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MODEL SYSTEMS AND EXPERIMENTAL CONDITIONS THAT LEAD TO EFFECTIVE REPOPULATION OF THE LIVER BY TRANSPLANTED CELLS

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Abstract

In recent years, there has been substantial progress in transplanting cells into the liver with the ultimate goal of restoring liver mass and function in both inherited and acquired liver diseases. The basis for considering that this might be feasible is that the liver is a highly regenerative organ. After massive liver injury or surgical removal of two-thirds or more of the liver tissue, the organ can restore its mass with completely normal morphologic structure and function. It has also been found under highly selective conditions that transplanted hepatocytes can fully repopulate the liver and cure a metabolic disorder or deficiency state. Fetal liver cells can also substantially repopulate the normal liver, and it is hoped in the future that effective repopulation will be achievable with cultured cells or cell lines, pluripotent stem cells from other somatic tissues, embryonic stem cells, or induced pluripotent stem cells, which can now be generated in vitro by a variety of methods. The purpose of this review is to present the major systems that have been used for liver repopulation, the variables involved in obtaining successful repopulation and what has been achieved in these various systems to date with different cell types.

Keywords

liver repopulation; hepatocytes; stem cells; progenitor cells; selective and non-selective conditions

INTRODUCTION

A method to isolate single hepatocytes that are viable in culture was first developed in the late 1960's (Berry and Friend, 1969). Subsequently, primary isolated hepatocytes were transplanted into the liver through the portal circulation, leading to transient reduction in serum bilirubin levels in the Gunn rat, a model for the human disorder, Crigler-Najjar Syndrome, Type 1 (Matas et al. 1976). In the 1980's, primary isolated hepatocytes were transplanted into a variety of ectopic sites, including the dorsal fat pad (Jirtle et al. 1980), spleen (Kusano and Mito, 1982), peritoneal cavity (Demetriou et al., 1986) and under the renal capsule (Ricordi et al, 1989). The cells survived to varying degrees in these sites and

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demonstrated hepatic morphology and function, including modest proliferation after inducing liver regeneration (Jirtle and Michalopoulos, 1982; Gupta et al, 1987). Studies during the past 15 years have clearly established that the liver can be repopulated by transplanted hepatic cells with restoration of normal structure and function and these studies will be the subject of this review.

Early Studies of Hepatocyte Transplantation into the Liver

Initially, it was shown that primary isolated hepatocytes transplanted into the spleen, traverse to the liver and engraft into the hepatic parenchyma (Gupta et al. 1990; Ponder et al. 1991). However, once the number of transplanted cells exceeded 1–2% of total hepatic mass, there was obstruction of the hepatic sinusoids, portal hypertension and hepatic infarction. Inclusion of a liver proliferative stimulus in conjunction with hepatocyte transplantation (two-thirds partial hepatectomy or acute liver injury through administration of CCl₄ or another hepatotoxin), or repeated infusions of hepatocytes, led to a modest increase in liver repopulation, but not to levels sufficient for hepatic cellular therapy (Rajvanshi et al. 1996a; Rajvanshi et al. 1996b). These limitations are reflected in several clinical studies in which hepatocyte transplantation has led to modest but only temporary improvement in hepatic function (Fox et al. 1998; Fisher and Strom, 2006).

Repopulation of the Liver by Transplanted Hepatocytes under Selective Conditions

A major breakthrough in hepatic cell transplantation occurred in the early to mid 1990's through the development of two mouse models for liver repopulation by transplanted hepatocytes. In the first model, Sandgren et al. (1991) developed a transgenic mouse in which a protease, urokinase plasminogen activator (uPA), is expressed exclusively in hepatocytes under control of the albumin promoter and is then secreted into the serum. However, small amounts of uPA remained in the liver tissue causing extensive injury. Most of the mice died between 4–6 weeks of age, but some survived, and in these mice, there were nodules of normal liver tissue of varying sizes scattered throughout the hepatic parenchyma (Fig. 1A). This occurred by deletion of the uPA transgene from individual hepatocytes, and these revertant hepatocytes expanded clonally into large clusters and replaced damaged tissue. This serendipitous finding subsequently led Rhim et al. (1994) to transplant normal hepatocytes (marked with a β -galactosidase transgene) into uPA mice after which they observed extensive liver repopulation (Fig. 1B). Assuming a 10% engraftment efficiency, Rhim et al. estimated that each transplanted hepatocyte that had become incorporated into the uPA host liver underwent ~12–14 cell divisions (Rhim et al. 1994).

Grompe and colleagues (Overturf et al., 1996) developed a second mouse model to repopulate the liver with mature hepatocytes by targeted disruption of fumarylacetoacetate hydrolase (Fah), the last gene in tyrosine catabolism. Deletion of Fah leads to accumulation of upstream intermediates in tyrosine catabolism, some of which (primarily fumarylacetoacetate) are toxic and cause extensive and continuous liver injury. Administration of 2 (2-nitro-4-trifluoromethylbenzoyl)-cyclohexane-1,3-dione (NTBC), a pharmacologic inhibitor of tyrosine catabolism upstream of homogentisic acid, prevents accumulation of fumarylacetoacetate in the liver of Fah null mice. NTBC was developed originally as an experimental agent to treat patients with hereditary tyrosinemia type 1 (HT1), an inherited metabolic disorder based on mutation of the Fah gene (Lindstedt et al. 1992) and is now being used clinically.

In Fah null mice, Grompe and colleagues demonstrated that liver repopulation by transplanted wt hepatocytes can be totally regulated by NTBC administration. When NTBC is administered, fumarylaceticate does not accumulate in the liver, liver injury and cell turnover are reduced markedly and there is no advantage for wt hepatocytes to survive over host Fah null hepatocytes. Therefore, when wt hepatocytes are transplanted into Fah null mice maintained on NTBC, only scattered small clusters of transplanted hepatocytes are detected (Fig. 1C), similar to results cited above when wt hepatocytes are transplanted into a normal host liver environment in conjunction with CCl₄ administration (Rajvanshi et al. 1996b). However, if NTBC treatment is discontinued at or shortly after cell transplantation, extensive liver injury resumes and transplanted cells proliferate extensively and the transplanted wt hepatocytes selectively repopulate the liver, forming large clusters replacing most of the hepatic mass within 6 weeks (Fig. 1D). Therefore, cyclic administration and withdrawal of NTBC can be used as a tool to control liver failure in Fah null mice and to modulate liver repopulation by transplanted cells. This is an advantage of the Fah null mouse model compared to uPA transgenic mice in which transplanted wt hepatocytes compete with revertant host hepatocytes for liver repopulation. Fah null mice with livers repopulated by wt hepatocytes remain healthy, have normal liver function tests and show a relatively normal liver structure for many months after hepatocyte transplantation (Overturf et al. 1996). Studies in the Fah null mouse were the first to show that liver repopulation can effectively cure a metabolic disease, namely, the mouse equivalent to HT1.

In Fah null mice, not only do transplanted wt hepatocytes replace Fah null hepatocytes, but the transplanted repopulating hepatocytes can also be serially transplanted through seven consecutive Fah null mice, while retaining full ability to proliferate and replace host hepatocytes (Overturf et al. 1997). In these studies, Grompe and colleagues calculated that each serially transplanted hepatocyte underwent an average of at least 69 cell divisions. Previously, it was thought that mature hepatocytes could undergo only two or three cell divisions after which they become terminally differentiated and cannot proliferate further, even under a liver regenerative stimulus. Thus, in both of these mouse models, a paradigm shifting result was obtained; namely, that hepatocytes exhibit extensive capacity to proliferate and restore liver mass and function under highly selective conditions; i.e., when there is massive and continuous injury to host hepatocytes leading to high cell turnover, together with a significant selective advantage for survival of transplanted hepatocytes compared to host hepatocytes.

