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Hepatocytes and Progenitor – Stem Cells in Regeneration and Therapy

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1. Introduction

The liver is a highly specialized detoxifying organ involved in: i) glucose homeostasis; ii) lipid homeostasis and ketone bodies production; iii) metabolism of amino acids. Most of the liver functions are carried out by the hepatocytes (about 70-75% of hepatic cells) that, together with cholangiocytes (10-5 % of hepatic cells), are of endodermal derivation and constitute the hepatic parenchyma.

The liver has a peculiar and fascinating ability: it is able to regenerate itself after loss of parenchyma for surgical resection or injuries caused by drugs, toxins or acute viral diseases. The ancient myth of Prometheus highlighted this capability: the Titan Prometheus was bound for ever to a rock as punishment by Zeus for his theft of the fire; each day a great eagle ate his liver and each night the liver was regenerated, only to be eaten again the next day.

The liver compensatory regeneration is a rapid and tightly orchestrated phenomenon efficiently ensuring the reacquisition of the original tissue mass and its functionality. Primarily, it involves the re-entry into cell cycle of parenchymal hepatocytes which are able to completely recover the original liver mass (Fausto, 2000). The liver anatomical and functional units reconstitution also requires non-parenchymal cells (endothelial cells, cholangiocytes, Kupffer cells, stellate cells). It is yet not clear if each cell histotype is involved in the proliferative process or if the regeneration requires the activity of a cell with multiple differentiation potential. Recently, the bipotentiality of the hepatocytes, able to divide giving rise to both hepatocytes and cholangiocytes, has been suggested. Furthermore, when injury is severe or the hepatocytes can no longer proliferate a progenitor cell population, normally a quiescent compartment is activated. A population of small portal cells named oval cells was first identified in 1978 by Shinozuka and colleagues (Shinozuka et al., 1978). Now as "oval cells" is indicated a heterogeneous population of bipotent transient amplifying cells, originating from the Canal of Hering (Dabeva & Shafritz, 1993). These cells are normally quiescent but, after injury, rapidly and extensively proliferate and differentiate in hepatocytes and cholangiocytes (Yovchev et al., 2008).

The observation that oval cells are a mixed precursor population suggests their differentiation from liver stem cells (Theise et al., 1999). Since the hepatocytes are able to

regenerate themself to compensate liver mass loss, the existence of a liver stem cell, able to drive regeneration in conditions of extreme toxicity affecting the same hepatocytes, has long been debated. Today, there is growing evidence that the liver stem cell exists and its isolation from the organ, its numerical expansion in vitro and its characterization are joint efforts in many laboratories around the world. The interest of the scientific community in the identification, isolation and manipulation of the hepatic stem cell also depends on the fact that the great hopes placed in the use of mature hepatocytes in cell transplantation protocols for the treatment of liver diseases have been disappointed. The basis of these unsatisfactory therapeutic approaches lie in the paradox, not yet resolved, of the inability of hepatocytes, which show *in vivo* a virtually unlimited proliferative potential, to grow *in vitro* to quantitatively and qualitatively amount suitable for cell transplantation in adults.

2. Hepatocyte and regeneration

Regeneration of the original liver mass after damage has been extensively studied in rodents after two-thirds partial hepatectomy (PH) (Bucher, 1963). Regeneration of the liver depends on both hyperplasia and hypertrophy of the hepatocytes, cells that in a normal adult liver exhibit a quiescent phenotype. Hypertrophy begins within hours after PH then hyperplasia follows (Taub, 2004). This occurs first in the periportal region of the liver lobule then spreads toward the pericentral region (Fausto & Campbell 2003).

The restoration of liver volume depends on three steps involving the hepatocytes: i) initiation, ii) proliferation and iii) termination phases.

The initiation step depends on the "priming" of parenchymal cells, mainly via the signaling pathways triggered by the cytokines IL-6 and TNF-α secreted by Kupffer cells, rendering the hepatocytes sensitive to growth factors and competent to replication.

