could be detected in the female livers. A further approach included transplantations of major histocompatibility complex class II L21-6 isozyme negative whole livers into positive enzyme expressing rats; after induction of liver damage, positive enzyme expressing cells could be detected. Alison *et al*. (84) investigated human female livers from patients who had received male bone marrow transplants. Y-chromosome positive cells that co-expressed CK8 were detected in the female livers. About 0.5 – 2% of all livers cells were Y-chromosome positive. Theise *et al*. described further *in vivo* experiments on the possible contribution of bone marrow cells towards hepatic lineages in mice (85) and humans (86). Whole bone marrow cells or $CD34^{+}lin^{-}$ sorted cells from male mice were transplanted into female recipients; up to 2.2% (bone marrow) or about 0.7% ($CD34^{+}lin^{-}$) Y-chromosome positive cells could be detected within the female livers. In human patients who had undergone cross-sex bone marrow transplantation, Y-chromosome positive cells could be observed in female livers. 4 – 43% of cholangiocytes and 4 – 38% of hepatocytes were positive for Y-

chromosome. Lagasse *et al*. (87) intravenously injected adult wild type bone marrow cells in FAH-/- mice, an animal model of tyrosinemia type I. The mice were rescued and biochemical functions were regained. Only purified hematopoietic stem cells gave rise to donor-derived hematopoietic and hepatic regeneration from total bone marrow cells. However, subsequently published studies revealed that the majority of those liver cells, which were assumed to be donor derived differentiated bone marrow cells, are instead rather the product of donor cells fusing with host liver cells (88, 89). Other studies demonstrated bone marrow cells contributed nothing or very little to liver lineages *in vivo* (90-92). Jang *et al*. (93) and Harris *et al*. (94) could show, however, that a minor percentage (up to 0.1%) of bone marrow cells can contribute to liver cells *in vivo* without fusion. Most evidence to date indicates that only a minority of the observed trans-differentiation events is actually due to differentiation of bone marrow cells into liver lineages and the majority of observed trans-differentiated cells are indeed fusion events.

Similar to the findings of the above described *in vivo* studies, *in vitro* studies of the hepatic differentiation potential of hematopoietic stem cells produced contradicting findings (95-99). Overall, results from *in vitro* studies suggest that bone marrow hematopoietic stem cells can differentiate only barely, if at all, into hepatic lineages.

Mesenchymal stem cells (MSCs), which have similar characteristics, have been isolated from various tissue sources; MSCs from sources such as bone marrow (100-105), skin (106), umbilical cord (107, 108) and adipose tissues (109-115) have been analyzed for the potential to differentiate towards hepatic lineages *in vitro* and *in vivo*. MSC markers from various tissues show similar surface marker expression profiles, described first as classical MSC markers by Pittenger *et al* (116), which were CD29, CD44, CD71, CD90, CD106, CD120a, and CD124. Culture selected clonal bone marrow derived MSCs expressed mesenchymal cell-specific markers (e.g. CD13, CD29, CD44, and CD90), and were negative for hematopoietic markers such as CD3, CD14, CD34, and CD45 (100). When transplanted in SCID mice, non-fused human cells could be detected in the liver. Adipose tissue derived stem cells were described to differentiate into hepatic lineages (109-115). Adipose tissue derived MSCs were characterized to potentially express CD9, CD13, CD29, CD44, CD49d, CD54, CD73, CD90, CD105, CD146, CD166, osteopontin and osteonectin, and to be negative for hematopoietic and endothelial markers such as CD45, CD34 and CD31. Marker expressions and hepatic potential are further summarized in current reviews (117, 118). In general, most *in vivo* transplantation studies using MSCs did not exclude donor cell fusion with host cells. Only one study (Aurich *et al*. (112)) demonstrated the integration of non-fused human adipose MSCs in the livers of mice that had undergone combined toxin induced liver damage and hepatectomy.

Lee *et al*. (119) transplanted green fluorescent protein mouse gallbladder epithelial cells into non-fluorescent SCID mice that had undergone retrorsine treatment and either partial hepatectomy before transplantation or carbon tetrachloride treatment following transplantation. Within one to four months after transplantation, green fluorescent protein positive cells could be detected within the recipient mice. These cells expressed mostly biliary markers, but cells positive for hepatic markers could be detected as well.

Zhao *et al*. isolated hematopoietic stem cells from peripheral blood (120) and demonstrated their *in vitro* multilineage differentiation potential; treatment of cultures with HGF induced cells to acquire a round or oval-like flattened morphology. Most of the cells were positive

for intracellular albumin and AFP expression; some cells demonstrated CK7 expression. Sun *et al*. (121) showed that human umbilical blood cells integrated into livers of rat chimeras, and these cells were positive for human hematopoietic, biliary, and hepatic proteins. Crema *et al*. isolated $CD133^+$ cord blood cells (122). Transplantation into liver-damaged SCID mice resulted in clusters of human-derived cells expressing human leucocyte antigen-class I, HepPar1, and OV6 antigens. Within these clusters, human albumin, AFP, and CK19 could be detected. Human umbilical blood cells demonstrated *in vitro* hepatocyte-like differentiation and expression of hepatic proteins when transplanted in rodents with induced liver damage (107, 123, 124).

Conclusively, it appears that extra-hepatic progenitors integrate into the liver only to a very minor percentage and only when severe liver damage is induced. The majority of these events appear to be due to fusion and not differentiation. The observed improvements of liver functions by mesenchymal cells could be attributed to their secretion of growth factors and cytokines and immunosuppressive properties (111, 125-127).

8. Conclusion

Although there is still some debate about the detailed characteristics that identify hepatic progenitors, much progress has been achieved during recent years in defining, isolating, characterizing, and transplanting various types of progenitors. This is especially the case for hepatic progenitors isolated from human livers. Hepatic progenitors represent a population with potential advantages over total liver cell suspensions or hepatocytes for cell transplantation in patients (29, 128), for review see (129). Because of their high proliferation and differentiation potential a major advantage for transplantation of stem cells over total liver cell suspensions would be the requirement for less cell numbers to inject, which would decrease the risks associated with transplanting high cell numbers. In addition, because of their proliferation and differentiation potential, progenitors could be used in applications such as extracorporeal liver support systems (130, 131), and may be used as an alternative cell source in pharmacological screening models. Cultures of progenitors also provide an easy *in vitro* tool to study principles of developmental biology.

9. References

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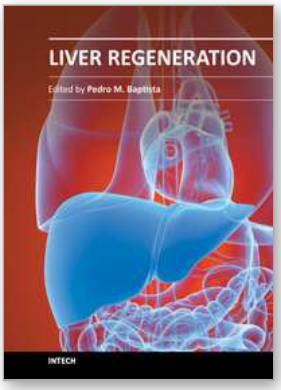

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Doctors and scientists have been aware of the "phenomenom" of liver regeneration since the time of the ancient Greeks, illustrated by the mythic tale of Prometheus' punishment. Nevertheless, true insight into its intricate mechanisms have only become available in the 20th century. Since then, the pathways and mechanisms involved in restoring the liver to its normal function after injury have been resolutely described and characterized, from the hepatic stem/progenitor cell activation and expansion to the more systemic mechanisms involving other tissues and organs like bone-marrow progenitor cell mobilization. This book describes some of the complex mechanisms involved in liver regeneration and provides examples of the most up-to-date strategies used to induce liver regeneration, both in the clinic and in the laboratory. The information presented will hopefully benefit not only professionals in the liver field, but also people in other areas of science (pharmacology, toxicology, etc) that wish to expand their knowledge of the fundamental biology that orchestrates liver injury and regeneration.

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