A third method to obtain a high level of liver repopulation by transplanted hepatocytes is to impair proliferation of endogenous hepatocytes, i.e., to render them incapable of cell division, and then transplant wt hepatocytes with normal proliferation potential in conjunction with a liver proliferative stimulus to the host liver. These conditions are also highly selective for liver repopulation by transplanted cells compared to endogenous host hepatocytes. This was first achieved in rats using retrorsine, a plant alkaloid that is taken up by hepatocytes, cross links cellular DNA, and blocks proliferation of hepatocytes by disrupting their progression through the cell cycle (Samuel and Jago, 1975; Laconi et al. 1998). When retrorsine or a closely related compound, monocrotaline, is administered to rats or mice (Laconi et al. 1998; Guo et al. 2002; Nierhoff et al. 2005; Witek et al. 2005), there is a long-lived inhibition of hepatocyte proliferation. However, essential metabolic functions are maintained in these DNA damaged hepatocytes and the animals survive. After the effects of acute chemical injury have subsided (2–4 weeks), the animals are subjected to two-thirds PH or CCl₄ administration and hepatocytes from normal animals are transplanted into the liver. This leads to a brisk regenerative response by transplanted hepatocytes and there is near total replacement of DNA damaged host hepatocytes within several months (Laconi et al. 1998; Guo et al. 2002; Nierhoff et al. 2005). To follow the fate of transplanted hepatocytes, we used a specific strain of inbred Fischer (F) 344 rats with a spontaneous

mutation in the dipeptidyl peptidase (DPP)IV gene, rendering the mutant protein enzymatically inactive. DPPIV is expressed on the apical (bile canalicular) domain of the hepatocyte cell surface membrane in a characteristic pattern that can be readily identified (Gossrau, 1979; Hubbard et al. 1985; Walborg et al. 1985). In 1991, Hixson and colleagues developed a model system for liver repopulation in which they transplanted normal (wt) F344 rat hepatocytes into syngeneic DPPIV⁻ mutant F344 rats and identified transplanted hepatocytes in the host liver by DPPIV enzyme histochemistry or immunohistochemistry (Thompson et al. 1991). Because wt and DPPIV⁻ mutant F344 rats are inbred, there is no rejection of transplanted cells. We have utilized this model extensively, as well as DPPIV^{-/-} mice and DPPIV^{-/-}/Rag2^{-/-} mice, to study repopulation of the liver by transplanted cells (Laconi et al. 1998; Oren et al. 1999; Dabeva et al. 2000; Sandhu et al. 2001; Menthena et al. 2004; Oertel et al. 2006a,b; Oertel et al. 2007; Oertel et al. 2008; Yuan et al. 2003; Gouon-Evans et al. 2006). We also developed a double label enzyme histochemistry assay to simultaneously detect DPPIV and Na⁺-K⁺ ATPase, another enzyme that is expressed on the bile canalicular domain of the hepatocyte plasma membrane (Dabeva et al. 1997). Therefore, we could follow the engraftment, proliferation and structural incorporation of transplanted hepatocytes into the host parenchyma (Laconi et al. 1998).

What was most surprising in the retrorsine/PH model is that the transplanted hepatocytes do not develop into hyperplastic nodules (Fig. 2A), the liver remodels and the transplanted hepatocytes become fully integrated into the hepatic plates with transplanted hepatocytes forming hybrid canaliculi with neighboring host hepatocytes (Fig. 2B). The liver structure becomes and remains essentially normal for many months after cell transplantation and 98–99% of host hepatocytes can be replaced in 6 to 9 months (Fig. 2C, D) (Laconi et al. 1998). Using the retrorsine/PH model, we have also transplanted wt allogenic hepatocytes into albumin deficient Sprague Dawley rats, i.e., the Nagase analbuminemic rat, under immunosuppression with cyclosporine. Under these conditions, there was 75–80% replacement of albumin-deficient host hepatocytes by wt albumin expressing hepatocytes (Oren et al. 1999), which led to a 7,000-fold increase in albumin production and restoration of serum albumin levels to the normal range. Other methods to achieve a selective advantage for transplanted cells include x-irradiation of the host liver to induce DNA damage (Guha et al. 1999; Malhi et al. 2002) or use of transgenic or knock-out hepatocytes with augmented proliferative potential or resistance to apoptosis to repopulate the liver (Mignon et al. 1998; Yuan et al. 2003). In these various models of host hepatocyte mitoinhibition, the liver proliferative stimulus can be supplied by two-thirds PH, CCl₄ administration, ischemic liver injury or treatment of the host with hormones (tri-iodothyronine) or growth factors (HGF) (Guha et al. 1999; Yuan et al. 2003; Malhi et al. 2002; Oren et al. 1999; Landis et al. 2006).

Repopulation of the Liver by Transplanted Hepatic Cells Under Non-Selective Conditions

A key feature in all the above models is that strong selection conditions existed in the host to enable transplanted hepatocytes to extensively repopulate the liver. Although transplanted hepatocytes can engraft into the liver in a normal host (Gupta et al. 1990; Ponder et al. 1991) and undergo several cell divisions in response to a proliferative stimulus, such as two-thirds PH or CCl₄ induced liver injury (Rajvanshi et al. 1996b), very little repopulation occurs under these conditions (Rajvanshi et al. 1996b; Sandhu et al. 2001). We refer to these circumstances as non-selective, because the basic liver function is normal and both host and transplanted hepatocytes can respond equally to a liver proliferative stimulus, i.e., neither cell type exhibits a selective advantage for proliferation, survival or function. Since the number of viable host hepatocytes remaining in the liver after two thirds PH or high dose CCl₄ administration is ~100-fold greater than the number of hepatocytes that can be transplanted and engrafted, the amount of liver repopulation that can be achieved by normal

hepatocytes under non-selective conditions is no more than 1–2%. Our goal was to identify cells that were capable of effectively repopulating the liver in a normal, non-selective, host environment.

A logical source for cells that might have high repopulation potential is the fetal liver. During liver development in the mouse, undifferentiated endodermal stem cells begin to proliferate on embryonic day (ED) 8.0 (Fig. 3) (Zhao and Duncan, 2005). Specification of endodermal stem cells to the hepatic lineage occurs at ED8.5 and requires fibroblast growth factor (FGF) and bone morphogenic protein (BMP) signaling (Jung et al. 1999; Rossi et al. 2001; Wandzioch and Zaret 2009). Cells expand from the foregut endoderm and by ED 9.0–9.5, these cells begin to bud and express GATA4 and liver-enriched, nuclear factor HNF4 α , as well as liver-specific genes, α -fetoprotein (AFP), followed by albumin (Alb). These hepatic-specified cells (now referred to as hepatoblasts) proliferate massively and invade the septum transversum mesenchyme.

Hepatoblasts continue to proliferate rapidly and begin to express other characteristic genes, including cytokeratins 7, 8, 18 and 19. Just prior to ED16, hepatoblasts diverge along two lineages, hepatocytes (expressing AFP and albumin) and cholangiocytes (expressing CK-19). In the rat, similar events occur one day later (Shiojiri et al. 1991; Marceau et al. 1992). This is associated with a massive and abrupt change in the gene expression profile of rat fetal liver epithelial cells to a more differentiated phenotype, which occurs between ED16 and ED17 (Petkov et al. 2004). After ED16, the percentage of bipotential cells, i.e., those expressing genes in both the hepatocytic and cholangiocytic lineages (i.e., AFP, Alb and CK-19) is markedly reduced (Dabeva et al. 2000; Sandhu et al. 2001). Most of the cells are now unipotential and committed to either the hepatocytic, AFP(Alb)⁺/CK-19⁻, or cholangiocytic, AFP(Alb)⁻/CK-19⁺, lineage. As organogenesis proceeds, intrahepatic bile ducts are formed in the vicinity of large portal vein branches, and the basic lobular structure is completed, although the parenchymal cords do not become fully mature until several weeks after birth.