After the G0/G1 transition in the initiation phase, the hepatocytes will enter into the cell cycle (Taub, 2004). Growth factors, primarily HGF, epidermal growth factor (EGF) and TGF-α, are responsible of this second step of regeneration in which the hepatocytes both proliferate and grow in cell size, activating the IL-6/STAT-3 and the PI3K/PDK1/Akt pathways respectively. The first signaling cascade regulates the cyclin D1/p21 and also protects against cell death, for example by up-regulating FLIP, Bcl2 and Bcl-xL. The latter pathway regulates cell size via mammalian target of rapamycin (mTOR) (Fausto, 2000; Serandour et al., 2005; Pahlavan et al., 2006; Fujiyoshi & Ozaki 2011). Numerous growth factors (for example HGF, TGF-α, EGF, glucagon, insulin and cytokines like TNF, IL-1 and -6 and somatostatin (SOM)) are implicated in the regeneration process.

The HGF is a potent growth factor mainly acting on hepatocytes in a paracrine manner binding to its specific trans-membrane receptor tyrosine kinase c-met. HGF is secreted as an inactive precursor and stored in the extracellular matrix (ECM), then activated by the fibrinolytic system (Kim et al., 1997). Plasmin and metalloproteinases (MMPs) degrade the ECM and release pro-HGF that, in turn, is cleaved into an activated form by the urokinase-type plasminogen activator (u-PA)(Kim et al., 1997). The HGF/met signaling is transduced to its downstream mediators, i.e. the Ras-Raf-MEK, ERK1/2 (Borowiak et al., 2004), PI3K/PDK1/Akt (Okano et al., 2003) and mTOR/S6 kinase pathways, resulting in cell cycle progression.

TGF- α is another growth factor relevant in liver regeneration (Tomiya et al., 2000). It belongs to the EGF family, of which all members (EGF, heparin binding EGF-like factor and amphiregulin) transduce trough the common receptor EGF receptor (EGFR) and exert overlapping functions (Fausto 2004). This factor acts in autocrine and paracrine fashions and its production and secretion are induced by HGF.

IL-6 induces mitotic signals in hepatocytes through the activation of STAT-3 (Cressman et al., 1996). The IL-6/STAT-3 signaling involves several proteins: the IL-6 receptor, gp130, receptor-associated Janus kinase (Jak) and STAT-3. The IL-6 receptor is in a complex with gp130, which, after recognition by IL-6, transmits the signal. Jak is responsible of gp130 and STAT-3 activation after IL-6 binding. The STAT-3 form released by gp130 dimerizes and translocates to the nucleus to activate the transcription. STAT3 controls cell cycle progression from G1 to S phase regulating the expression of cyclin D1. In fact, in the liver-specific STAT3-KO model mice, mitotic activity of hepatocytes after PH is reduced significantly (Li et al., 2002).

The PIK/PDK1/Akt signaling pathways are activated by receptor tyrosine kinases or receptors coupled with G proteins by IL-6, TNF-α, HGF, EGF, TGF-α and others (Desmots et al., 2002) (Koniaris et al., 2003). An important downstream molecule of Akt for cell growth is mTOR (Fingar et al., 2002). The activation of this pathway coexists with STAT-3 signaling. In STAT-3-KO mice no significant differences were observed macroscopically in liver regeneration in comparison to control animals, reaching the liver of these mice after PH an equal size. This observation may be explained considering the increase in size of the hepatocytes. Increase in cell size corresponds to marked phosphorylation of Akt and its downstream molecules $p70^{S6K}$, mTOR and GSK3beta (Haga et al., 2005).

The third phase in liver regeneration is the termination step. A stop signal is necessary to avoid an inappropriate liver functional size but the molecular pathways involved in this phenomenon are not yet clear. A key role is exerted by the cytokine TGF-β, secreted by hepatocytes and platelets, that inhibits DNA synthesis (Nishikawa et al., 1998). In fact, within 2-6 hours after PH, the insulin growth factor (IGF) binding protein-1 (IGFBP-1) is produced to counteract its inhibitor effects (Ujike et al., 2000).

3. Liver progenitor cells and regeneration

When liver parenchyma damage is particularly serious and hepatocytes are no longer able to proliferate, liver regeneration can occur through the intervention of bipotent progenitor cells that can proliferate and differentiate into hepatocytes and bile duct cells. It was 1950 when Wilson and Leduc, studying the regeneration of rat liver after severe nutritional damage, observed for the first time these particular cells, located within or immediately adjacent to the Canal of Hering, and their differentiation into two histological types of liver epithelial cells (Wilson & Leduc, 1950). In 1956 Faber called these cells, which are found in the liver of mice treated with carcinogens (Farber 1956), "oval cells" for their morphology.

