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Hepatocyte transplantation: potential of hepatocyte progenitor cells and bone marrow derived stem cells

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Summary

The liver has a large regenerative capacity in response to injury. However, in severe cases of liver injury, its regenerative capacity may prove insufficient and the liver injury may progress to liver failure, and in such situations liver transplantation is the only treatment option. An alternative, less invasive approach may be transplantation of hepatocytes or hepatocyte precursor cells. In the adult liver two candidate progenitor cells have been identified: oval cells and small hepatocytes. The former are induced by liver injury under conditions preventing cell division of mature hepatocytes, while the latter are present in small numbers in normal liver. Both cell types have the capacity to expand and differentiate into hepatocytes. In recent years evidence has been presented that bone-marrow derived stem cells can also be expanded and differentiated into hepatocyte progenitor cells. Such cells may be a source for hepatocyte transplantation and hence have the potential to offer a novel therapy for liver failure.

Key words: liver; regeneration; failure; stem cell; transplantation; differentiation

The liver constitutes about 1.5% of our body mass and is located between the gut and the body's systemic circulation. It simultaneously performs a variety of different functions, such as the regulation of energy homoeostasis, biosynthesis of numerous plasma proteins, biotransformation and excretion of endogenous metabolic end-products and xenobiotics, and the production and secretion of bile [1]. The liver consists of a variety of different cell types, the vast majority of which are hepatocytes. Others are endothelial cells, stellate cells and Kupffer cells. The strategic interposition of the liver between the gut and the systemic circulation, and its key role in biotransformation and detoxification, exposes liver cells continuously to many potential threats and toxic insults. The liver is therefore an organ with a tremendous capacity for self-regeneration. In most cases repair of injured liver parenchyma is taken care of by proliferation of mature adult cells, particularly hepatocytes. This great regenerative capacity of the liver has been demonstrated most impressively in the tyrosinaemic mouse model, where wild type hepatocytes were serially transplanted 6 times, resulting in at least 69 doublings [2].

In severe cases of liver injury, the proliferative capacity of liver cells is not sufficient to successfully restore organ function. In such situations, hepatocyte progenitor cells and stem cells of intrahepatic and/or extrahepatic origin may come into play in organ regeneration. In humans, liver transplantation is sometimes the only therapeutic option in fulminant liver failure or endstage liver disease, e.g. liver cirrhosis. However, liver transplantation has several disadvantages: First, it is compromised by a severe shortage of suitable donor organs. Second, after liver transplantation, patients require lifelong drug treatment for immunosuppression and hence lifelong medical supervision. Third, liver transplantation and lifelong medical follow-up are very costly. Therefore, alternative therapeutic options, e.g. hepatocyte transplantation, are important therapeutic approaches in overcoming the problems of whole organ transplantation. The advantage of hepatocyte transplantation is that it is less invasive than whole organ liver transplantation. At present potentially the most promising diseases for treatment with hepatocyte transplantation are inherited metabolic disorders [3]. In fact, proof in principle that hepatocyte transplantation can be a therapeutic option for the cure of liver disease has been presented [4, 5].

In the adult liver, two progenitor cell candidates have been isolated: First, *oval cells* were observed several decades ago at the very early stages