Studies were conducted to determine whether fetal liver cells could effectively repopulate the normal adult liver under non-selective conditions (i.e., in a basically normal liver with no underlying hepatic dysfunction) and to compare the results to those obtained with adult hepatocytes under both non-selective vs. highly selective conditions. For highly selective conditions, rats were pretreated with retrorsine to block proliferation of host hepatocytes. Under both non-selective and selective experimental conditions, two-thirds PH was performed at the time of cell transplantation and in each case comparable numbers of hepatic cells were infused directly into the portal vein. As shown in Fig. 4, under non-selective conditions, there was significant repopulation by ED14 fetal liver cells (Fig. 4A), but much less repopulation with ED16 fetal liver cells (Fig. 4C) and almost no repopulation with adult hepatocytes (Fig. 4E) (data shown at 4 months after cell transplantation because repopulation proceeds at a slower pace under non-selective conditions). Using the same numbers of cells under selective conditions (2 doses of retrorsine 30 mg/kg, two weeks apart, followed by a one month recovery period), there was a 10–100 fold greater liver repopulation with all three cell types, even as soon as 2 months after cell transplantation (Fig. 4B, D & F). These studies demonstrated that after lineage divergence in the fetal liver, there is a dramatic reduction in the repopulation potential of hepatic progenitor cells when transplanted into a normal liver (i.e., under non-selective conditions). However, both ED14 (early) and ED16 (later, more differentiated) liver progenitor cells, as well as fully differentiated, mature hepatocytes exhibit high repopulation potential under highly selective conditions. These studies also demonstrate that there is an enormous difference in the repopulation potential of hepatic cells under selective vs. non-selective conditions. Therefore, one cannot compare the repopulation potential of different cell types when the

repopulation studies are performed under different experimental circumstances. Finally, Ott and coworkers have recently reported that for mouse or human cells transplanted into Alb-uPA/Rag2^{-/-}/γc^{-/-} mice (highly selective conditions), repopulation with adult hepatocytes is more efficient than with ED 11.5–13.5 fetal liver cells (Haridass et al. 2009). Our results with adult hepatocytes vs. ED 12.5 mouse fetal liver cells in retrorsine/CCl₄ treated mice (Nierhoff et al. 2005) are consistent with the findings of Ott and coworkers. Whether this is due to more efficient engraftment of mature hepatocytes compared to hepatoblasts in the mouse liver or some other factor(s) remains to be determined.

What is a stem cell, and how does this cell differ from a progenitor cell?

During development of the mammalian embryo, stem cells originate from the inner cell mass of the blastocyst (Thomson et al. 1998). These cells, termed embryonic stem (ES) cells, are totipotent and give rise to somatic stem cells that differentiate further into multipotent, tissue-specified stem cells. The latter differentiate further into lineage committed progenitor cells and ultimately into adult differentiated cells, comprising the various organs. Tissue specific (or somatic) stem cells have been identified in studies of tissues with high turnover, such as hematopoietic cells of the bone marrow and epithelial cells of the skin and intestinal mucosa. Continuous proliferation and differentiation of stem cells in these organs maintains tissue mass at a steady state as part of normal cellular turnover (Potten, 1997).

Tissue specified stem cells are generally considered to exhibit four basic properties: (1) capacity for self-renewal or self-maintenance; (2) multipotency, producing progeny in at least two lineages; (3) functional, long-term tissue reconstitution; and (4) serial transplantability. Progenitor cells are the progeny of stem cells. These cells may be comprised of distinct subpopulations, some with multilineage potential (multipotent progenitor cells) and others that have differentiated further and give rise to progeny in only a single lineage (unipotent progenitor cells). In contrast to stem cells, progenitor cells do not self-renew; they divide rapidly but are capable of only short-term tissue reconstitution. Progenitor cells cannot be serially transplanted (under normal circumstances), and they have been termed “transit amplifying cells” (Potten, 1997).

Properties of Fetal Liver Epithelial Cells as Hepatic Stem/Progenitor Cells

As implied above, the ultimate test for a putative stem cell is to demonstrate its ability to functionally repopulate a tissue or organ, long-term. Sandhu et al. (2001) reported 5–10% repopulation of DPPIV⁻ mutant F344 rat liver by transplanting wt ED14 fetal liver epithelial cells into the normal adult liver in conjunction with two-thirds PH. The transplanted cells were integrated into the host parenchyma, proliferated extensively, and the majority of repopulating clusters contained both hepatocytes and mature bile duct cells (Sandhu et al. 2001). Liver repopulation by transplanted ED14 fetal liver cells increased progressively and remained stable over six months. These results were obtained in a normal tissue environment, requiring only a two-thirds PH to initiate the repopulation process. These findings are comparable to those obtained in the hematopoietic system, where extensive ablation is required for bone marrow replacement by transplanted hematopoietic stem cells.

In ED14 rat fetal liver, there are three distinct populations of epithelial cells, those positive for AFP and Alb but negative for CK-19, those positive for AFP, Alb and CK-19 and those positive for CK-19 but negative for AFP and Alb (Dabeva et al. 2000; Sandhu et al. 2001; Oertel et al. 2006a). The number of AFP⁺/Alb⁺/CK-19⁺ cells decreases dramatically at ED16, after which liver repopulation potential of rat fetal liver cells also decreases dramatically (Sandhu, 2001). The level of liver repopulation by rat ED14 fetal liver cells under non-selective conditions can also be increased to 20–25% by increasing the number of

ED14 fetal liver cells transplanted (Fig. 5A & B) (Oertel et al. 2006a). A frequently asked question is whether these repopulation results represent a general phenomenon or a unique property of DPPIV⁻ mutant rats which is not apparent, since the liver appears normal morphologically. To address this question, we have reversed the model, so that ED14 fetal liver cells from DPPIV⁻ mutant F344 rats have been transplanted into normal wt (DPPIV⁺) F344 hosts. In these studies, essentially the same results have been obtained (Fig. 5C & D).

Repopulating fetal liver cells have been enriched to 95% homogeneity by immunoselection for Dlk-1, an early fetal liver epithelial cell surface marker. The enriched cells retain the full repopulation potential of unselected fetal liver cells in the normal adult rat liver (Fig. 5E & F) (Oertel et al. 2008). Repopulation continues to increase for up to one year, reaching an average of ~30% for the total liver (Oertel et al., unpublished observations). Repopulation remains stable for the life of the animal, which is consistent with the slow turnover of parenchymal cells in this organ. In this normal rat model, there is a several thousand fold amplification of transplanted fetal liver epithelial cells in the repopulated host liver (Oertel et al. 2006b). Both hepatic parenchymal cords and mature bile ducts are formed by transplanted fetal liver cells, as well as whole new liver lobules, and the progeny of the transplanted cells express normal levels of hepatocytic and cholangiocytic genes in the respective cell types (Oertel et al. 2006b; Oertel et al. 2008). Thus, transplanted rat ED14 fetal liver epithelial cells exhibit three major properties of stem cells: 1) extensive proliferation, 2) differentiation into the two mature epithelial cell phenotypes in the liver, i.e., hepatocytes and cholangiocytes, and 3) long-term, in vivo functional reconstitution of the tissue. Since serial transplantation (an indicator of self-renewal) has not yet been demonstrated with fetal liver epithelial cells, they are referred to as fetal liver stem/progenitor cells (Oertel et al. 2006a,b; Oertel et al. 2008).