The first characterization of oval cells has shown the simultaneous expression of bile ducts (CK-7, CK-19 and OV-6) and hepatocytes (alpha-fetoprotein and albumin) markers (Lazaro et al., 1998). Subsequent studies have shown the activation, during oval cell compartment proliferation, of stem cell genes such as c-kit (Fujio et al., 1994), CD34 (Omori et al., 1997) and LIF (Omori et al., 1996) .

Stable lines of oval cells, useful for *in vitro* and *in vivo* studies of differentiation and of liver colonization, were obtained from normal rat liver F-334 (Hixson et al., 1990), or from rats fed with DL-ethionine (Sells et al., 1981) or treated with allyl alcohol (Yin et al., 1999). In addition, these precursors were stabilized starting from liver explants of animal models of Wilson disease (Yasui et al., 1997) of transgenic mice expressing Ras (Braun, et al., 1987) of p53 knockout mice fed with choline-free diet and finally of human liver (Dumble et al., 2002).

The oval cell is currently the best characterized liver progenitor cell although several studies have demonstrated the presence of precursors/stem cells either residing in the liver or coming from blood.

Regardless of the species in which were observed and the name that was given to them, the progenitor cells of the liver have common characteristics:

- they are very few and hardly recognizable in the healthy liver, but clearly evident as a result of chronic liver injury near the terminal trait of biliary duct;
- they express cholangiocyte and hepatocyte markers;
- they are basophilic, with a high ratio of nucleus/cytoplasm and are smaller than mature hepatocytes (10 μM in diameter compared to 50 of hepatocytes);
- they are immature and have a great proliferative capacity.

Further than oval cells, other bipotential precursor cells able to differentiate and colonize diseased liver in animal models have been isolated from rodent and human livers, allowing the study of molecular mechanisms triggering their differentiation. The identification and characterization of an immortalized bipotent precursor cell was firstly described by Spagnoli and coworkers (Spagnoli et al., 1998) in MMH cell lines. MMHs (Met Murine Hepatocyte) are immortalized cell lines derived from explants of embryonic, fetal and new-born livers derived from transgenic mice expressing a constitutively active truncated human Met receptor (cyto-Met) (Amicone et al., 1997). All of the MMH lines are not tumorigenic and show a differentiated phenotype judging from the retention of epithelial cell polarity and the expression of liver enriched transcriptional factors (LETF). In addition, many of them express hepatic functions. MMHs have been found to contain a cell subpopulation constituted by fibroblastoid cells, called "palmate cells" for their morphology, showing characteristics of a bipotent progenitor. The palmate cells are not polarized, do not express liver specific transcription factors or liver products, but retain the ability to divide and differentiate into hepatocytes and bile duct cells. Unequivocal demonstration that palmate cells can give rise to epithelial-hepatocytes is provided by cloning of individually fished cells and characterization of their progeny. Moreover, as true stem cells, palmate cells are diploid whereas their epithelial progeny is hypotetraploid. All of these findings demonstrate that palmate cells are the precursors of hepatocytes in MMH cell lines. These bipotential liver cells are also able to *in vivo* differentiate into hepatocytes and colonize diseased livers in mice (Spagnoli et al., 1998). Using the same methods of isolation and selection, Strick-Marchand and Weiss subsequently isolated, from mouse embryos wild-type, bipotent cells able to regenerate livers of mice uPA/SCID mice (Strick-Marchand & Weiss 2002). Bipotent progenitors were isolated and stabilized also from pig liver (Strick-Marchand et al., 2004), monkey (Talbot et al., 1994) and human fetal liver (Allain et al., 2002).

The identification of precursor cells has increasingly strengthened the idea that in the liver there are also real stem cells with a wide differentiation potential (capable of explaining many processes not yet fully understood such as liver development and regeneration) and which may give rise, by asymmetric division, to the same bipotent precursor cells.