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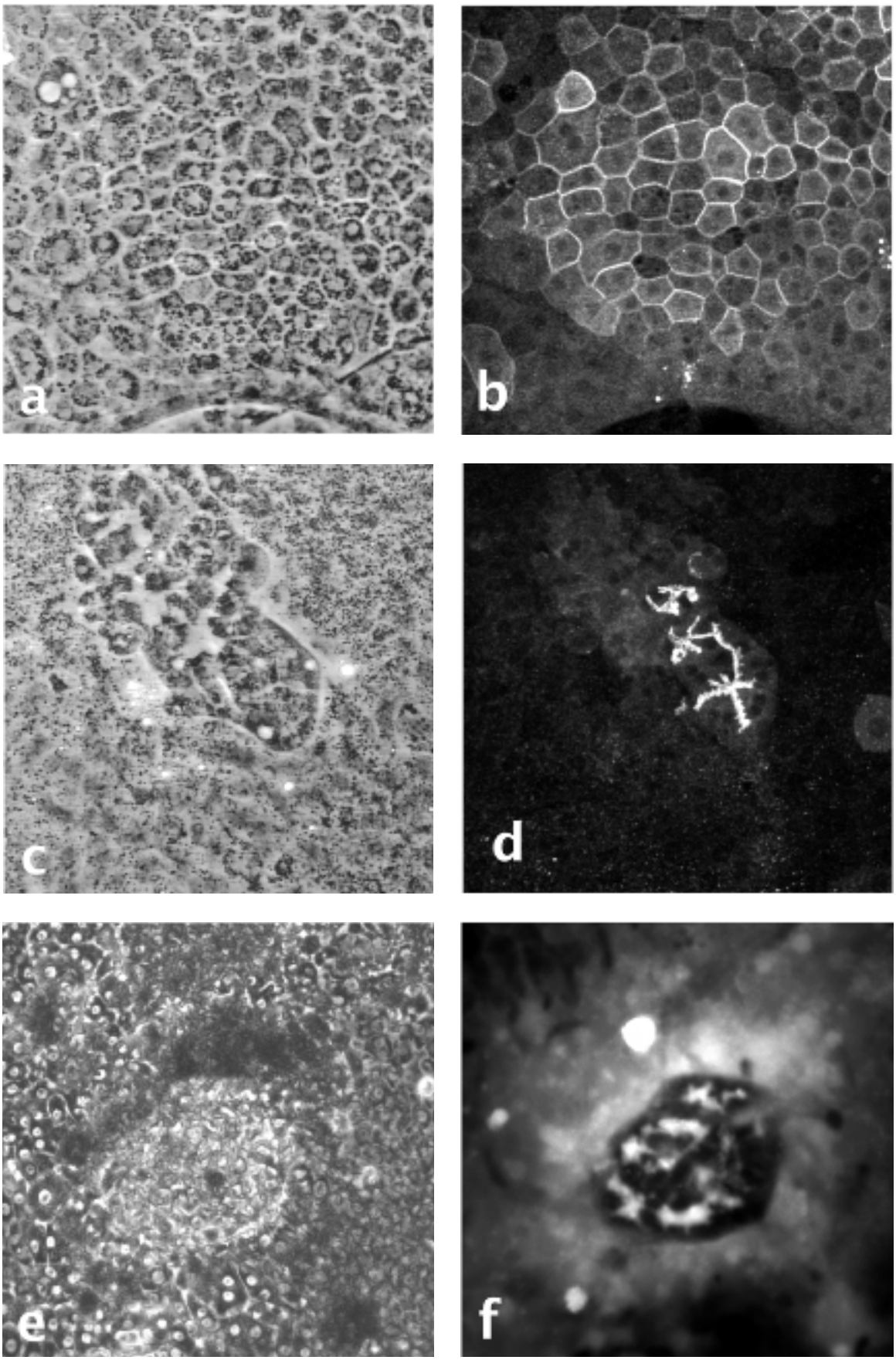

Figure 1

Functional differentiation of long-term cultured rat small hepatocytes. Small hepatocytes were isolated from rat liver and cultured for 11 (panels a to d) and 9 weeks (panels e and f) and subsequently processed as described [22, 23]. Hepatocyte, identified by hexagonally shaped cells, were observed as colonies of different size, which developed during the culture time. Phase contrast pictures show a large colony in (a) and two smaller ones, located in the centre of the pictures (c,e). Expression of hepatocellular organic anion transporters, namely the basolateral organic anion transporting polypeptide Oatp1b2 (b) and the canalicular multidrug resistance-related protein Mrp2 (d) is demonstrated by immunofluorescence. Functional competence of long term cultured hepatocytes is demonstrated by canalicular secretion of fluorescein-diacetate (f), a substrate for Mrp2.

of chemically induced liver carcinogenesis [6]. This animal model has been refined over the years and treating animals with an inhibitor of hepatocyte proliferation such as acetaminofluorene followed by induction of liver regeneration (e.g. carbon tetrachloride treatment or partial hepatectomy) is now a frequently used model for induction of oval cell proliferation [7, 8]. Oval cells have the capacity to replace diseased liver cells, as evidenced by transplantation into the livers of fumarylacetoacetate hydrolase deficient mice. After transplantation, oval cells repopulate the diseased

Table 1

Observed frequencies of transdifferentiated hepatocytes with extrahepatic origin.