The mechanism for liver repopulation by rat fetal liver stem/progenitor cells has been shown to be cell competition between the transplanted cells and host hepatocytes (Oertel et al. 2006b), a process originally described in *Drosophila* during wing development (Moreno and Basler, 2004; de la Cova et al. 2004). Rat fetal liver stem/progenitor cells have been cryopreserved with full ability to repopulate the normal adult liver after thawing (Oertel et al. 2006a).

Induction and Properties of “Oval” (Progenitor) Cells

The term “oval cells” was introduced by Farber in 1956 to describe non-parenchymal cells in the periportal region that were present after treating rats with carcinogenic agents, such as ethionine, α (alpha)-acetaminobenzene [2-AAF] and 3-methyl-4-diethylaminobenzene (Farber, 1956). Other methods to induce proliferation of “oval cells” are treatment of rats with D-galactosamine (Lemire et al. 1991; Dabeva and Shafritz, 1993), a choline deficient/ethionine substituted diet (Sells et al. 1981; Akhurst et al. 2001) or allyl alcohol (Yin et al. 1999) or treatment of mice with dipin (Factor et al. 1994) or 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) (Preisegger et al. 1999). Cells that are induced in each of these models have a small oval-shaped, pale-stained blue nucleus and very scant, lightly basophilic cytoplasm. Farber did not believe that “oval cells” are hepatocyte progenitors (Tatematsu et al. 1984), but Thorgeirsson and coworkers (Evarts et al. 1987) demonstrated that “oval cells”, induced to proliferate in the periportal region after treatment of rats with 2-AAF followed by two-thirds PH, subsequently differentiate into distinct clusters of basophilic hepatocytes in the mid-parenchyma (Evarts et al. 1987; Evarts et al. 1989). They subsequently demonstrated that “oval cells”, induced to proliferate by 2-AAF/PH, are derived from undifferentiated cells in the Canals of Hering (Paku et al. 2001). Other evidence that “oval cells” are hepatic progenitors came from reports that “oval cells” express c-kit (Fujio et al. 1994), CD34 (Omori et al. 1997), flt3 receptor (Omori et al. 1997) and LIF

(Omori et al. 1996), all known markers for hematopoietic stem cells or their immediate derivatives. Sca-1, another cell surface protein expressed by hematopoietic stem cells in the mouse, is also expressed in fetal liver epithelial cells (Nierhoff et al. 2005) and in “oval cells” of the adult mouse liver (Petersen et al. 2003; Wright et al. 2008). Most recently, using lineage tracing studies in a double transgenic mouse expressing a β (beta)-galactosidase reporter gene under control of the Foxl1 promoter, Greenbaum and coworkers (Sackett et al. 2009) showed that “oval cells”, induced to proliferate by bile duct ligation or feeding mice a DDC containing diet, can differentiate into both hepatocytes and bile duct epithelial cells, and thus represent bipotent progenitor cells.

The term “oval cells” is used to identify a highly heterogeneous population of cells, and many authors use the terms, oval cells, oval/progenitor cells and hepatic progenitor cells interchangeably. Attempts to establish specific markers for “oval cells” to distinguish them from mature hepatocytes and bile duct epithelial cells and to determine their lineage origin (mesoderm or endoderm) have led to conflicting findings. All investigators agree that “oval cells” express common liver epithelial progenitor cell markers, such as AFP and albumin for hepatocyte progenitors and CK-19 (and OV6 in the rat) for bile duct progenitor cells. However, it is not clear whether “oval cells” from different animal species or from different hepatic injuries are in fact the same. Although “oval cells” were initially thought to express hematopoietic stem cell markers, c-kit, CD34 and Thy-1 (Fujio et al. 1994; Omori et al. 1997; Crosby et al. 2001; Petersen et al. 1998), several recent studies have shown that both fetal liver progenitor cells and “oval cells” are negative for c-kit, CD34 and Thy-1 (Suzuki et al. 2000; Tanimizu et al. 2003; Dezso et al. 2007; Yovchev et al. 2007).

Liver Repopulation by “Oval Cells”

As indicated previously, since hepatocytes do not effectively repopulate the normal adult liver, an obvious alternative would be to transplant progenitor (“oval”) cells that should have a higher proliferative potential than adult hepatocytes. “Oval cells” isolated from the liver of rats treated with D-galactosamine also proliferate and differentiate into hepatocytes after transplantation into rats undergoing two-thirds PH (Dabeva et al. 1997). However, in these studies, which were conducted in a non-selective tissue environment, liver repopulation by transplanted D-galactosamine induced “oval cells” was low. Duct-like epithelial cells, isolated from the atrophic pancreas of rats treated with a copper deficient diet supplemented with a copper chelating agent (Rao et al. 1988; Dabeva et al. 1995), also proliferate modestly after transplantation into the normal rat liver in conjunction with two-thirds PH (Dabeva et al. 1997). Isolated pancreatic cells from normal mice also repopulate the liver of Fah null mice (Wang et al. 2001). “Oval cells” isolated from the liver of DDC-fed mice also repopulate the liver of Fah null mice, although with a reduced efficiency compared with mature hepatocytes (Wang et al. 2003). Similarly, “oval cells” from GFP transgenic mice maintained on a DDC diet also repopulate the liver of wt mice treated with monocrotaline in conjunction with PH (Song et al. 2004). Other recent studies have shown effective repopulation of the liver by purified “oval cells” in retrorsine treated rats (Yovchev et al. 2008).

Most recently, “oval cells” have been isolated from normal mouse and dog liver (Wright et al. 2008; Suzuki et al. 2008; Arends et al. 2009). These cells exhibit properties characteristic of hepatic progenitor cells in culture, but in vivo repopulation data are very limited. Numerous studies have reported the isolation of “oval cell” lines or hepatic progenitor cell lines from rats, mice and humans (Grisham 1980; Yasui et al. 1997; Coleman et al. 1997; Suzuki et al. 2002; Strick-Marchand et al. 2004; Fougere-Deschatrette et al. 2006; Dan et al. 2006; Suzuki et al. 2008). These cells are clonal, bipotent and exhibit other stem and progenitor cell properties in vitro and in vivo. However, liver repopulation by “oval” or

hepatic progenitor cell lines has generally been very low, even under highly selective conditions.

Liver Repopulation by Extrahepatic and Embryonic Stem Cells

Various studies have reported that cells released from the BM into the circulation, migrate to the liver and differentiate into hepatocytes. However, the extent to which this occurs and the mechanism(s) involved remain controversial (for reviews, see Goodell, 2003; Wagers and Weissman, 2004; Fausto, 2004; Thorgeirsson and Grisham, 2006). Originally, Petersen and coworkers reported that BM stem cells isolated from DPPIV⁺ F344 rats and transplanted into sublethally irradiated DPPIV⁻ F344 rats repopulate the BM and then migrate to the liver and “transdifferentiate” into hepatocytes through the liver “oval cell” progenitor pathway (Petersen et al. 1999). These findings were subsequently confirmed by studies in both mice and humans (Theise et al. 2000a,b; Alison et al. 2000). However, studies by Wang et al. (2003) showed that BM cells did not enter the “oval cell” pool in mice treated with DDC. Menthen et al. (2004) also showed that DPPIV⁺ BM cells transplanted into DPPIV⁻ rats contributed <1% to the “oval cell” pool in three different models of “oval cell” activation: 1) 2-AAF/PH, 2) retrorsine/PH or 3) D-gal induced liver injury. These studies (Wang et al., 2003; Menthen et al., 2004) and others in both mice and rats (Wagers and Weissman, 2004; Fausto, 2004; Thorgeirsson and Grisham, 2006) have demonstrated that “transdifferentiation” of hematopoietic stem cells into “oval cells” is at best a rare event that probably does not have physiologic significance. More recently, Oh et al. (2007) reported that DPPIV⁺ oval cells derived from bone marrow cells and transplanted into DPPIV⁻ mutant rats treated with 2-AAF/PH, represent 20% of total activated “oval cells”. However, after secondary transplantation of these DPPIV⁺ “oval cells” into secondary DPPIV deficient hosts under highly selective conditions (monocrotaline/PH), less than 0.5% of hepatocytes in the repopulated liver were DPPIV⁺. Therefore, bone marrow cells that had “transdifferentiated” into hepatic “oval cells” contributed very little to liver repopulation under highly selective circumstances.