The immunophenotypic characterization of the heterogeneous oval cell population, in which there are cells expressing hematopoietic stem cells (HSC) (eg, c-kit, CD34 and Thy-1) markers, had initially led to believe that oval cells could originate from the recruitment and differentiation of circulating HSC. In fact, many studies have demonstrated the ability of HSCs to differentiate into hepatocytes *in vitro* and their mobilization from the marrow and recruitment in the liver during regeneration. Two independent works (Wang et al., 2003; Vassilopoulos et al., 2003) however, have shown that stem cells derived from murine bone marrow and transplanted in FAH-/- mice, were involved in the regeneration of the damaged liver tissue through a process of cell fusion with endogenous hepatocytes rather than through a trans-differentiation process. The new hepatocytes in fact had both host and donor genetic markers. The events of trans-differentiation of HSC precursors into oval cells or hepatocytes documented to date are in fact extremely rare (Menthena et al., 2004; Grompe, 2003; Fausto, 2004; Thorgeirsson & Grisham, 2006). Mesenchymal-like cell population, depicting high level of proliferation and possessing a broad differentiation potential, has been isolated from adult human liver (Herrera et al., 2006; Najimi et al., 2007).

The efforts of different research groups is still directed towards the identification and isolation of a cell "resident" in the liver with stem cell characteristics, namely the ability to regenerate itself (self-renewal) and, more importantly, to divide asymmetrically, generating a cell identical to itself and a bipotent progenitor.

Reid and colleagues focused on human hepatic stem cells and highlighted as liver is comprised of different maturational lineages of cells both intrahepatically in periportal zone by the portal triads and extrahepatically in the hepato-pancreatic common duct (Turner et al., 2011). More in dectail, the intrahepatic stem cell niches have been located in the canals of Hering (for pediatric and adult livers) and in the ductal plates (for fetal and neonatal livers) (Schmelzer et al., 2007; Turner et al., 2011; Zhou et al., 2007). The extrahepatic niche was recently unveiled by the Reid's research group that demonstrated the presence of multipotent stem/progenitors in human peribiliary glands, deep within the duct walls, of the extrahepatic biliary trees (Cardinale et al., 2011 and 2012). These cells, which self-replicate, are positive for transcriptional factors typical of endoderm and surface markers typical of stem/progenitors and may express genes of liver, bile duct and pancreatic genes.

Conigliaro and colleagues recently reported the identification, the isolation from fetal and neonatal murine livers, the characterization and the reproducible establishment in line of a non-tumorigenic "liver resident stem cell" (RLSC), that proved to be a useful tool to study liver stem cell biology (Conigliaro et al., 2008). The immunophenotype of this cell (CD34- and CD45-) indicates a not hematopoietic origin and the transcriptional profile highlights the expression of a broad spectrum of 'plasticity-related genes' and 'developmental genes', indicating a multi-differentiation potential. Indeed, RLSCs not only differentiate spontaneously into hepatocytes and cholangiocytes (suggesting their partial endodermal determination), but can be induced *in vitro* to differentiate into osteocytes, chondrocytes and

cells of neuroectodermal derivation (astrocytes, neurons). The ability of RLSCs to differentiate spontaneously in hepatocytes, the lack of albumin and the wide differentiation potential place these liver stem cells at the pre-hepatoblast/liver precursor hierarchical position. Notably, RLSCs are also a model to *in vitro* study liver zonation. This term indicates the typical distribution into hepatic lobule of several functions. Most of the main metabolisms of the liver, in fact, are not uniformly distributed over the hepatic lobule but follow gradients of enzymatic activities along the centrolobular/portal axis. Coherently, adult hepatocytes undergo into a post-differentiation patterning resulting into a zonal heterogeneity of gene expression and functions defined "metabolic zonation". Specific enzymatic/metabolic activities, i.e. carbohydrate metabolism, ammonia detoxification, bile formation/transport/secretion and drug biotransformation, are confined to the perivenular (PV, i.e. near the centrolobular vein) or periportal (PP, i.e. near the portal vein) zones of the hepatic lobule (Gebhardt, 1992). The elucidation of the mechanisms responsible for induction and maintenance of the hepatocyte heterogeneity remains one of challenge in experimental hepatology. Intriguingly, inversion of the blood flow direction changes the enzymatic gradients and, consequently, the zonation of some, but not all, the liver metabolisms, thus revealing the influence exerted by the oxygen and circulating molecules on this phenomenon (Kinugasa & Thurman, 1986). For the bloodstream independent gradients, cell-cell and cell-extracellular matrix interactions and paracrine signaling have been suggested as instructive stimuli (Gebhardt & Reichen, 1994). Recently, concerning soluble factors, a key role of the Wnt/β-catenin pathway has been unveiled. Within the hepatic lobuli, Wnt signaling has been proposed to originate from endothelial cells of the central vein and follows a stable gradient that decrease toward the PV–PP axis. In the liver, Benhamouche and collaborators observed a mutually exclusive localization of activated β-catenin and its negative regulator APC in the PV and in PP hepatocytes, respectively. Moreover, these authors demonstrated that genetic manipulation of APC expression and adenoviral delivery of the extracellular antagonist of Wnts DKK allowed to switch the phenotype from PP into PV and vice versa (Benhamouche et al., 2006).