Species	Method of analysis	Frequency	Reference
Mouse	Chromosome analysis	1.5% hepatocytes	[28]
Human	Chromosome analysis	5 to 64% hepatocytes	[29]
Human	Short tandem repeat analysis	41% hepatocytes 91% cholangiocytes	[43]
Human	Microsatellite analysis	0.6% hepatocytes 64 to 75% macrophages and Kupffer cells	[44]
Mouse	Chromosome analysis	1:30 000 to 1:140 000 hepatocytes	[45]

liver and were found to be as efficient as mature hepatocytes in liver regeneration [9]. This highlights their potential as hepatocyte progenitor cells. Oval cell induction protocols have also demonstrated their ability to differentiate into biliary and hepatocytic cells, suggesting bipotential properties of oval cells [10]. Additional evidence for the bipotential properties of oval cells is provided by in vitro studies in which permanent cell lines with oval cell-like protein expression patterns were established [11, 12]. Furthermore, bone marrow transplantation experiments have furnished evidence that oval cells may be derived from bone marrow stem cells, which migrate into damaged liver [13]. However, the latter view has also been disputed in recent studies, which found no evidence for the extrahepatic origin of oval cells [9, 14]. While the ultimate source of oval cells is still an open question, there is agreement that proliferating oval cells are first detected within the liver in the vicinity of small bile ducts, which in humans are termed canals of Hering [7, 8, 15–20].

Besides oval cells, *small hepatocytes* have been identified in primary cultures of liver cells as cell types with high potential for proliferation and differentiation into mature hepatocytes [21, 22]. This is in contrast to "regular" hepatocytes, which cannot be expanded in primary culture unless they are dedifferentiated. Isolation of hepatocytes for primary culture involves the centrifugation of the cell suspension obtained after the two-step collagenase perfusion of a liver at very low g-forces. The remaining supernatant contains numerous different cell types, among them cells resembling hepatocytes but much smaller in size, which is about one third that of mature hepatocytes. This cell mixture is cultured on collagen in the presence of nicotinamide. After an initial lag-phase, small hepatocytes can be expanded in culture at a slow growth rate. After several weeks, the small hepatocytes form small colonies, which show differentiation into mature hepatocytes based on the following criteria [23]: First, they express and secrete albumin. Second, they express transferrin, cytokeratins 8 and 18 and connexin 32. And third, they form canaliculi and are able to secrete fluorescein into the canaliculi [22]. Some of the colonies are also positive for cholangiocyte markers such as cytokeratins 7 and 19 as well as connexin 42 [23]. Whether the cholangiocyte-like cells are the progeny of small hepatocytes or originate from other cell types present in the culture is an open question. Interestingly, after 20 days in culture, alphafoetoprotein positive cells appear. These studies were extended by us to investigate the expression of hepatocellular transport systems [24]. After 9 weeks' culture the vast majority of small hepatocyte derived colonies showed polarised expression of the basolateral Na^+-dependent taurocholate cotransporting polypeptide (Ntcp) and the organic anion transporting polypeptide 1b2 (Oatp1b2), as well as of the canalicular ATP-dependent bile salt export system (Bsep) and the multidrug resistance-associated protein 2 (Mrp2) [25]. Furthermore, these colonies formed a three-dimensional network of interconnected bile canaliculi and were able to secrete the fluorescent bile-salt derivative cholylglycyl-fluorescein into the interconnected canaliculi [24]. Hence these hepatocyte colonies derived from small hepatocytes expanded into organoid-like structures with a fully differentiated transporter phenotype (figure 1). Interestingly, the time sequence of the transporter expression mirrored the ontogenesis of transport systems in the developing rat liver [26]. This recapitulation of the ontogenesis of transporter expression in the developing rat liver and the expression of alphafoetoprotein supports the concept that small hepatocytes may be derived from embryonic hepatoblasts [21, 27].

In recent years, evidence has been presented that adult non-hepatic stem cells, which constitute a subpopulation of bone-marrow derived stem cells, can differentiate in vivo into hepatocytic precursor cells or even hepatocytes. Thus, Petersen and coworkers demonstrated in rats that after bone marrow transplantation hepatocytes appeared in the liver which originated from the donor bone marrow cells [13]. This was achieved by transplanting bone marrow cells from normal male rats into dipeptidylpeptidase IV deficient female recipient animals. The results were subsequently confirmed in mice [28] and human liver specimens [29, 30]. However, other more recent observations indicate that the hepatocytes seemingly originating from donor bone marrow derived stem cells actually arose by cell fusion [31, 32]. Fusion of bone marrow derived stem cells with hepatocytes in the liver may result in cells expressing genetic markers from the donor as well as hepatocyte specific gene products. Finally, a further study presented evidence for transdifferentiation of transplanted

blood cells into hepatocytes in the absence of cell fusion [33]. This transdifferentiation arose by altering the genetic programme of the bone marrow derived donor cells to cells expressing the genetic programme of hepatocytes. The mechanisms involved in this genetic reprogramming are not yet understood. Hence this area remains highly controversial, as is supported by the fact that highly variable proportions of hepatocytes display a non-hepatic phenotype in animal models and human liver biopsies (table 1).