In Fah^{-/-} mice and other model systems, cell fusion has been shown to be the predominant mechanism by which hematopoietic cells acquire an hepatocytic phenotype. Initial studies in cell culture showed that BM and neuronal cells can fuse with ES cells (Terada et al. 2002; Ying et al. 2002). Wang et al. (2003) and Vassilopoulos et al. (2003) subsequently showed that hematopoietic stem cells fuse with hepatocytes in Fah null mice to produce cells that express the deficient enzyme, which then expand massively to restore liver tissue. Fusion also occurs between hematopoietic cells and neurons or muscle cells (Alvarez-Dolado et al. 2003; Weinmann et al. 2003) and it has been shown that myelomonocytic cells can fuse with hepatocytes (Camargo et al. 2004; Willenbring et al. 2004) or muscle cells (Camargo et al. 2003) to produce somatic hybrids expressing genes from both parental cell types.

Other studies have reported that fusion is not required for BM-derived cells to differentiate into hepatocytes (Newsome et al. 2003; Harris et al. 2004; Jang et al. 2004; Oh et al. 2007). Unfractionated or CD34⁺ enriched cells from human cord blood (Danet et al. 2002; Wang et al., 2003; Kakinuma et al. 2003; Kollet et al. 2003), multipotent adult progenitor cells (MAPC) (Schwartz et al. 2002; Jiang et al. 2002) or mesenchymal stem cells from human cord blood or bone marrow (Lee et al. 2004; Aurich et al. 2007; Brulport et al. 2007; Kuo et al. 2008) have been transplanted into the liver of immunodeficient mice. These transplanted cells express a differentiated hepatocytic phenotype (Danet et al. 2002; Wang et al. 2003; Kakinuma et al. 2003; Kollet et al. 2003; Schwartz et al. 2002; Jiang et al. 2002; Lee et al. 2004; Kogler et al. 2004; Lee et al. 2004; Anjos-Afonso et al. 2004; Sato et al. 2005; Aurich et al. 2007), but liver repopulation was once again very low. Several studies have reported that mesenchymal stem cells isolated from adipose tissue can also engraft in the liver

parenchyma and contribute to liver regeneration (Banas et al. 2007; Sgodda et al. 2007). One of these studies (Sgodda et al. 2007) reported large repopulation clusters with hepatocyte-differentiated mesenchymal stem cells, but this required retrorsine pretreatment of the recipients and the overall level of liver repopulation was not reported.

Many studies have demonstrated that ES cells in culture can be induced along the endodermal and hepatocytic lineages by addition of specific cytokines and growth factors (Hamazaki et al. 2001; Jones et al. 2002; Yamada et al. 2002; Yamamoto et al. 2003; Rambhatla et al. 2003; Kubo et al. 2004; Guon-Evans et al. 2006; Heo et al. 2006; Basma et al. 2009). This typically involves the generation of embryoid bodies, followed by the induction of definitive endoderm using activin A. The endodermal population is further specified towards the hepatic lineage using BMP-4 and basic FGF. Cells produced in this fashion express typical hepatocytic markers, such as AFP and albumin. They have been transplanted into the liver under highly selective conditions and differentiate into both mature hepatocytes (Gouon-Evans et al. 2006; Heo et al. 2006; Basma et al. 2009) and bile duct epithelial cells (Heo et al. 2006). However, the level of liver repopulation obtained with hepatocyte-differentiated ES cells is very low, although somewhat higher when the cells are transplanted into MUP-uPA/SCID mice (Heo et al. 2006). To date, all ES differentiation protocols generate “hepatocyte-like” cells, but not fully mature hepatocytes. More mature “hepatocyte-like” cells have been selected using surface markers, such as the asialoglycoprotein receptor, and selected cells show higher levels of differentiated function after their transplantation (Basma et al. 2009).

Induced Pluripotent Cells (iPS)

Because of their extensive proliferative capacity, pluripotent stem cells are an attractive potential source of transplantable hepatocytes. Not only can these cells divide extensively, but they also retain the ability to differentiate into many different mature cell types (Slack, 2008). Pluripotent cells can now be derived by direct genetic reprogramming of somatic cell types, such as dermal fibroblasts (Takahashi et al. 2007; Takahashi and Yamanaka, 2006; Yu et al. 2007). Initially, this required the introduction and ectopic expression of four genes, Oct4, Klf4, Sox2 and c-myc (Takahashi et al. 2006; Takahashi et al. 2007), but more recently the same effect has been produced using recombinant proteins (Zhou et al. 2009) and some of these genes have been induced in somatic cells by treatment with small molecules (Shi et al. 2008; Ding 2009). A substantial literature is now evolving concerning the potential use of iPS cells to treat monogenic metabolic disorders. In two most recent studies (Sullivan et al. 2010; Si-Tayeb et al. 2010), iPS cells, derived from human skin fibroblasts, can be differentiated into hepatocyte-like cells in vitro at very high efficiency (80–90%), using differentiation protocols similar to those used for ES cells. In one of these studies (Si-Tayeb et al. 2010), hepatocyte differentiated hiPS cells were injected directly into the hepatic parenchyma of newborn mice and exhibited human albumin expression 7 days later. These studies are just a beginning but have major implications concerning the potential feasibility of using hepatocyte-differentiated iPS cells for therapeutic liver cell repopulation.

Hepatic Stem Cells from Human Liver

Several investigators have isolated, cultured and/or passaged human fetal liver epithelial cells with bipotential properties, and several of these studies have demonstrated their differentiation into hepatocytes after transplantation into SCID or nude mice (Malhi et al. 2002; Dan et al. 2006; Mahieu-Caputo et al. 2004). Schmelzer et al. (Schmelzer et al. 2006) have identified two populations of hepatic progenitor cells from human fetal, neonatal and pediatric liver that exhibit stem cell properties. One population is thought to represent an

hepatic stem cell (AFP⁻/Alb⁺) and the other a slightly more differentiated hepatoblast (AFP⁺/Alb⁺). More recently, Schmelzer et al. (2007) reported data suggesting that these cells may reside in the Canals of Hering. One unexpected difference from their rodent counterpart is that human hepatic-specified stem cells are Alb positive but AFP negative. This is opposite to what one might expect based on expression of AFP before Alb during liver development (Shiojiri et al. 1991). Nonetheless, these studies suggest that a human liver somatic stem cell might exist and hopefully future studies will demonstrate in vivo self-renewal and long-term repopulation of the liver by these cells, proving that they are indeed stem cells.