A second key element in controlling hepatic zonation was identified in the transcriptional factor HNF4α: Stanulovic and colleagues have recently shown that this orphan nuclear receptor regulates the zonal expression of some genes, including Cyp7, UDP-glucuronyltransferase and apolipoprotein E (Stanulovic et al., 2007). Their analysis of HNF4α knock-out mice revealed in PV hepatocytes a maintenance of PV genes expression and in PP hepatocytes the inhibition of a PP gene (PEPCK) coupled to the activation of PV genes. These observations led to the conclusion that HNF4α exerts a dual role of activator of PP genes and inhibitor of PV genes in PP hepatocytes. In frame with these observations Colletti and colleagues showed as RLSCs spontaneously differentiate into periportal hepatocytes that, following Wnt pathway activation, switch into perivenular hepatocytes. Moreover, they gathered evidences showing a direct convergence of the canonical Wnt signaling pathway and HNF4α in controlling the hepatocyte heterogeneity. HNF4α and Wnt signaling pathway have been proposed as active members of the same machinery that controls the transcription of differentially zonated HNF4–dependent genes (Colletti et al., 2009).

In conclusion we can say that there are no more doubts about the existence of liver stem cells residing in the liver although there is still much to do especially with regard to the identification and characterization of specific microenvironments able to define the corresponding tissue stem- niche.

4. Molecular mechanisms controlling liver stem cell fate

A stem cell "niche" is believed to maintain the liver progenitor cells in a native state and allows their activation when required. It is conceived as a restricted area in an adult organ that regulates, by means of micro-environmental signaling, stem cell maintenance and differentiation. Stem cell behavior, in particular the balance between self-renewal and differentiation, is ultimately controlled by the integration of autocrine and paracrine factors supplied by the surrounding microenvironment. Stem cells respond to these instructive signals from the niche by changing their expression profile in a reversible manner. In particular, instructive signals received from the niche influence the so-called stem cell "metastable" phenotype. The metastability, currently considered a common characteristic of embryonic and adult stem cells and a manifestation of cell plasticity (McConnell &Kaznowski, 1991; Hay, 1995; Thomson et al., 1998; Blau et al., 2001; Burdon et al., 2002; Reddy et al., 2002; Prindull & Zipori, 2004), consists essentially in the cell capability to change the expression profile in a reversible manner and it is characterized by the co-expression of both epithelial and mesenchymal traits. This highly dynamic cell state may be considered as a balance between epithelial-mesenchymal and mesenchymal-epithelial transitions (EMT/MET). Both the EMT and the reverse process MET are typical events of development, tissue repair and tumor progression. The EMT is the process by which polarized cells, closely attached to each other, gradually lose epithelial features and acquire mesenchymal characteristics, including invasiveness and motility (Thiery et al., 2009). MET refers to the reverse phenomenon often occurring in a secondary site, by which the epithelia-derived mesenchymal cells reacquire their epithelial phenotype.

The observation that a number of stem cells are restricted to a specific differentiation fate suggests that elements pivotal for their metastability and for the coordinated execution of opposite processes, such as self-renewal and differentiation, may be tissue specific. A simple and direct molecular mini-circuitry of master elements of mutually exclusive biological processes, able also to reciprocally influence their own expression, may provide the best device to trigger such complex phenomena.

The availability of a stable stem cell line executing specific differentiation programs discloses a unique possibility to investigate mechanisms regulating alternative cellular choices.