Bone marrow derived stem cells have also been transdifferentiated in vitro into a hepatocyte lineage. Oh and coworkers demonstrated the upregulation of mRNA for albumin in rat bone marrow cells cultivated in the presence of hepatocyte growth factor (HGF) [34]. Avital and coworkers, using a coculture system of rat hepatocytes and a subpopulation of Thy1 positive and β_2-microglobulin negative rat bone marrow derived stem cells in the presence of cholestatic rat serum, were able to demonstrate urea production by the cultivated bone marrow cells as well as expression of alpha-fetoprotein and albumin [35]. Schwartz et al. succeeded in isolating from mice, rat and human bone marrow so-called multipotent adult progenitor cells, which could be expanded without signs of differentiation in vitro over many doublings [36]. By cultivating these cells in a hepatocyte-differentiation medium on Matrigel, these investigators were able to transdifferentiate bone marrow derived stem cells into hepatocyte-like cells expressing albumin and cytokeratin18. Importantly, these cells also displayed functional characteristics of hepatocytes, namely production of urea, secretion of albumin and phenobarbital-inducible activity of cytochrome P450 of the 2b/2B family. Trans-differentiation of stem cells derived from umbilical cord blood into hepatocyte-like cells in vitro in the presence and absence of feeder layers has also been reported [37, 38]. These transdifferentiated cells expressed, among other markers, albumin and cytokeratin18. Along these lines, starting with mononuclear cells from umbilical cord blood, we were able to generate stromal cells, to expand these cells with growth factors and to subsequently differentiate them into hepatocyte-like cells expressing mRNA for albumin, the basolateral bile salt transport system NTCP and the canalicular bile salt export pump BSEP (Peters, Meier and Stieger, unpublished). Very excitingly, generation of hepatocyte-like cells, so-called neohepatocytes, from human monocytes has been reported [39, 40]. These neohepatocytes express a variety of hepatocyte markers and display albumin secretion, urea production and phase I and phase II biotransformation reactions. In contrast to cells derived from subpopulations of bone marrow derived stem cells, which are obtained in very small numbers, monocytes are easily obtained in significant numbers from peripheral blood, which makes this approach very attractive.

In conclusion, while many findings on liver stem cells as well as transdifferentiation of subpopulations of bone marrow derived stem cells in vivo or in vitro remain controversial, research in this area has a high potential both with respect to future application in patients with end stage liver disease and in learning more about the biology of liver development and regeneration [41, 42]. To arrive at a state where clinical application of hepatic progenitor cells is possible will require a better understanding of the fundamental molecular and genetic mechanisms of transdifferentiation.

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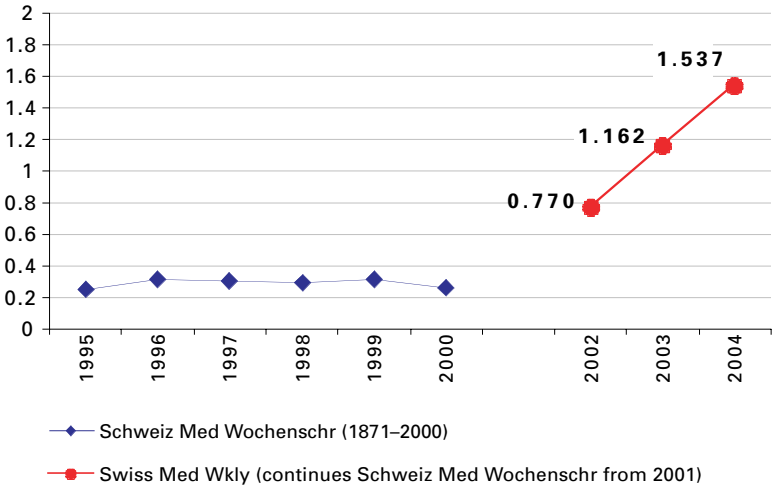

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