Xenorepopulation Models

Two models demonstrating repopulation of the mouse liver with human hepatocytes have been reported. In 2001, Dandri et al. (2001) showed that immune-deficient uPA transgenic mice could be engrafted with human hepatocytes and they used this system as a model for hepatitis B virus infection; Mercer et al. (2001) used a similar model for hepatitis C virus infection. Subsequently, this model has been developed further to permit extensive repopulation, reaching levels as high as 90% human cells (Tateno et al. 2004; Meuleman et al. 2005). Highly immunodeficient Fah null mice can also be repopulated extensively with human hepatocytes (Azuma et al. 2007). This model has an advantage over uPA transgenic mice in that the level of hepatic injury can be titrated by NTBC. An additional difficulty in using the uPA transgenic mouse model has been poor breeding efficiency. However, this has recently been overcome by producing an immunodeficient, alb promoter driven-rt TA/SCID mouse that can be transduced at very high efficiency with an adenovirus TRE-uPA vector to produce an hepatocyte specific, doxycycline regulatable-uPA mouse that can be used for liver repopulation studies with xenographic cells (Song et al., 2009).

Summary of Repopulation Results from Studies Transplanting Isolated Primary Cells and Cell Lines into the Liver

Table 1 summarizes the results of selected cell transplantation/liver repopulation studies reviewed above and indicates the model systems and specific physiologic or pathophysiologic circumstances, i.e., selective or non-selective conditions, under which liver repopulation has been achieved. Under non-selective conditions, transplanted hepatocytes engraft into the liver and undergo a few cell divisions, but they do not effectively repopulate the liver. However, under highly selective conditions, hepatocytes transplanted into mice or rats can massively repopulate the liver. In most instances, the liver needs to be undergoing a high level of injury/cell turnover, but similar results can be achieved in other selective models in which the proliferation of host hepatocytes is disrupted, while transplanted exhibit normal proliferative activity. In the latter host mitoinhibitory models, a proliferative stimulus substantially accelerates liver repopulation by transplanted cells (Laconi et al 1998) but is not required (Laconi et al. 2001). "Oval cells" transplanted into the liver under non-selective conditions engraft but also undergo only limited expansion. In contrast, under strong selective conditions, "oval cells" highly repopulate the liver. Fetal liver epithelial (stem/progenitor) cells before divergence into the hepatocytic and biliary lineages exhibit substantial repopulation potential in the adult rat liver under non-selective conditions. This results from cell competition between transplanted fetal liver cells and host hepatocytes in which the fetal liver cells induce apoptosis of host hepatocytes, while they are resistant to apoptosis and continue to proliferate (Oertel et al. 2006b). After lineage divergence, liver repopulation by fetal liver cells is markedly reduced (Sandhu et al. 2001).

Studies with cultured hepatic epithelial cells and cell lines have led to only very limited liver repopulation under either non-selective or selective conditions. The same is true for

hematopoietic stem cells (with one notable exception), embryonic stem cells or mesenchymal stem cells. Under most highly selective conditions, Lagasse et al. (2000) showed extensive repopulation of *Fah*^{-/-} mouse liver by hematopoietic cells, which was later shown to result from fusion of hematopoietic progenitor cells with host hepatocytes (Wang et al. 2003; Vassilopoulos et al. 2003).

Figure 6 is a schematic diagram that highlights the inverse relationship between the proliferative/repopulation potential of fetal liver epithelial cells and their differentiation. After endodermal cells become specified to the hepatic lineage, there is a progressive decrease in the repopulation potential as the cells differentiate. Initially, the cells (early progenitor cells or stem/progenitor cells) are capable of repopulating a normal liver tissue environment, but as the cells differentiate further and diverge along the hepatocytic or cholangiocytic lineage, their repopulation potential declines substantially and they do not effectively repopulate the normal liver under non-selective conditions. Both unipotential hepatic progenitor cells and fully differentiated hepatocytes repopulate the liver only under highly selective conditions. Results obtained with rat fetal liver “progenitor cells” after ED16 are comparable to those obtained with “oval cells” isolated from the adult liver (see Table I). In both instances, some of the cells are bipotential (expressing hepatocytic and cholangiocytic genes) and can be clonally propagated. However, they have lesser repopulation potential than fetal liver stem/progenitor cells (ED14) isolated before lineage divergence. This is a potential area for future investigation. Genetic signals in the transplanted cells and paracrine signals emanating from the host liver environment in which the cells have engrafted undoubtedly contribute to the differences in repopulation potential under non-selective vs. selective conditions, and this is also an important area for future investigation.

Future Perspective

From many recent studies, it is clear that mammalian cells exhibit a high degree of phenotypic plasticity and that previous distinctions between stem cells, progenitor cells and mature differentiated cells are somewhat arbitrary. Based on the experimental conditions under which cells are tested for proliferative and differentiated functions, either in vitro or in vivo, mammalian cells exhibit different patterns of gene expression, resulting in different cellular behavior. Since the functional properties of the cells depend upon the context in which the cells are maintained and the extracellular signals that they receive from their surrounding environment, the ability of such cells to repopulate a tissue or organ will depend not only on their innate properties, but also on the tissue environment in which the cells are located. Therefore, for effective tissue repopulation to be achieved, it will be important to identify the signals and mechanisms that regulate the specific properties of the cells and to determine how the behavior of the cells can be modulated by external signals.

The emphasis of this review was to indicate the importance of the tissue environment in determining how a specific cell type might behave in terms of specific tissue repopulation. The presentation focused on whether the host tissue environment was either selective or non-selective for repopulation by a particular transplanted cell, at what stage in specific lineage progression was the transplanted cell at the time it was transplanted and how does this impact on the level of tissue repopulation achieved. The next steps will be to determine how these processes are regulated and to identify methods to modulate these processes and enhance the repopulation potential of a given cell type. Effective modulation of these processes in vivo may seem like science fiction today, but at some time in the future, this will become routine medical practice.

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Figure 1.

Alb-uPA transgenic and $Fah^{-/-}$ mouse models for liver repopulation.

A. Spontaneous regeneration of the uPA transgenic mouse liver by endogenous revertant hepatocytes (Sandgren et al. 1991). Hepatocytes that have deleted the uPA transgene replace uPA transgenic hepatocytes because they are not undergoing liver injury and have a selective advantage over uPA expressing hepatocytes that are continually being destroyed.

B. Repopulation of the liver of a uPA transgenic mouse by transplanted wt hepatocytes expressing a marker gene, β -galactosidase (Rhim et al. 1994). Transplanted β -gal expressing, but otherwise normal, mouse hepatocytes have a selective advantage for survival compared to endogenous uPA expressing hepatocytes that are continuously undergoing destruction by expression of the toxic uPA transgene.

C. $Fah^{-/-}$ mouse transplanted with wt FAH^{+} hepatocytes while on continuous treatment with NTBC (Overturf et al. 1996). Note the presence of small scattered cultures of FAH^{+} hepatocytes, but no significant liver repopulation by FAH^{+} cells.

D. $Fah^{-/-}$ mouse transplanted with wt FAH^{+} hepatocytes followed by withdrawal of NTBC (Overturf et al. 1996). Under these conditions, which are highly selective for survival of FAH expressing hepatocytes, there is massive replacement of the liver by FAH^{+} hepatocytes at 6 weeks after cell transplantation.

Figure 2.

Retrorsine/PH model for liver repopulation.

A. Active repopulation of the liver by transplanted wt DPPIV⁺ hepatocytes without evidence for tissue compression or destruction of the surrounding hepatic DPPIV⁻ parenchyma (Laconi et al. 1998).

B. Transplanted hepatocytes are completely integrated into the host liver parenchymal, forming DPPIV⁺ hybrid bile canaliculi with adjacent DPPIV⁻ host hepatocytes (Laconi et al. 1998).