Recently, RLSCs and hepatocytes derived from their differentiation (RLSCdH) permitted to identify a simple cross-regulatory circuitry between HNF4α (master regulator of hepatocyte differentiation and MET inducer) and Snail (master regulator of the EMT), whose expression is mutually exclusive due to their direct reciprocal transcriptional repression (Cicchini et al., 2006; Santangelo et al., 2011). In particular, Cicchini and co-workers showed that Snail represses the HNF4α transcription through the direct binding to its promoter (Cicchini et al., 2006) and that Snail over-expression is sufficient i) to induce EMT in hepatocytes with change of morphology, down-regulation of several epithelial adhesion molecules, reduction of proliferation and induction of matrix metalloproteinase 2 expression and, ii) most relevantly, to directly repress the transcription of the HNF4α gene. These findings demonstrated that Snail is at the crossroads of the regulation of EMT in hepatocytes by a dual control of epithelial morphogenesis and differentiation. More recently, Santangelo and colleagues collected evidence that HNF4α has a direct master role in the MET process of the

hepatocyte and that its differentiation role is intrinsically linked to an active repression of mesenchymal program expression (Santangelo et al., 2011). Their data highlight as both, key EMT regulators (Snail and Slug) and mesenchymal genes, have to be included among the target genes relevant for HNF4α1 master function in controlling epithelial phenotype. Their main finding was to ascribe to HNF4α1 a general "anti-EMT" role through the orchestrated repression of both master EMT regulators and mesenchymal markers. HNF4α-mediated repression of mesenchymal gene program, moreover, is executed not only in the dynamic EMT/MET processes but also in the stable maintenance of the hepatocyte epithelial phenotype. In fact, they found that: in dedifferentiated hepatomas HNF4α1 ectopic expression was sufficient to down-regulate Snail, Slug, HMGA2, Vimentin and Fibronectin genes. In addition, in differentiated hepatocytes, HNF4α1 was found stably recruited to the promoters of EMT inducers and its knockdown caused the upregulation of these genes.

Consistent with these observations Garibaldi and colleagues (Garibaldi et al., 2011)Garibaldi et al., in press demonstrated that the same molecular players in an epistatic mini-circuitry are pivotal for the RLSC maintenance. In particular they observed that hepatic stem cells constitutively express Snail and that their spontaneous differentiation into hepatocytes is underlined by negative regulation of Snail expression. Snail silencing causes down-regulation of stemness markers and its ectopic expression in hepatocytes is sufficient to restore their expression. In RLSC Snail stably represses HNF4 and miR-200a-b-c and miR-34a, known as stemness inhibiting microRNAs and distinctive of epithelial cells. This latter activity is probably due to a direct mechanism as suggested by the binding of endogenous Snail to miR-200c and 34a promoters in RLSC. In terms of conceptual advances, these data allow to extend the role of Snail from EMT inducer to stemness stabilizer.

In the light of the previously demonstrated reciprocal repression between Snail and HNF4α these observations have been extended: Garibaldi and colleagues described that HNF4α is required for miR-200a-b-c, and miR-34a expression in hepatocytes and that HNF4α silencing in hepatocytes and its targeting in KO mouse models correlates with a strong down-regulation of their expression. This is probably due to a direct mechanism as suggested by the fact that endogenous HNF4α was found recruited on miR-200a-b, miR-200c and miR-34a promoters in both differentiated hepatocytes and mouse liver. Notably, in HNF4 KO mouse models miRs down-regulation correlates to a strong up-regulation of the stemness markers SCA1 and FOXA1. Thus HNF4α, first identified as a positive regulator of hepatocyte differentiation and recently located at the crossroad of other cellular functional categories (i.e. cell cycle, apoptosis, stress response) appears to participate also in the active repression of stemness.

The proposed mechanism implies that the execution of a stemness program requires the active repression of a differentiation program while the maintenance of the hepatocyte one requires the active repression of stemness traits. These observations, focusing on epithelial differentiation, are centered on a HNF4α/Snail/epithelial-miRs circuitry, however may be conceivable that other differentiation pathways could be regulated by similar mechanisms. In this light Snail can probably be considered as a general factor counteracting (and counteracted by) tissue-specific regulators. This is further suggested by studies indicating that Snail family members repress the expression of tissue-specific inducers as the pro-neural genes sim and rho (Xu et al., 2010) and the skeletal muscle master regulator MyoD (Kosman et al., 1991).

5. Hepatocyte transplantation in cell-based therapeutic

Animal models in which transplanted cells show a selective advantage over resident hepatocytes have been used to study transplantation, proliferation and reconstitution potential of the hepatocytes. Liver animal models belong to three groups (Palmes & Spiegel 2004): i) hepatotoxin-induced models; ii) surgical models; iii) animal models of hereditary liver defects.

Normal adult hepatocytes can be serially transplanted and single hepatocyte can be clonally amplified, showing stem-like properties, and serially passaged to repopulate almost 70% of the liver of (Fah)-deficient mice (Overturf et al., 1999). Excellent results have been obtained by using transgenic *Rag2$^{-/-}$/Il2rg$^{-/-}$* mice (deficient for the recombinant activation gene-2 and the common γ-chain of the interleukin receptor) (Traggiai et al., 2004) or the *Alb-uPA(tg(+/-)* mice (expressing the uroplasminogen activator (uPA) under the transcriptional control of the albumin promoter) (Sandgren et al., 1991)) or mice obtained by the crossing of the above reported genotypes (Haridass et al., 2009; Azuma et al., 2007).

Hepatocyte transplantation protocols in humans have been proposed as an alternative to orthotropic liver transplantation in patients and used for some metabolic disorders i.e. familial hypercholesterolemia, glycogen storage disease type 1a, urea cycle defects and congenital deficiency of coagulation factors (Quaglia et al., 2008). Currently, the liver transplantation is the treatment of choice for acute and chronic end-stage liver failure and for diseases refractory to other treatments; but the limited availability of donor organs is the major limiting factor in this therapeutic procedure. Although different techniques of implants using either complete liver, liver reduced or hyper-reduced "split liver" (liver for two) have tried to overcome the shortage of organs, liver transplantation remain an unsufficient approach to satisfy the needs of patients with liver disease.

In recent years, hepatocyte transplantation has emerged as a potential alternative or complementary procedure to liver transplantation, at least in certain circumstances. The application of this therapeutic modality is based on the concept that cell transplantation would replace the function of the affected organ, either temporarily, allowing the recovery of the organ functionality or the availability of a liver for the transplant, or permanently, preventing need for this last procedure.

The development of this therapeutic approach could provide a new opportunity for patients with liver disease, particularly for children suffering from some metabolic diseases, with certain advantages over liver transplantation. In fact it is a less invasive and risky procedure and it has a lower cost. There is also a greater availability of material to be transplanted and that could be used as a source of cells (organs considered "marginal", material resulting from organ reductions, from partial hepatectomy and cadaveric livers unsuitable for transplantation) and the possibility of using a donor to several recipients.

Despite these advantages, a number of critical issues are still unresolved: the rejection of transplanted hepatocytes, their correct localization and functionality and, mostly, cells availability at the right time. The latter remains a problem that would be definitively solved with the cultivation and the preservation of large scale culture of hepatocytes. Nevertheless, these cells in culture, contrary to what happens *in vivo* during liver regeneration, have a very low proliferative potential and quickly lose their differentiated characteristics.

This implies that cell therapy can be carried out only with freshly isolated cells, not expanded *in vitro*. The number of cells that can be achieved with this approach is usually not sufficient to colonize adult livers, while there is more chance of success in pediatric patients with metabolic diseases of genetic origin since they can be treated with a limited number of hepatocytes.

6. Conclusion

Liver stem cells may represent an important tool for the treatment of the liver diseases. They could be an alternative source of functional hepatocytes aimed at cell transplantation, tissue engineering and bio-artificial liver. Manipulation of stem cells will be more efficient since we know the factors controlling their biology. Only by dissecting the molecular events underlying the stemness, the differentiation choice and the maintenance of the differentiated phenotype can we control stem cell behavior for therapeutic purposes. The translation of *in vitro* studies in *in vivo* experimental models and, finally, in humans is one of the major challenges of experimental hepatology. Moreover, better understanding the mechanisms that control the proliferation of stem and progenitor cells will shed new light on the molecular and cellular basis of liver cancer.

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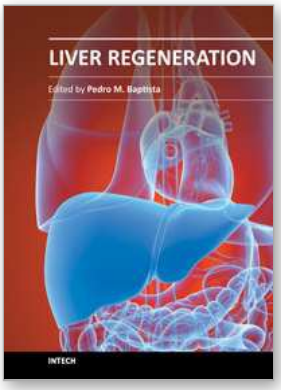

Liver Regeneration

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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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