C. Liver of DPPIV⁻ mutant host removed by two-thirds PH just prior to hepatocyte transplantation (Laconi et al. 1998).

D. Liver of the same DPPIV⁻ mutant rat at 9 months after transplantation of 1×10^6 wt (DPPIV⁺) hepatocytes, exhibiting 99% replacement of the host liver by DPPIV⁺ cells (Laconi et al. 1998).

Figure 3.
Schematic diagram of fetal liver development in the mouse.

Figure 4.

Photomicrographs showing the level of liver repopulation by rat ED14 and ED16 fetal liver cells and adult rat hepatocytes under non-selective (normal/PH) vs. highly selective (retrotransine/PH) host conditions. In each case, the number of transplanted hepatic epithelial cells was $\sim 4 \times 10^5$ cells. For non-selective conditions (**A, C & E**), animals underwent two-thirds PH at the time of hepatocyte transplantation and were sacrificed 4 months after cell transplantation. For highly selective conditions (**B, D & F**), animals were treated with retrotransine/PH prior to hepatocytic transplantation and were sacrificed 2 months after cell transplantation. **A & B**, ED14 fetal liver cells, **C & D**, ED16 fetal liver cells and **E & F**,

adult hepatocytes (Sandhu et al. 2001 and previously unpublished). All photomicrographs at 100X original magnification.

Figure 5.

High levels of liver repopulation by rat ED14 fetal liver cells and highly enriched rat ED14 fetal liver stem/progenitor cells under non-selective conditions. **A & B**, 50×10^6 unfractionated rat ED14 fetal liver cells from a DPPIV⁺ donor were transplanted into a DPPIV⁻ mutant rat under non-selective conditions (PH without retrorsine pretreatment) (Oertel et al. 2006b). **A**, whole liver section scanned without magnification and **B**, photomicrograph at 100X original magnification. **C & D**, reversal of the DPPIV model system in which 20×10^6 ED rat fetal liver cells from a DPPIV⁻ donor were transplanted into a wt (DPPIV⁺) host liver under non-selective conditions (PH without prior retrorsine treatment) (Oertel et al., previously unpublished). **C**, whole liver section scanned without magnification and **D**, photomicrograph at 100X original magnification. **E & F**, 1.9×10^6 purified ED14 fetal liver stem/progenitor cells (enriched to 95% purity by immunoselection for Dlk-1 and equivalent to 35×10^6 unfractionated ED14 fetal liver cells) from a DPPIV⁺ donor transplanted into a DPPIV⁻ mutant rat under non-selective conditions (PH without retrorsine pretreatment) (Oertel et al. 2008). **E**, whole liver section scanned without magnification and **F**, photomicrograph at 100X original magnification.

Figure 6. Schematic diagram illustrating the relationship of fetal liver hepatic lineage progression to the repopulation potential of isolated hepatic cells under non-selective vs. highly selective repopulation conditions.

TABLE 1

Liver repopulation by transplanted cells of hepatic and non-hepatic origin

REPORTING GROUP	SPECIES OF TRANSPLANTED CELLS	HOST MODEL SYSTEM	% REPOPULATION	COMMENTS
ADULT HEPATOCYTES				
Rhim et al.1994	mouse	Alb-uPA mouse (highly selective)	massive (30–80%)	first study reporting large clusters of repopulating hepatocytes throughout liver parenchyma
Overturf et al.1996	mouse	Fah ^{-/-} mouse (highly selective)	massive (> 80%)	normal regenerated liver structure and function
Rajvanshi et al. 1996b	rat	DPPIV ⁻ rat/CCl ₄ (non-selective)	low	scattered single cells and small clusters of DPPIV ⁺ hepatocytes, repopulation modestly increased after 3 doses of CCl ₄ at weekly intervals
Laconi et al.1998	rat	PH alone (non-selective)	no significant repopulation	isolated single cells or very small clusters of hepatocytes
		DPPIV ⁻ rat retrorsine/PH (highly selective)	massive (up to 99%)	normal regenerated liver structure
Mignon et al.1998	Bcl2 transgenic mouse	normal adult mouse/ anti-Fas Ab (8–12 weekly treatments) (moderately selective)	moderate 3–16%	% repopulation increased progressively with the number of anti-Fas Ad treatments
Guha et al.1999	rat	DPPIV ⁻ rat x-irradiation/PH (highly selective)	massive	similar to results obtained with retrorsine/PH
Dandri et al.1999 2001	Woodchuck human	Alb-uPA/Rag2 ^{-/-} mouse Alb-uPA/Rag2 ^{-/-} mouse (highly selective)	massive (up to 90%) 1–15%	humanized mouse liver can be infected with hepatitis B virus
Malhi et al. 2002	rat	DPPIV ⁻ rat x-irradiation/ischemic injury (highly selective)	massive	similar to results obtained with either retrorsine/PH or x-irradiation/PH
Yuan et al. 2003	P27 Kip1 null	normal adult mouse/ CCl ₄ x 4–8 weekly treatments (weakly selective)	modest 2–6%	number and size of repopulation cell clusters increased with number of CCl ₄ treatments
Tateno et al. 2004	human	Alb-uPA/SCID mouse (highly selective)	massive (up to 90%)	improved results based on use of Futhon, an anti-human complement factor antibody, to prevent cell rejection and kidney failure
Azuma et al. 2007	human	Fah ^{-/-} /Rag2 ^{-/-} /Il2rg ^{-/-} mouse (highly selective)	7/43 recipients highly repopulated (30–90%)	level of liver repopulation can be modulated by NTBC administration
Haridass et al. 2009	mouse human	Alb-uPA/Rag2 ^{-/-} /γ _c ^{-/-} mouse (highly selective)	46% 10%	human cells less effective than mouse cells in this model system

REPORTING GROUP	SPECIES OF TRANSPLANTED CELLS	HOST MODEL SYSTEM	% REPOPULATION	COMMENTS
Song et al. 2009	mouse	rt TA/SCID mouse transduced with Ad. TRE-uPA (highly selective)	highly variable	new TET-regulatable model system with improved breeding compared to Alb-uPA mouse
OVAL/PROGENITOR CELLS				
Dabeva et al. 1997	D-gal activated rat liver	DPPIV ⁻ rat/PH (non-selective)	very low (< 1%)	scattered clusters of hepatocytes and ductular cells
Wang et al. 2003	DCC treated mouse liver	Fah ^{-/-} mouse (highly selective)	high	somewhat lower than adult hepatocytes
Song et al. 2004	DDC treated mouse liver	mouse/monocrotaline/CCl ₄ (highly selective)	high (~40% β -gal ⁺ , 10% α_1 -AT ⁺)	β -gal transgenes mouse oval cells transduced ex vivo with human α_1 -antitrypsin gene
Oh et al. 2007	rat BM-derived, 2-AAF/PH activated oval cells	DPPIV ⁻ mutant rat, monocrotaline/PH (highly selective)	<0.5%	BM-derived oval cells exhibit much lower repopulation potential than liver-derived oval cells
Yovchev et al. 2008	2-AAF or D-gal activated rat liver oval cells	DPPIV ⁻ rats retrorsine/PH (highly selective)	high (60–80%)	very large clusters of repopulating hepatocytes throughout liver parenchyma
FETAL HEPATOBLASTS (STEM/PROGENITOR CELLS)				
Sandhu et al. 2001	ED14 rat	DPPIV ⁻ rat PH alone (non-selective)	5–10%	hepatocytes and bile ducts, whole new liver lobules;
Malhi et al. 2002	cultured human	SCID mice/CCl ₄ (non-selective)	not determined	scattered cells and small cell clusters
Mahieu-Caputo et al. 2004	cultured human	nude mouse or Rag2 ^{-/-} / γ_c ^{-/-} /50% PH (non-selective)	up to 10%, mean ~1%	small clusters of transplanted human cells expressing Hep Par1, albumin and other liver specific genes
Nierhoff et al. 2005	ED12.5 fetal mouse	DPPIV ^{-/-} mouse CCl ₄ (non-selective)	<1%	scattered hepatocytic and endothelial cell clusters
		retrorsine/CCl ₄ (highly selective)	up to 80% av. 10–30%	large clusters of transplanted hepatocytes and endothelial cells
Oertel et al. 2006	ED14 rat	DPPIV ⁻ rat PH alone (non-selective)	20–25%	repopulation increases with number of cells transplanted; cell competition between transplanted fetal liver cells and normal host hepatocytes is mechanism responsible for liver repopulation in normal host environment
Haridass et al. 2009	ED13.5 fetal mouse	Alb-uPA/Rag2 ^{-/-} / γ_c ^{-/-} mouse (highly selective)	range 3–16% (mean 12.1%)	detailed study comparing results

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	human 13–17 weeks gestation	Alb-uPA/Rag2 ^{-/-} /γc ^{-/-} (highly selective)	2.7 ± 1.1%	with hepatocytes, fetal liver cells and ES cells of mouse and human origin cells or small clusters of cells with hepatoblast morphology throughout the liver
“STEM” CELLS (FETAL, NEONATAL AND ADULT LIVER)				
Schmelzer et al. 2007	human	NOD/SCID mice/± CCl ₄ (non-selective)	not determined	short-term repopulation (2–8 days) similar results with or without CCl ₄
CULTURED HEPATIC EPITHELIAL CELLS AND CELL LINES				
Yasui et al. 1997	Long-Evans Cinnamon (LEC) rat	Nagase Analbuminemic Rat (non-selective)	not determined	cells injected into the liver parenchyma, scattered cells and small cell clusters expressing β-galactosidase or albumin
Coleman et al. 1997	WB-344 rat epithelial cell line	DPPIV- rats (non-selective)	not determined	cells injected into liver parenchyma
Suzuki et al. 2002	mouse liver cell line	mouse/CCl ₄ (non-selective)	not determined	scattered clusters of transplanted hepatocytes and bile duct clusters
Strick-Marchand et al. 2004	mouse fetal liver cell line	Alb-uPA/SCID mouse (highly selective)	0.8–2.1%	cells with either an hepatocytic or bile ductular phenotype reported
Fougère-Deschatrette et al. 2006	adult mouse liver cell line	Alb-uPA/SCID mouse (highly selective)	0.3–0.4%	hepatocytes and bile ducts
Dan et al. 2006	cloned human fetal liver cell line	Rag2 ^{-/-} /γc ^{-/-} /retrosine/CCl ₄ (highly selective)	0.8–1.7%	in vitro, cloned cells differentiate into hepatocytes or bile duct epithelial cells; in vivo, clusters of cells with hepatic morphology and human albumin expression observed, as well as human albumin in mouse serum
Suzuki et al. 2008	cloned CD133 ⁺ cell lines from DDC treated mice	Fah ^{-/-} mice (highly selective)	0.03%	moderately sized hepatocyte clusters
EMBRYONIC STEM (ES) CELLS				
Gouon-Evans et al. 2006	mouse hepatocyte-differentiated cells	DPPIV ^{-/-} /Rag2 ^{-/-} (highly selective) Fah ^{-/-} mouse (highly selective)	not determined	scattered DPPIV ⁺ hepatocytic and endothelial cell clusters

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Heo et al. 2006	mouse hepatocyte-differentiated cells	MUP-uPA/SCID (highly selective)	1.94%	small hepatocytic and bile duct cell clusters; increased cluster size with time
Duan et al. 2007	human hepatocyte-differentiated cells	NOD/SCID mouse (non-selective)	not determined	cells injected under liver capsule
Basma et al. 2009	human hepatocyte-differentiated cells	Alb-uPA/SCID (highly selective)	not determined	clusters of human Alb ⁺ cells throughout parenchyma
		Nagase Analbuminemic Rat/retrorsine/PH (highly selective)	not determined	clusters of human Alb ⁺ cells throughout parenchyma
Haridass et al. 2009	mouse and human hepatocyte-differentiated cells	Alb-uPA/Rag2 ^{-/-} /γc ^{-/-} (highly selective)	no significant repopulation	mouse, a few scattered cells with hepatocytic phenotype; human, no cells observed with hepatocytic phenotype
INDUCED PLURIPOTENT STEM (iPS) CELLS				
Si-Tayeb et al. 2010	human	newborn mouse	Not studied	hepatocyte-differentiated hiPS cells injected directly into the hepatic parenchyma expressed human albumin
NON-HEPATIC CELLS AND CELL LINES – BM OR CORD (HEMATOPOIETIC) STEM CELLS				
Petersen et al. 1999	rat	2-AAF/CCl ₄ (highly selective)	~0.1–0.2%	scattered cells expressing transplanted cell makers
Theise et al. 2000	mouse	bone marrow ablation (non-selective)	0.37–2.2%	data corrected for 50% efficiency of Y chromosome detected by FISH in mouse tissue
Theise et al. 2000	human	females transplanted with male BM cells	5–40%	5/6 cases studied from liver needle biopsy specimens
		or males transplanted with a female liver		data corrected for 20% efficiency of Y chromosome detection by FISH in human tissue
Alison et al. 2000	human	BM transplant recipients	0.5–2%	scattered cells and small clusters of Y chromosome+ hepatocytes
Lagasse et al. 2000	mouse	Fah ^{-/-} mouse (highly selective)	~30–50%	selection of FAH expressing cells produced by fusion of hematopoietic cells to hepatocytes; (40–70 very large clusters scattered throughout liver parenchyma)
Danet et al. 2002	human	NOD/SCID/CCl ₄ (non-selective)	<0.1%	% repopulation increased by CCl ₄ administration
Kakinuma et al. 2003	human	SCID mice/2-AAF/1/3 PH	0.1–1.0%	scattered cells and small cell

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		(highly selective)		clusters
Wang et al. 2003	human	NOD/SCID/+ B ₂ M ⁺ /CCl ₄ (non-selective)	0.05–0.5%	scattered cells and small cell clusters
Newsome et al. 2003	human	NOD/SCID mouse (non selective)	0.01–0.02%	~50 human Hepar1 Ab ⁺ cells, cell fusion not observed
Kollet et al. 2003	human	NOD/SCID mice (non-selective)	≤0.01%	rare Alb ⁺ cells or cell clusters
MESENCHYMAL STEM CELLS				
Jiang et al. 2002	cultured mouse	NOD/SCID mice (non-selective)	5–10% in selected periportal regions in 25% of sections examined	human/mouse cell fusion not observed
Aurich et al. 2007	cultured/ differentiated human	Pfp/Rag2 ^{-/-} mice/CCl ₄ (non-selective)	very low	isolated Alb ⁺ cells and small clusters
Brulport et al. 2007	human adherent, proliferating cord blood cells	NOD/SCID or uPA/ Rag2 ^{-/-} (non-selective or selective)	not studied	human Alb ⁺ cells were not hepatocytic
Kuo et al. 2008	cultured/ differentiated human	NOD/SCID mice/CCl ₄ (non-selective)	not measured directly	rare small clusters of human Alb ⁺ cells
ADIPOSE STEM CELLS				
Banas et al. 2007	cultured human CD105 ⁺ cells	Balb/c nude mice/CCl ₄ (non-selective)	not studied	rare human Alb ⁺ cells seen one day after cell transplantation
Sgodda et al. 2007	cultured/ differentiated rat	DPPIV ⁻ rat/retrorsine (highly selective)	not studied	large clusters of Alb ⁺ or α ₁ -AT ⁺ human cells